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RESEARCH ARTICLE

Population genetics of a widely distributed small freshwater fish with varying conservation concerns: the southern purple-spotted gudgeon, *Mogurnda adspersa*

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Abstract Genetic variation plays a pivotal role in species viability and the maintenance of population genetic variation is a main focus of conservation biology. Threatened species often show reduced genetic variation compared to non-threatened species, and this is considered indicative of lowered evolutionary potential, compromised reproductive fitness, and elevated extinction risk. The southern purple-spotted gudgeon, *Mogurnda adspersa*, is a small freshwater fish with poor dispersal potential that was once common throughout the Murray–Darling Basin (MDB) and along the central east coast of Australia. Its numbers and distribution have shrunk dramatically in the MDB due to flow alteration, degradation of habitat, decreasing water quality, and introduction of alien species. We used microsatellite DNA markers to assess population structure and genetic variation at both large (i.e. across basin) and fine (i.e. within river catchments) spatial scales using a substantial sampling effort across the species range (n = 579 individuals; 35 localities). The results consistently indicated very low levels of genetic variation throughout, including along the east coast where the species is relatively common. At the broader scale, three highly differentiated groups of populations were found, concordant with previously reported genealogical distinctiveness. Hence we propose each group as a distinct Evolutionarily Significant Unit. We also inferred a minimum of 12 management units in *M. adspersa*, with no appreciable gene flow between them. Our study discloses findings relevant for both long- and short-term management, as it informs on the geographic context in which conservation priorities should be defined and specifies biological units for population monitoring and translocations.

Keywords Conservation genetics · Freshwater fish · Endangered biodiversity · Ecological genetics · Phylogeography · Population connectivity

Introduction

Genetic diversity is known to play a pivotal role in species viability, and thus the maintenance of genetic variability at the population level is a key focus of conservation biology (Avise 2004; Frankham et al. 2002). Genetic diversity reflects variability in heritable characters, including phenotypes and DNA sequence variation (Frankham et al. 2002). Many factors and processes influence spatial and temporal variations in levels of genetic diversity. In particular, gene flow and genetic drift are two of the main microevolutionary processes affecting levels of genetic diversity between and within populations (Hedrick 2009).

Gene flow strongly influences pattern and process of population genetic structure and can in principle be

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quantified by assessing levels of genetic differentiation between populations. Gene flow can introduce new alleles to genetically different populations, and hence increase genetic diversity (Frankham et al. 2002). Gene flow also shapes metapopulation dynamics in which local extinction can be rescued by recolonization (Hanski 1998). Obviously, rates of gene flow vary among species and are critically dependant on life history, local environment and biogeography.

For isolated populations, genetic drift is perhaps the crucial factor shaping levels of genetic diversity and evolutionary potential (Hedrick 2009). A completely isolated population i.e. where there is no gene flow between populations, is the most severe form of fragmentation (Frankham et al. 2002). Populations in such a fragmented state may suffer strongly from a loss of genetic diversity due to the stochastic effects of genetic drift, whereas increased allelic similarity due to inbreeding may lead to population extinctions (Frankham et al. 2002). The rate of such effects is faster in smaller populations because the likelihood of allele loss is higher when effective population sizes (*Ne*) are small (Hughes et al. 1999; Frankham et al. 2002; Hedrick 2009). The resultant inbreeding may lead to reductions in fecundity, survival of young, and body size (Amos and Balmford 2001; Weiss 2005; Thrower and Hard 2009) due to deleterious allele combinations. Small populations under the influence of the above effects are also susceptible to disease and stochastic events (Frankham 2003) and, as a consequence, the number of reproductive individuals (*Ne*) decreases even more. Importantly, the residual consequences due to reduction in *Ne* will persist for a long time, even if the original cause of population fluctuation is eliminated (Frankham 2003).

Understanding the potential genetic effects of isolation is important for the conservation and management of species, and is a particular challenge for freshwater organisms due to the highly structured and variable nature of the environment. Unlike terrestrial or marine environments, rivers form a dendritic network in which the movement of species is restricted (Fagan 2002; Hughes et al. 2009). Correspondingly, freshwater species such as fishes often display low intra-population and high inter-population genetic diversity (e.g. Poissant et al. 2005; Neville et al. 2006; Faulks et al. 2010a), especially when compared to marine fishes (Beheregaray and Sunnucks 2001; Sala-Bozano et al. 2009; Jones et al. 2010). In addition to natural heterogeneity, rivers have been changing as a result of human use (Strayer and Dudgeon 2010) and rapid climate change (Cai and Cowan 2008; Coumou and Rahmstorf 2012). Extensive flow regulation through the widespread construction of dams and weirs has resulted in greatly increased fragmentation, through both direct barriers and pervasive alteration to flow regimes (Nilsson et al. 2005), while drought exacerbates this loss of connectivity and habitat loss for many freshwater species (Cai and Cowan 2008).

Reflecting a continent with comparatively low rainfall and a high proportion of its population and agriculture concentrated in the drier southern half, Australia's temperate freshwater systems have been heavily impacted by all of the above-mentioned causes of habitat fragmentation. Most Australian temperate catchments have experienced extensive river modifications (Lloyd et al. 2004), and recent extreme droughts have cumulated in critical impacts, especially in the heavily modified Murray–Darling Basin (MDB) (e.g. Hammer et al. 2013; Ellis et al. 2013; Kingsford et al. 2011). To explore the consequences of fragmentation on the aquatic fauna of this region, we investigated population connectivity and genetic diversity in a small Australian freshwater fish, the southern purple spotted-gudgeon, *Mogurnda adspersa*, as a model species to link broad- and fine-scale patterns of population isolation to species and habitat conservation.

Mogurnda adspersa is a habitat specialist that requires dense cover in the form of physical elements and aquatic vegetation in off-channel wetland habitats (Hammer et al. 2015). The species displays an idiosyncratic distribution pattern of being a widespread species with very limited dispersal ability (Hughes et al. 2012; Shipham et al. 2013). The species as currently defined (Allen and Jenkins 1999) occurs in tropical to temperate coastal drainages in Eastern Australia, and into the inland MDB. However, there is genetic evidence of a species level divide north and south of the Burdekin Gap on the east coast (Adams et al. 2013), and additional strong phylogeographic signal within the southern candidate taxon suggesting that populations in the MDB were separated from coastal populations approximately 1.4–1.8 million years ago (Faulks et al. 2008). There are contrasting conservation concerns between these coastal and inland lineages, with *M. adspersa* considered common on the east coast (apart from possible local extinction in the Richmond and Clarence rivers, New South Wales: Pusey et al. 2004), but endangered in the MDB following a dramatic decline in numbers and distribution being listed as endangered in New South Wales, critically endangered in South Australia and presumed extinct in Victoria (Wager and Jackson 1996; Lintermans 2007; Hammer et al. 2009). In the MDB scattered populations remain in northern Darling River tributaries, while the species was considered extirpated across a large area of former habitat in the southern Murray and Murrumbidgee rivers until the recent discovery of a single remnant population in the lower portion of the system (Hammer et al. 2015). This population was nonetheless impacted by over abstraction of water and prolonged drought and its habitat completely dried in 2007. Fortunately, the Lower Murray

M. adspersa was rescued before "re-extirpation" and included within a "Drought Action Plan" for fishes at very high risk of extirpation in the lower MDB that conducted several ex situ and in situ management actions (described in Hammer et al. 2013, 2015).

Given its wide distribution and patchy regional decline, *M. adspersa* might be expected to show different spatial levels of population connectivity and genetic 'health' across its range. We therefore predict that endangered MDB populations will exhibit even lower rates of gene flow than the low rates previously found for non-threatened east coast populations (Hurwood and Hughes 1998; Hughes et al. 2012), even at very local scales (Shipham et al. 2014). Comparisons of population connectivity and genetic diversity between endangered and non-endangered populations should provide new insight into the spatial structure in this species (Hughes et al. 2012). Our study aims to apply novel microsatellite markers developed for the MDB taxon to conduct two distinct but complementary surveys of genetic variation in *M. adspersa*. Firstly, the study originally assessed patterns of genetic variation and differentiation by comprehensively sampling across three major river basins. This is expected to inform on levels of genetic isolation and conservation value of populations at a river basin scale, plus assist with identification of management units at particular risk of extinction. Secondly, comparisons of replicated samples within particular river basins will assess whether there is any relationship between contemporary genetic connectivity and riverine distance. These large- and fine-scale surveys of bi-parental genetic variation are expected to clarify aspects of life-history in the species and improve conservation management practices over a range of spatial scales, including ongoing captive breeding and restoration genetic programs of *M. adspersa.*

Materials and methods

Sampling

A total of 583 individual samples were collected from 35 locations spread across three regions: the Murray–Darling Basin, Southeast coast division and Northeast coast division (Fig. 1; Table 1). Sampling was done by seine net, dip net or bait traps. Fin clips or entire individuals were either stored in 95 % ethanol or in liquid nitrogen at the field. From the total of 583 fish, muscle tissues of 488 fish were dissected from frozen specimens curated at the South Australian Museum, whereas 95 were obtained from a previous study (Faulks et al. 2008). The study areas covered most of the known distribution of the *M. adspersa* "southern" taxon of Adams et al. (2013) i.e. south of Townsville, including Queensland, New South Wales and South Australia (Table 1). Samples from South Australia (SA—site #1, Table 1) include specimens from a captive breeding program based on rescued wild populations from Jury Swamp in the lower MDB (Hammer et al. 2015).

Genetic methods

Total DNA was extracted using a modified salting-out protocol (Sunnucks and Hales 1996). Microsatellite polymorphism was quantified at 14 newly-characterized loci (Carvalho et al. 2011). The microsatellite polymerase chain reactions (PCRs) consisted of approximately 3 ng/ul of template DNA, 1 uL of 5X reaction Buffer, 15 mM of MgCl2, 0.2 mM of dNTPs, 0.02 uM of forward primer, 0.1 uM of reverse primer, 0.1 uM of M13 fluorescent primer, 0.05 Units of Mango Taq DNA polymerase, 0.1 mg/ml of BSA and 1.45 uL of dH_2O (total of 5 ul reaction). Amplification was carried out using the modified touchdown cycling of Beheregaray and Sunnucks (2000); starting with 94 °C for 3 min and followed by 94 °C for 20 s, annealing for 45 s and 71 °C for 1 min. Annealing temperature of first cycle starts with 63 °C and decreases by 2 °C until 55 °C. The first four cycles were run once and the last cycle was run 35 times to maximise the specificity of amplification. Amplification products were detected in an automated DNA sequencer ABI 3130 (Applied Biosystems). Microsatellite profiles were examined using GENEMAPPER 4.0 (Applied Biosystems) and peaks scored manually. Samples with poor amplification were repeated with increasing template DNA to 30 ng. Scoring errors due to null alleles and large allele dropout were checked with MICROCHECKER (Van Oosterhout et al. 2004).

Analysis of genetic variation

Statistical assessments of observed genotype frequencies versus those expected under Hardy–Weinberg equilibrium (HWE) were undertaken for each population and over all loci using GENEPOP v4 (Rousset 2008). Sequential Bonferroni correction was employed to adjust probability values to reflect the use of multiple statistical tests (Rice 1989). Genetic variation at the population level was quantified by estimating the number of alleles (*NA*), the percentage of polymorphic loci (% poly loci), and expected (*He*) and observed (*Ho*) heterozygosity in GENALEX 6.4 (Peakall and Smouse 2006). Allelic richness taking rarefaction into an account (*AR*) was estimated by HP-RARE 1.1 (Kalinowski 2005). Inbreeding index (*Fis*) was estimated in FSTAT 2.9.3 (Goudet 2001).

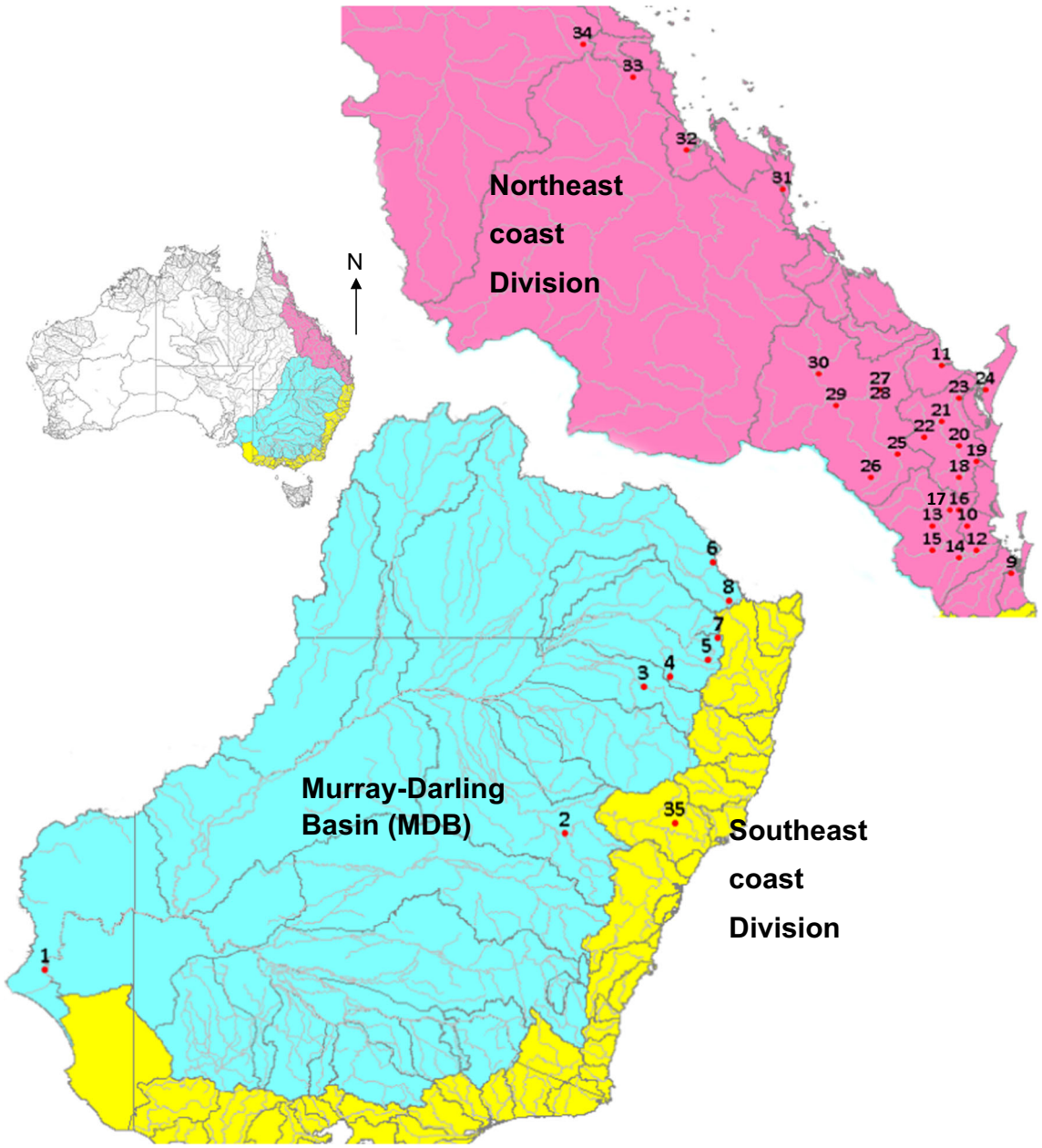

Fig. 1 Map of Australia (*inset*) showing the sampling locations for *Mogurnda adspersa* in the Southeast and Northeast coast divisions and in the Murray–Darling Basin. The *number* associated with each location can be found in Table 1

Analysis of population structure

We used SPAGEDI 1.5 (Hardy and Vekemans 2002) to assess whether Fst or Slatkin's *R*st (1995) was the most appropriate index to estimate population differentiation. When observed *R*st is significantly greater than permuted *R*st (p*R*st), mutation is likely to contribute to the genetic diversity, and hence Fst values are likely to be upwardly biased and therefore overestimate genetic differentiation (Hardy and Vekemans 2002; Hardy et al. 2003). Because SPAGEDI revealed that *R*st was significantly smaller than p*R*st (see Results), genetic differentiation among populations was estimated as pairwise Fst in ARLEQUIN 3.5.1.3 (Excoffier et al. 2005) using 1000 permutations of the data.

A hierarchical analysis of molecular variance (AMOVA) was carried out in ARLEQUIN to estimate genetic differentiation among defined groups, among populations within defined groups, among individuals within populations and within individuals. We first ran AMOVA for all populations as one group, then we divided them as eastern and western side of the Great Dividing Range (GDR) following Faulks et al. (2008) finding of a

Table 1 Information about the 35 sampling sites of *Mogurnda adspersa* used in this study

Basin	Rivers	Site	Map ID	Abbr	Latitude	Longitude
MDB						
	Lower Murray River	Jury Swamp	1	SA	35°06′08″S	139°18′32″E
	Macquarie-Bogan Rivers	Wuluuman Ck	2	WC	32°34′55″S	149°4′45″E
	Gwydir River	Bingara	3	BING	29°52′50″S	150°36′52″E
	Border Rivers	Inverell R	4	INV	29°41′50″S	151°06′01″E
	Border Rivers	Deepwater R	5	DPW	29°22′50″S	151°46′52″E
	Condamine-Culgoa Rivers	Toowoomba	6	TOOM	27°36′40″S	151°52′40″E
	Border Rivers	Tenterfield Ck	7	TEN	28°59′50″S	152°01′01″E
	Condamine-Culgoa Rivers	Farm Ck	8	FAM	28°17′50″S	152°11′52″E
Northeast coast basin						
	South Coast	Pimpama R	9	PIM	27°46′50″S	153°17′55″E
	Pine River	Pine R	10	PINE	27°11′40″S	152°47′50″E
	Burrum River	Gregorys Ck	11	GRE	25°10′55″S	152°30′24″E
	Brisbane River	Gap Ck	12	GAP	27°29′30″S	152°55′8″E
	Brisbane River	Esk Ck	13	ESK	27°11′40″S	152°24′20″E
	Brisbane River	Warril Ck	14	WRL	27°35′40″S	152°41′59″E
	Brisbane River	Laidley Ck	15	LAI	27°31′20″S	152°25′8″E
	Brisbane River	Delaney Ck	16	DEL	26°58′45″S	152°41′50″E
	Brisbane River	Kilcoy Ck	17	KIL	27°00′48″S	152°36′00″E
	Mary River	Little Yabba Ck	18	LYC	26°35′50″S	152°40′50″E
	Mary River	Cooroora Ck	19	COO	26°23′30″S	152°52′50″E
	Mary River	Cunningham Ck	20	CUN	26°12′50″S	152°38′50″E
	Mary River	Mary R	21	MR	25°55′55″S	152°30′24″E
	Mary River	Wide Bay Ck	22	WBC	26°03′55″S	152°14′24″E
	Mary River	Tinana Ck	23	TNN	25°30′55″S	152°40′24″E
	Fraser Island	Fraser Is	24	FRS	25°39′30″S	152°59′48″E
	Burnett River	Barambah Ck	25	BRB	26°15′55″S	152°00′55″E
	Burnett River	Reedy Ck	26	REE	26°31′48″S	151°42′14″E
	Burnett River	Sunday Ck	27	SUN	25°29′50″S	151°48′30″E
	Burnett River	Bin Bin Ck	28	BIN	25°29′50″S	151°48′30″E
	Burnett River	Boyne R	29	DCB	25°40′37″S	151°05′51″E
	Burnett River	Burnett R	30	BUR	25°15′55″S	151°07′24″E
	Water Park Creek	Maryvale Ck	31	MVC	22°55′40″S	150°40′30″E
	Styx River	Amity Ck	32	AMI	22°32′40″S	149°37′30″E
	Fitzroy River	Prospect Ck	33	PRO	21°35′40″S	149°01′30″E
	Burdekin River	Broken Ck	34	BRO	21°10′55″S	148°24′45″E
Southeast coast basin						
	Hunter River	Upper Hunter R	35	HUN	32°23′39″S	151°11′14″E

Map ID refers to numerical ID used in Fig. 1. Abbr is the code given to each sample site

deep mtDNA split between the MDB lineage (western group) and coastal lineage (eastern group). Eastern group contained SA, WC, BING, INV, DPW, TOOM, TEN and FAM and western group consisted of the remaining populations (Table 1).

Population genetic structure was further estimated without prior knowledge of sample location by the Bayesian clustering method implemented in STRUCTURE 2.3.4 (Pritchard et al. 2000). Ten independent STRUCTURE runs were conducted for each putative genetic cluster K (i.e. K tested between 1 and 35). Each run used a burn in of 100,000 and a Monte-Carlo Markov Chain (MCMC) of 1000,000. Resulting runs were analysed in STRUCTURE HARVESTER (Earl and vonHoldt 2011) to estimate appropriate K following Evanno et al. (2005). Since the samples were collected across a very broad distributional range, primary population clusters were expected to be identified first by STRUCTURE. For this reason,

each primary cluster from the first run was further analysed by STRUCUTRE using the same parameters.

We also used an assignment test in GENECLASS 2.0 (Piry et al. 2004) to assess the reliability of the STRUCTURE results. This spatial assignment test uses frequencies of multilocus genotypes of an individual to compute its likelihood to be assigned to the reference population based on simulated likelihood of genotypes (Piry et al. 2004). This is regarded as a valuable complementary approach to STRUCTURE, as it employs different algorithms and increases the accuracy of individual assignment to a population (Manel et al. 2002; Negrini et al. 2008). The Bayesian criterion (Rannala and Mountain 1997) with the number of simulated individuals being 10,000 and an alpha of 0.01 and the leave-one-out method (Paetkau et al. 2004) were used.

An UPGMA tree using the chord genetic distance *D*ce (Cavalli-Sforza and Edwards 1967) was also constructed. *D*ce was selected because it is known to perform well in constructing tree topology in populations largely impacted by drift i.e. in cases where geographic isolation largely dictates population structure (Takezaki and Nei 1996). UPGMA was employed as it is useful for populations with similar evolutionary rates (Nei 1987), as expected within species comparisons. A matrix of *D*ce genetic distances was first estimated using GENDIST in PHYLIP 3.69 (Felsenstein 1989), and used to construct an unrooted UPGMA tree in NEIGHBOR, also in PHYLIP 3.69. The original data set was bootstrapped 1000 times in SEQBOOT in PHYLIP 3.69, a UPGMA tree of 1000 replicates was generated, and the program CONSENSE in PHYLIP 3.69 to create a consensus tree averaged over 1000 replicates. The resulting tree was visualised in FIGTREE v1.4.2 (Rambaut 2009).

Comparison of genetic diversity among regions

Prior knowledge about *M. adspersa* including (i) lower haplotype diversity in the MDB populations (Faulks et al. 2008) and (ii) critically endangered status of the MDB populations, allowed us to test for differences in genetic diversity between MDB and coastal lineages. The mean of *A*R and *He* from the groups identified at regional scale by STRUCTURE was compared (IBM SPSS version 20). Test of normality and homoscedasticity of data indicated that one-way ANOVA and Kruskal–Wallis test were appropriate tests for *A*R and *He* respectively.

Analysis of isolation by distance

Mantel tests (Mantel 1967) were applied to investigate whether genetic differentiation between populations is related to spatial distance between their locations. This was conducted in GENALEX 6.501 using linearized Fst [Fst/(1−Fst)] (Rousset 1997) and riverine/linear distances between populations. Riverine distances were calculated in DIVA GIS 7.5.0.0 (Hijmans et al. 2001). When all sites were included in the analysis, the Euclidean distance was calculated from coordinates because it is not possible to obtain riverine distance between sites from different drainages. The test for isolation by distance was carried out on the whole dataset and for populations within the same river, when five or more sites were sampled as fewer sites may affect statistical accuracy.

Genetic diversity versus genetic uniqueness

Theoretically, an increase in genetic differentiation (and uniqueness) between populations should lead to a reduction in within-population genetic diversity due to increased genetic drift and reduced gene flow. Prioritising management based on differentiation alone may be detrimental to the maintenance of genetic diversity because of the strong negative relationship usually found between population differentiation and diversity (Coleman et al. 2013). If this pattern is observed, a '*local is best*' management strategy would not be ideal for translocation efforts because local populations might not contain sufficient genetic diversity (Sgrò et al. 2010). In order to assess for relationships between genetic diversity and uniqueness in *M. adspersa* and to optimize conservation planning for MDB populations, we followed the approach of Coleman et al. (2013) with modifications. This was done by regressing expected heterozygosity and allelic richness against population specific Fst for each regional group independently. Although Coleman et al. (2013) used the mean number of alleles, we chose allelic richness because our sample size varies substantially among populations (Table 2). Population specific Fst was estimated in GESTE v2 using default settings (Foll and Gaggiotti 2006). Subsequently, contribution of genetic diversity of each population towards regional groups was assessed using METAPOP 1.0.3 (Pérez-Figueroa et al. 2009). This program uses genotypes to calculate (i) the contribution of each sub-population to the total genetic diversity of the population (regional group in this study), (ii) gain/loss of genetic diversity of the population after removing a sub-population from the regional population and (iii) the contribution of each sub-population to the population with higher genetic diversity. Datasets were divided into three regional groups based on STRUCTURE results and separate analyses were run for each group. Each group was considered as a whole population while each site in a group was treated as a sub-population. This complements the previous analysis of the relationship between genetic diversity and uniqueness and enables strategic selection of populations for conservation management.

Table 2 Genetic parameters for 579 individuals of *Mogurnda adspersa* sampled from 35 populations

SITE	N	NA	AR	% Poly loci	Ho	He	Fis
SA	56	3.36	1.88	78.57	0.41	0.41	0.025
WC	18	1.50	1.22	28.57	0.11	0.11	0.051
BING	24	2.43	1.58	64.29	0.29	0.29	0.004
INV	2	1.50	1.50	42.86	0.32	0.21	−0.286
DPW	4	2.14	1.83	50.00	0.46	0.32	−0.322
TOOM	7	1.64	1.35	42.86	0.17	0.17	0.064
TEN	26	2.00	1.47	57.14	0.23	0.23	0.053
FAM	21	2.57	1.66	71.43	0.32	0.31	−0.010
PIM	5	2.14	1.73	71.43	0.39	0.32	−0.093
PINE	33	5.14	2.28	100.00	0.54	0.55	0.031
GRE	7	3.57	2.25	85.71	0.53	0.52	0.049
GAP	20	3.00	1.86	85.71	0.41	0.40	0.022
ESK	10	2.00	1.53	71.43	0.25	0.26	0.097
WRL	20	3.71	2.06	92.86	0.49	0.48	0.002
LAI	16	2.86	1.87	78.57	0.36	0.40	0.148
DEL	22	4.93	2.22	100.00	0.50	0.53	0.081
KIL	10	4.79	2.41	100.00	0.61	0.58	0.008
LYC	5	2.93	2.09	78.57	0.39	0.42	0.183
COO	20	5.36	2.47	85.71	0.60	0.59	0.015
CUN	7	3.00	1.99	78.57	0.46	0.43	0.024
MR	10	3.21	2.02	78.57	0.44	0.43	0.048
WBC	15	6.79	2.64	92.86	0.64	0.64	0.040
TNN	30	6.07	2.30	85.71	0.51	0.53	0.066
FRS	28	4.86	2.00	92.86	0.39	0.44	0.125
BRB	30	8.00	2.46	92.86	0.61	0.59	−0.018
REE	27	7.29	2.65	100.00	0.59	0.68	0.144
SUN	10	6.79	2.88	100.00	0.71	0.73	0.075
BIN	24	8.93	2.83	100.00	0.65	0.72	0.113
DCB	5	5.43	3.08	100.00	0.73	0.74	0.123
BUR	8	5.79	2.81	100.00	0.70	0.69	0.063
MVC	3	3.50	2.72	92.86	0.60	0.59	0.187
AMI	6	4.43	2.55	92.86	0.55	0.60	0.175
PRO	10	5.07	2.34	85.71	0.51	0.52	0.070
BRO	10	4.71	2.22	85.71	0.45	0.48	0.113
HUN	30	2.14	1.61	78.57	0.31	0.32	0.038

Number of samples used for genetic analysis (*N*), mean number of alleles (*NA*), allelic richness (*AR*), percentage polymorphic loci (*% Poly loci*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), mean inbreeding index (*Fis*)

Results

Genetic variation

All 14 microsatellite loci were amplified for all 35 populations, resulting in a final dataset comprising the multilocus genotypes of 579 fish. MICROCHECKER showed no evidence for scoring errors or large allele dropouts and suggested null alleles in two loci (Mog4 and Mog10), but this result was observed in only four out of 35 population datasets. After Bonferroni correction, 13 out of 35 populations exhibited deviations from HWE in only one or two loci. All loci were kept for further analyses since deviations were not consistent across populations.

Number of alleles ranged from 1.5 (WC and INV) to 8.93 (BIN) and levels of allelic richness were consistently very low across populations, ranging from 1.22 (WC) to 3.08 (DCB) (Table 2). The percentage of polymorphic loci ranged from 28.57 % in MDB's WC, to 100 % in eight east coastal sites (Table 2). Mean expected heterozygosity and observed heterozygosity ranged from 0.11 and 0.11 respectively in WC, to 0.74 and 0.73 respectively in the coastal DCB. Most sites exhibited positive *F*is suggestive of inbreeding, except INV, DPW, FAM, PIM and BRB (Table 2).

Population genetic differentiation

The comparison of observed *R*st and p*R*st revealed that overall p*R*st was significantly higher than *R*st (Table S1), indicating that genetic drift is likely to contribute more to genetic diversity than mutation in this dataset and that Fst is a more appropriate statistic for assessing population differentiation.

Remarkably high genetic differentiation was observed between most population pairs based on Fst (Fig. 2; Table S2). The lowest pairwise Fst of 0.034 ($P < 0.05$) was found between sites BIN and SUN within the Burnett River; the highest (Fst = 0.774; $P < 0.05$) was between WC (MDB) and ESK (Brisbane River) (Table S2). Overall, MDB populations showed much higher population structure than coastal populations (Table S2). Strong population structure was also supported by AMOVA, with 40.88 % of genetic variation explained by differences among populations (Table 3A). When populations were grouped on each side of the GDR, the variation between regions was also substantial, 17.15 %, as well as among populations within each region, 29.66 % (Table 3B).

Bayesian and hierarchical clustering of populations

The Bayesian cluster approach in STRUCTURE initially detected three distinct genetic clusters of populations (Fig. 3a). This represents the strongest hierarchical split in the species, and includes two coastal groups [South East Queensland (SEQ—yellow cluster) and Central East Queensland (CEQ—orange cluster)] and one west of the GDR (the MDB—blue cluster). Unexpectedly, the geographic isolate Hunter River population (HUN) grouped with the CEQ cluster. Further splits at smaller regional

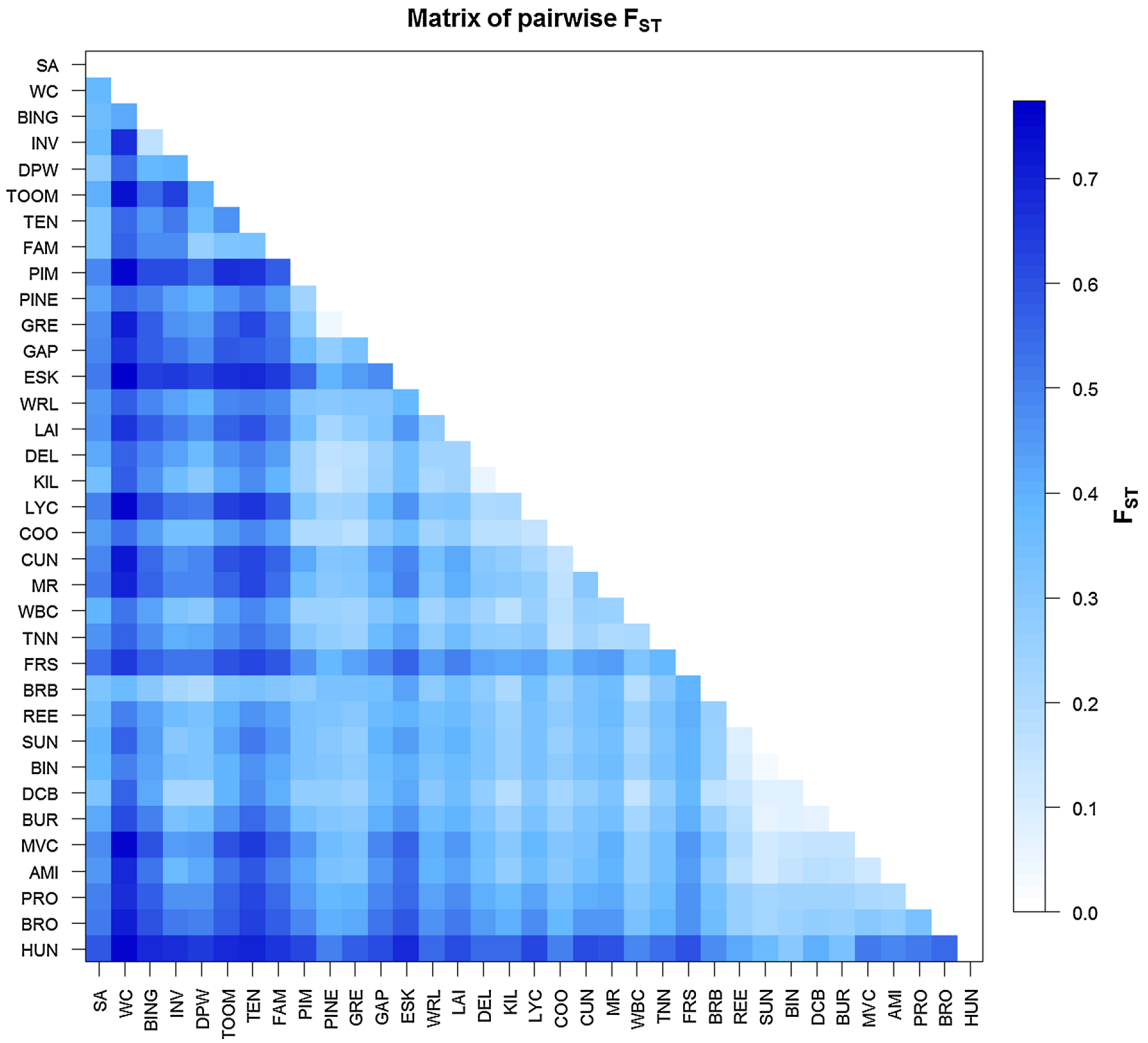

Fig. 2 Heat map of population differentiation measured as pairwise F_{ST} for all population pairs of *Mogurnda adspersa*

Table 3 AMOVA for *Mogurnda adspersa* based on Fst. A) Analysis for all population as one group and B) analysis for two groups: east and west of the Great Dividing Range

Source of variation	% variation	*F* statistic	*P* value
A			
Among pops	40.87723		
Within pops	59.12277	*F*st = 0.40877	<0.05
No group was formed			
B			
Among region	17.15479	*F*ct = 0.17155	<0.05
Among pops within region	29.65500	*F*sc = 0.35796	<0.05
Within pops	53.19023	*F*st = 0.46810	<0.05

Populations were grouped east and west of the Great Dividing Range

scales were identified when STRUCTURE was run within each cluster separately. The latter suggested three distinct populations in the MDB, six in SEQ and three in CEQ (Fig. 3b). In most cases, more than one cluster was found even within the same river (e.g. within the Burnett River), indicating population structure within river catchments.

The spatial assignment test in GENECLASS strongly corroborates previous findings of remarkably high population structure in this species. GENECLASS assigned 88.9 % of individuals to their sampling site (100 % assignment for some rivers; Table S3), with little to no overall assignment to other rivers. Nonetheless, individuals

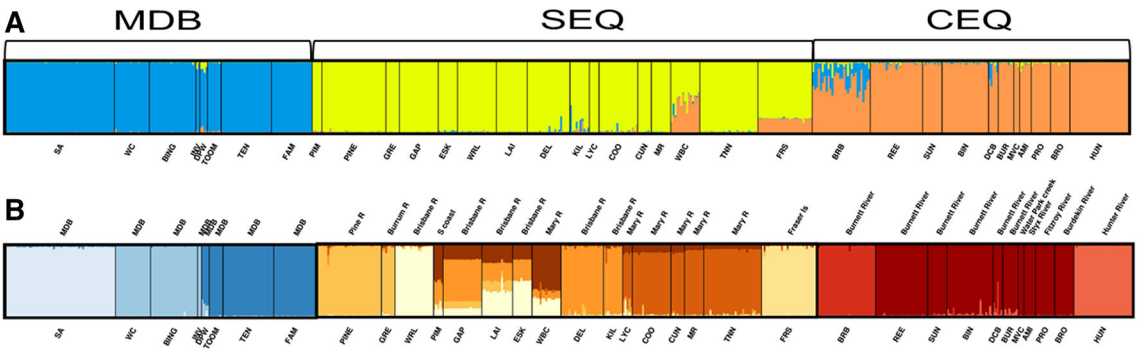

Fig. 3 STRUCTURE bar plots showing genetic clusters (K) selected for *Mogurnda adspersa*. Individuals are represented by vertical lines and each population is divided by a *black line*. Names above the plot are rivers and below are sites abbreviated as in Table 1. **a** Most probable outcome of $\Delta K = 3$ from the initial run, and **b** $\Delta 3$ (MDB), $\Delta 6$ (SEQ) and $\Delta 3$ (CEQ) from the subsequent run within each cluster independently

from some sites (WC, INV, DPW, TOOM, GRE, DEL, SUN, BIN, BUR, MVC and PRO) had probability of assignment lower than 80 % (Table S3). Individual assignment to its reference population was extremely high throughout the system, but it was relatively lower between GRE and PINE, DEL and KIL, SUN, BIN and DCB, BUR and DCB, and PRO and MVC—where STRUCTURE showed admixture (Fig. 3b). In addition, WC, BING, INV, DPW and TOOM all had individuals assigned to DCB (Table S3).

The UPGMA tree illustrated a clear pattern of genetic divergence, confirming the existence of deep regional splits between the MDB, SEQ and CEQ population groups (Fig. 4). It also showed affinities of WBC, FRS and BRB with other clusters, as also demonstrated by STRUCTURE.

Regional differences in genetic diversity

Regional groups showed statistically significant differences in mean expected heterozygosity and allelic richness (Table S4). Heterozygosity in the MDB cluster was significantly lower than that of both coastal groups, yet the difference between coastal groups was not significant (Table S4). As for allelic richness, the difference was significant across all comparisons (Table S4). The lowest genetic diversity was found in the MDB in both cases, following by SEQ and CEQ.

Isolation by distance

A weak but significant correlation between genetic differentiation and linear distance was obtained across all population samples ($P = 0.014$; Fig. 5). In addition, weak but marginally significant correlation between riverine distance and genetic differentiation was found within the Burnett River ($P = 0.051$; BRB, SUN, BIN, REE, DCB and BUR) (Fig. S1). No significant correlations were obtained when populations from the MDB ($P = 0.429$), Brisbane River ($P = 0.475$) and Mary River ($P = 0.319$) were analysed separately (Fig. S1).

Genetic diversity versus genetic uniqueness

Linear regressions of population specific Fst and genetic diversity (*He* and *A*R) strongly demonstrated that population uniqueness was negatively correlated with genetic diversity in all regional groups (Fig. S2). The highly divergent populations showed the lowest levels of genetic diversity, possibly reflecting the influence of genetic drift in small populations. According to this analysis, genetically differentiated populations SA, WBC and DCB displayed the highest diversity in the MDB, SEQ and CEQ clusters, respectively. This pattern was congruent with the METAPOP results, which indicated that SA, WBC and DCB are the populations that have the highest contribution of genetic diversity to their cluster (Table S5).

Discussion

Analysis of microsatellite DNA variation enabled us to carry out a detailed investigation of the current genetic diversity, structure and distribution of *M. adspersa* populations. This small freshwater fish shows contrasting conservation concerns along its vast range, which spans multiple river basins. Our work is the first to obtain information from nuclear DNA markers for MDB populations and to assess population genetic variation across both large (i.e. between river basins) and fine (i.e. within river catchments) spatial scales in *M. adspersa*. Our findings generally agree with previous

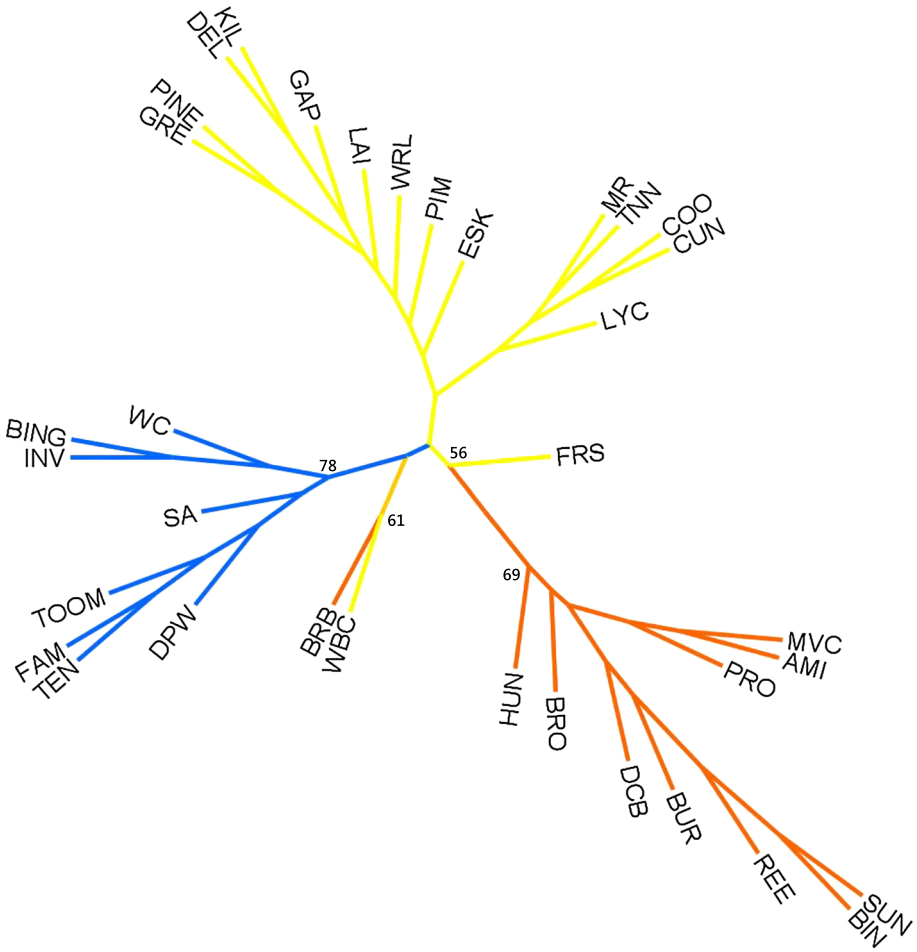

Fig. 4 UPGMA tree for *Mogurnda adspersa* based on genetic chord distance (*D*ce). Branch colour was attributed from STRUCTURE Δ3 result (Fig. 3). Bootstrap values greater than 50 are shown for the major divisions. (Color figure online)

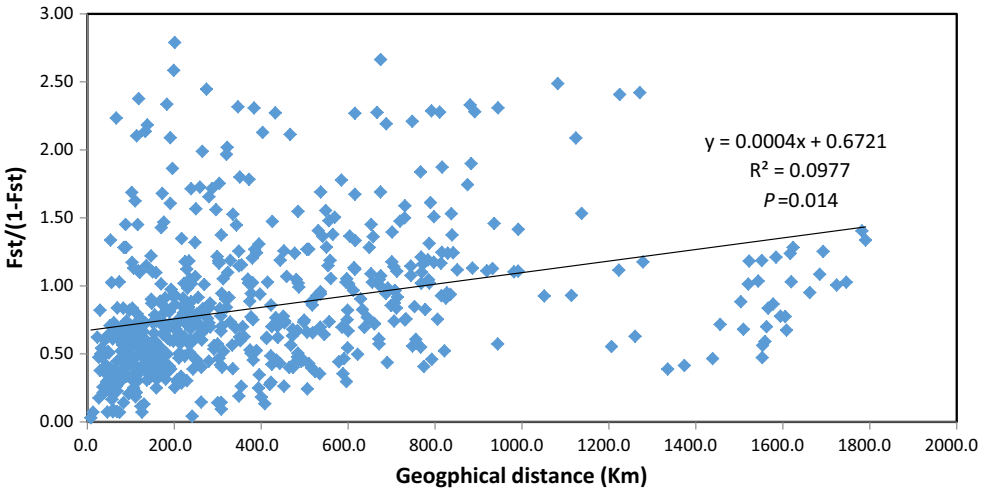

Fig. 5 Analysis of isolation by distance for all populations of *Mogurnda adspersa*

molecular studies in this species (Faulks et al. 2008; Hughes et al. 2012; Adams et al. 2013; Shipham et al. 2013; Hammer et al. 2015), but also disclose novel information relevant for both long- and short-term management and for prioritising conservation actions along the vast geographic distribution of this species.

Murray–Darling Basin and coastal populations

Overall, very low levels of population genetic diversity were found in *M. adspersa*. Both the average heterozygosity (0.46) and average allelic richness (2.12) (Table 2) were lower than values from a review of microsatellite

DNA studies of freshwater fishes (0.54 and 9.1, respectively) (DeWoody and Avise 2000). Most notably, all *M. adspersa* populations exhibited strikingly low allelic richness ($AR < 3.08$), one of the lowest reported to date for a fish (e.g. DeWoody and Avise 2000; Beheregaray et al. 2002; Gouin et al. 2006; Faulks et al. 2010a, b; Brauer et al. 2013). Furthermore, levels of genetic diversity in populations from the MDB were significantly lower than those from populations found in SEQ and CEQ regions, and lower than previous microsatellite studies of coastal *M. adspersa* from the Pioneer River (Hughes et al. 2012) and the Brisbane River (Shipham et al. 2013), an outcome that appears as independent of sample sizes. In addition, both our nuclear and a previous mtDNA study (Faulks et al. 2008) found remarkably strong population structure for *M. adspersa* in the MDB. Unlike coastal populations, MDB populations have all declined to the point where they are now regarded as endangered and conservation listed in three out of four jurisdictions (i.e. South Australia, Victoria and New South Wales; Hammer et al. 2009; Morris et al. 2001; Wager and Jackson 1993). Given that the highly fragmented MDB is one of the largest drainage basins in Australia (Beare and Heaney 2002; NLWRA 2002), it is inevitable that the few remaining populations are likely to be geographically well separated from each other. Reflecting this, riverine distances between MDB population pairs were much longer than between coastal populations. Importantly, a larger sample size for a few localities targeted in this study is needed to improve estimates of population genetic diversity and genetic differentiation.

It is highly unlikely that small and habitat specialized species such as *M. adspersa* would be capable of regular dispersal across the riverine distances that now separate the few remaining MDB populations. In fact, *M. adspersa* exhibits the highest known degree of differentiation between hydrologically connected creeks relative to what is known for other Australian freshwater fishes (Shipham et al. 2013). A genetic analysis of mean natal dispersal distance (i.e. the average distance travelled by juveniles from birthplace to breeding site) in this species showed isolation by distance between sites with relatively continuous habitat within the same creek, with dispersal often occurring over less than 1 km (Shipham et al. 2013). These results are consistent with mark-recapture studies (Boxall et al. 2002) and with genetic surveys that assessed population differentiation between creeks in *M. adspersa* (Hughes et al. 2012).

Broad surveys in the MDB (Harris and Gehrke 1997; Gilligan 2005; Smith et al. 2009) have found *M. adspersa* to be extremely rare or absent, indicating that discovering new populations is increasingly unlikely (Hammer et al. 2015). As a consequence, restricted gene flow appears inevitable, since all remaining populations lack geographic neighbours with which to exchange alleles. Species with no or little dispersal capacity will be restricted to small, isolated and disconnected populations, with genetic drift rather than gene flow being the primary determinant of genetic diversity (Meffe and Vrijenhoek 1988). Comparison of *R*st and p*R*st (Table S1) also indicated that drift, rather than the mutation, has probably contributed the most to broad pattern of genetic differentiation seen in this species. Under such circumstances, genetic differentiation between populations is predicted to be remarkably high, without spatially-correlated genetic structure over large scales, as generally found in the present study for MDB *M. adspersa*.

Given the low genetic diversity, putative inbreeding (the latter evidenced by the many positive values of *F*is), and very small size of local populations (e.g. Hammer et al. 2015), it appears that MDB populations are highly susceptible to stochastic events and to any decline in habitat quality in particular. Accordingly, decreasing rainfall and increasing temperature has been shown to lead to reduction of inflow in the MDB (Cowan and Cai 2009), while habitat degradation has clearly been impacting wild *M. adspersa* populations (Morris et al. 2001; Hammer et al. 2015). These observations further highlight concerns over the long-term viability of MDB populations.

Despite the fact that levels of genetic divergence were similarly high among coastal populations, these typically displayed different genetic architecture to those within the MDB. Generally, coastal populations showed higher levels of within-site genetic diversity and evidence for spatially-mediated gene flow within rivers (e.g. genetic differentiation was positively correlated with riverine distance in the Burnett River). Therefore, it appears that coastal populations are arranged as metapopulations at the drainage level and are genetically more diverse than MDB ones. Nevertheless, the very low levels of allelic richness across all populations does warrant concerns about the species' long term viability. This is particularly the case given the ongoing habitat degradation along coastal Queensland, including river modification and construction of impoundments (NLWRA 2002).

An unexpected outcome was the grouping of the geographic isolate Hunter River population (HUN, site #35)—the only sample from the Southeast coast division—with the Central East Queensland (CEQ) cluster (Figs. 3, 4). This obvious major geographic anomaly may or may not reflect a human-mediated translocation event, indicating that further genetic assessments of the CEQ lineage are needed to assess the native status of the highly isolated Hunter population.

Conservation implications

Human-induced habitat transformation and fragmentation are now recognized as leading threats to ecosystems worldwide (Kingsford et al. 2009). Anthropogenic activities impacting on habitat quality and availability can occur at a much faster rate than several natural processes (e.g. population recolonizations), and therefore can rapidly make populations threatened and vulnerable to local or global extinctions. Critically, populations of threatened species typically show reduced levels genetic variation when compared to non-threatened species and this reduction is considered indicative of lowered evolutionary potential, compromised reproductive fitness, and elevated extinction risk in the wild (Spielman et al. 2004). Over the last decades a plethora of human-induced threats have impacted both on local populations and across the range of *M. adspersa*, such as agricultural and industrial practices that reduced water quality and availability, especially in the MDB (Harris and Gehrke 1997; Pusey et al. 2004; Hammer et al. 2013). Additional pressure from introduced alien species such as eastern gambusia *Gambusia holbrooki* (Wager and Jackson 1993) and redfin perch *Perca fluviatilis* (Larson and Hoese 1996) have also impacted native populations. These disruptive anthropogenic activities have also been reported as major causes of population collapses and extinctions for freshwater fishes elsewhere in Australia and in the world (Cadwallader 1978; Allan and Flecher 1993; Maitland 1995). In the light of results from the present study, we offer recommendations to improve current conservation practices and to implement new management policies for the species.

Firstly, three major genetic lineages identified by this study (MDB, SEQ and CEQ) are congruent with distinct mitochondrial lineages (Faulks et al. 2008; Adams et al. 2013) and therefore meet the criteria usually applied for their classification as distinct Evolutionarily Significant Units (ESUs) (Moritz 1994; Bernatchez 1995; Crandall et al. 2000). The overall nuclear and mtDNA divergences strongly indicate that these three lineages have evolved independently from one another. The delineation of ESUs is important for long-term management issues since it provides a geographic context in which conservation priorities and strategies should be defined (Moritz 1994). At the same time, it has consequences for short-term conservation management since it informs that translocations of individuals between ESUs must be avoided (Moritz 1994).

Secondly, the microsatellite dataset identified 12 isolated management units (MUs) in *M. adspersa* that show little to no gene flow between them (Fig. 3). The MUs are populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles (Moritz 1994). Being genetically isolated or partially isolated, one expects that MUs are also functionally independent. Thus, a MU is important for short-term management (compared to the broader ESU) since it is a population unit for which more feasible population monitoring and demographic considerations can be obtained (Moritz 1994).

Thirdly, more effort towards the conservation of threatened MDB populations, which showed the lowest genetic variation and marked divergence to coastal non-endangered populations should be made in order to avoid extinction of irreplaceable (and likely locally adapted) biodiversity (Hammer et al. 2015). Of particular concerns are Wuluuman Ck (WC) and Toowoomba (TOOM), populations that showed some of the lowest levels of variability in *M. adspersa*, including by far the lowest percentage of polymorphic loci (28.6 and 42.3 % respectively), allelic diversity (1.22 and 1.35, respectively) and heterozygosity (0.110 and 0.171, respectively, Table 2)—results that suggest a local population of very small size. Loss of genetic diversity of these populations was negatively correlated with their legacy (population uniqueness measured by population-specific Fst), meaning that these populations have diverged from others in the MDB by losing genetic diversity. This suggests that maintaining populations based on their uniqueness—for example by conserving populations though translocation within an MU—could be inappropriate as a long-term solution because the same MU will likely display similarly low genetic diversity. Instead, we propose genetic rescue of WC and TOOM by translocating individuals sourced from populations with higher diversity such as Farm Creek (FAM) and Lower Murray (SA). Substantial improvements in fitness and evolutionary potential can be made by augmenting gene flow into small populations, a management action that should be done under low risk of outbreeding depression (Frankham 2015), such as translocating within an ESU as proposed here.

As a follow-up, the effective population size of these populations should be raised, preferably to around 1000, to maintain genetic diversity and promote evolution (Sgrò et al. 2010). If this cannot be achieved in situ (e.g. stream fencing and revegetation, addition of habitat structure, environmental flow programs), an ex situ approach such as releasing captive bred individuals from wild stocks could also be used. Although captive breeding may reduce fitness due to genetic adaptation to captivity, breeding using equal family size can minimise this issue (Frankham 2008). Captive breeding followed by reintroduction to the wild was recently attempted for five endangered fishes in the lower MDB, including for the single known regional population of *M. adspersa* (Hammer et al. 2013). This program was particularly successful with two species of pygmy perches, where genetic diversity was maintained

after one generation of captive breeding, a few thousand offspring were reintroduced, and subsequent recapturing events demonstrated initial survival and recruitment of captive-born individuals (Attard et al. 2016). Having regular immigration from the wild into captivity to refresh broodstock would also be useful for reducing adaptation to captivity if populations are to be maintained for longer periods (Frankham and Loebel 1992).

Another important aspect, besides establishing large effective population size, is securing gene flow through various environments to allow populations to undergo adaptive evolution (Sgrò et al. 2010). This could not only provide more opportunities for evolution to occur, but also to assist populations to form a metapopulation system, which would theoretically improve population resilience and recover local extinction by recolonization. This leads to another important conservation action for coastal populations. Some populations in the region appeared to be capable of maintaining connection between nearby sites, as shown in the isolation by distance analyses here and elsewhere (Hughes et al. 2012; Adams et al. 2013; Shipham et al. 2013) and by their relatively higher genetic diversity. Conservation actions for coastal populations should consider protection and restoration of habitat throughout catchments, both actions being relevant for maintaining metapopulation processes in coastal *M. adspersa*.

The regions inhabited by *M. adspersa* are increasingly affected by extreme weather events. At the same time that some areas in the MDB experienced long-term drought (Cai and Cowan 2008; Hammer et al. 2013), coastal areas suffered from severe rainfall and flooding (Coumou and Rahmstorf 2012). Endangered and non-endangered populations, such as those surveyed here, differ both in levels of genetic diversity and in habitat conditions, which leads to the expectation that they are locally adapted. Future work in this species would benefit from including information from locally adapted alleles, which could be identified from genome scans and transcriptome sequencing (Luikart et al. 2003). These measures would inform on functional, ecologically relevant genetic diversity and on evolutionary resilience. Information from adaptive diversity, in conjunction with findings from this study, would prove invaluable in assessing the conservation value of isolated populations and informing ongoing conservation breeding and restoration programs.

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