A Molecular Genetic Appraisal of Biodiversity and Conservation Units in Freshwater Fishes from Southern Australia

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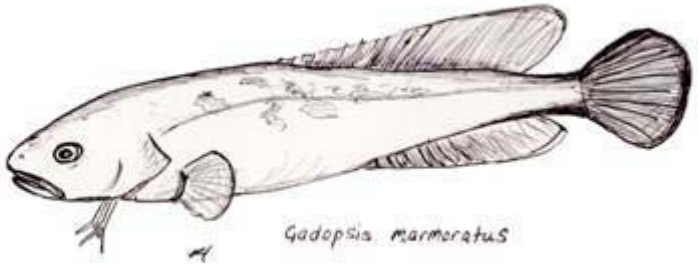

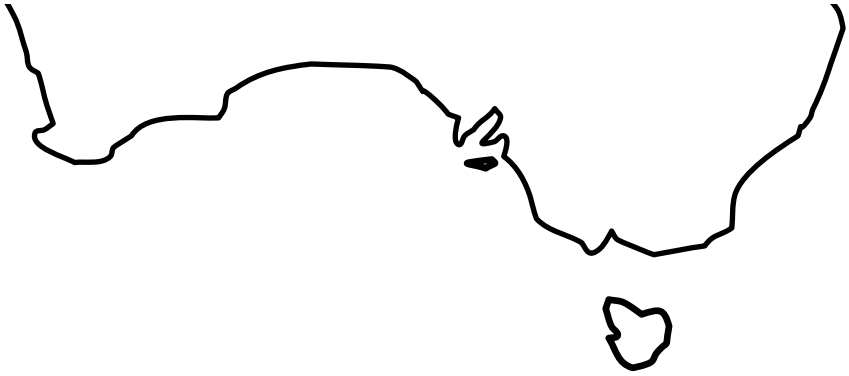

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Retropinna semoni s.l.

Nannoperca obscura

Nannoperca australis s.l.

Mogurnda adspersa

Philypnodon sp. (*macrostomus* complex)

Philypnodon grandiceps s.l.

Gadopsis marmoratus s.l.

Fishes from the lower River Murray, southern Australia (MH)

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SUMMARY

The freshwater fish fauna of southern Australia is characterised by low species richness and high endemism in groups displaying southern temperate, temperate-subtropical or temperate-tropical distributions. Comparatively few studies in Australia have incorporated modern molecular techniques to delineate species boundaries and define within-species conservation units. This is problematic because freshwater fishes are likely to show high levels of cryptic speciation and marked spatial sub-structure, and is information which is needed to conserve biological diversity and maintain the integrity of ecological communities and processes. The current study uses a 'combined evidence' approach, led principally by a set of nuclear genetic markers (allozymes), to assess species boundaries, spatial sub-structure and conservation units in obligate freshwater fishes from southern Australia.

A literature review (Chapter 2) concerns the nature and effects of fragmentation in freshwater environments. It considers the implications for freshwater fishes and the types of extrinsic and intrinsic characteristics, both natural and human accelerated, that might drive population fragmentation and divergence. This theoretical framework is then applied to a suite of six largely co-occurring species groups with contrasting biological characteristics, and derive hypotheses about expected levels of genetic divergence across and within different drainages.

Major findings

Species of *Retropinna* (Chapter 3) are widespread and generally regarded as 'common' and mobile. Allozyme analyses revealed species-level and population-level sub-divisions, including five distinct species with contiguous ranges and no evidence of genetic exchange. Three occur along the eastern seaboard (including three instances of sympatry), another in coastal and inland southeastern Australia and Tasmania, and a fifth in the Lake Eyre Basin. There is no indication of a simple '*tasmanica*' *versus* '*semoni*' dichotomy, but instead a complex pattern involving discrete clusters for the Upper Murray plus Darling rivers, Lower Murray, Glenelg River and Tasmanian regions. These findings have implications for biodiversity, conservation and ecology. This chapter has been published in modified form (*Marine and Freshwater Research* 58, 327-341).

Nannoperca obscura (Chapter 4) is a small demersal fish with specialised habitat requirements. It is under threat of extinction, particularly in the western section of its range. Combined nuclear and matrilineal genetic data identified congruent within-species sub-structure, divided by patterns

of distribution and biogeography. Four monophyletic mtDNA lineages, each distinct at multiple nuclear loci, indicate four Evolutionarily Significant Units (ESUs), namely (1) Lake Alexandrina in the Murray-Darling Basin (MDB), (2) Glenelg River, Millicent Coast River Basin and the outlying Mt Emu Creek, (3) Merri River and associated coastal streams, and (4) the eastern range section. Additional genetic and ecological data support multiple Management Units (MUs) within ESUs for individual or groups of river basins separated by marine barriers.

Nannoperca australis (Chapter 5) has a similar character to its aforementioned congener, except that it occurs across a much wider area. Although generally common, particular populations are threatened, especially in the MDB. Allozyme analyses of 57 populations confirm the presence of two divergent species, with an eastern species containing two ESUs: (1) Gippsland and Flinders Island, and (2) Ansons River in northeastern Tasmania. The western species shows sub-structure across its range, including a separation of MDB and coastal populations as two heterogenous ESUs. The Lower Murray region (Mount Lofty Range streams and the Lower Lakes) harbours a remarkable level of between- and within-population diversity, underscoring its importance for conserving evolutionary potential.

Mogurnda adspersa (Chapter 6) has been presumed extinct in South Australia since the early 1970s and has also been assumed lost from the southern MDB. This chapter reports on the rediscovery of *M. adspersa* from a wetland near the terminus of the Lower Murray, some 2500 river kilometres from the nearest known population. The nature and basic ecology of this population is documented, but the combined effects of drought and water abstraction recently have led to the probable extirpation of the wild population. A combined allozyme and mtDNA dataset confirmed the 'nativeness' of the population as a distinct sub-population (and MU), with a moderate level of allele heterogeneity. This information provides a platform for captive breeding as a conservation measure.

The endemic genus *Philypnodon* (Chapter 7) contains two nominal species: *P. grandiceps* and the long recognised but only recently described *P. macrostomus*. The former is considered widespread and common (near ubiquitous), whereas the latter is more patchily distributed. Some tolerance to marine conditions is indicated, suggesting that there may be less sub-structure, but allozyme analyses of 269 individuals indicate the presence of multiple, species-level taxa within both described species. This obscures interpretations of existing ecological data. Although the presence of genetically-similar populations within and across some drainage divides indicates higher levels of gene flow, the pattern is complex and suggests historic genetic exchange between some but not other geographically-adjacent taxa.

The freshwater blackfish genus *Gadopsis* (Chapter 8) has been a problem group for taxonomists, and it is unclear where the group is placed phylogenetically and how many species occur. Northern and southern forms on respective sides of the Great Dividing Range have been proposed, but with limited supporting evidence. Its dispersal ability (hence predicted genetic structure) is obscured by opposing life-history traits, including large body size (i.e. good swimming ability) *versus* habitat specialisation, demersal larvae and restricted home ranges. This chapter provides a genetic overview incorporating 61 locations across the range, and demonstrates unequivocally the presence of distinct northern and southern species of *G.* '*marmoratus*'. Moreover, distinct genetic discontinuities involving geographically abutting lineages indicate the likely presence of multiple ESUs within each species. A comparison of the allozyme data with previous mtDNA studies also identified two ESUs within *G. bispinosus*.

Overall, considerable complexity is demonstrated signalling the need for a review of how the southern Australian fish fauna should be viewed, studied and protected. The genetic data also provide insight into the interplay of intrinsic biological characters (e.g. dispersal ability, population ecology) with historic and contemporary extrinsic environmental factors (e.g. fragmentation, biogeographic processes). Comparisons between and within traditionally-defined species are problematic, however, owing to multiple species-level splits and other genetic divisions that may have matching biological counterparts. Together with other reports in the literature, the findings presented herein have significant conservation implications, particularly given the rapid pace of human-mediated change in some regions that house high species and genetic diversity and unique evolutionary components, notably southeastern Queensland (especially the Mary River) and the lower River Murray in South Australia. Other regions displaying high genetic substructure or divergent populations include the Clarence River and Lachlan River in New South Wales; Gippsland, Goulburn River, Glenelg River and Mt Emu Creek in Victoria, and the Macquarie River and Ansons River in Tasmania.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Signed: **Date:** 3rd September 2008

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Field collection of tissues for the various studies (chapters) represented a collaboration with Peter Unmack, involving separate or combined trips to form holistic coverage of study groups. Many other people helped with aspects relative to obtaining specimens, in particular T. Raadik (Arthur Rylah Institute, Victoria), J. Jackson (Inland Fisheries, Tasmania), M. Lintermans (Wildlife ACT), D. Gilligan (New South Wales Fisheries), J. Lyon and J. Lieschke (Arthur Rylah Institute), Q. Ye (SARDI Aquatic Sciences), S. Ryan and B. Bostock (Deakin University), D. Bray (Victorian Museum) and M. McGrouther (Australian Museum) who supplied specimens collection records from their local jurisdiction and/or field equipment, with field assistants including numerous Native Fish Australia (SA) members: Maree Hammer, S. Eastley, S. Westergaard, T. Ristic, R. Gannon, S. Wedderburn, J. Rowntree, J. Pritchard, T. Steggles, S. Doyle, P. Horne, C. Kemp, R. Foster and M. Baltzly, and R. Remmington. A special thanks and encouragement to T. Goodman. All collections were obtained under permit from various state fisheries agencies and with University of Adelaide animal ethics approval.

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1. INTRODUCTION

1.1. MOLECULAR SYSTEMATICS AND SPECIES CONSERVATION

Molecular genetic techniques offer insights into a variety of sub-disciplines in biology, including systematics, comparative evolution, conservation, ecology, animal husbandry, and embryonic development (Avise 2004). This thesis is concerned primarily with the role of these techniques in systematics and conservation, specifically the examination of genetic variation characterising entities and affinities within the evolutionary hierarchy: phylogenetic relationships, species boundaries, within species sub-structure, and heterogeneity within populations (i.e. the full gamut of biological diversity, hereafter 'biodiversity').

Molecular genetic information is a valuable supplement to taxonomic studies, especially in little studied or morphologically conservative groups (e.g. Bickford *et al*. 2006; Buhay *et al*. 2007); it is central to modern biogeographic analyses (Avise 2000), and provides understanding of biological process such as dispersal and gene flow, both natural and anthropogenic (e.g. Moritz 2002). Molecular techniques are also capable of identifying diverging evolutionary trajectories, within species variation or sub-division, and the within-population elements that support future adaptation (Moritz *et al*. 1995; Avise 2004). Importantly, spatial genetic information often provides a contrasting perspective to that of existing species recovery planning, since the latter may undervalue or overemphasise the evolutionary significance, and hence conservation value, of particular regional populations (e.g. Firestone *et al*. 1999). Consequently, a genetic framework provides a foundation for assessing conservation units and priorities (Vogler and Desalle 1994; Moritz *et al*. 1995; Soltis and Gitzendanner 1998).

There is no straight-forward method for recognising species boundaries. Some key issues involve how to address 'fuzzy' species boundaries and speciation in allopatry, how to accommodate hybridization and introgression, and the mismatch of genes and genealogy (e.g. through gene duplication, incomplete lineage sorting, horizontal transfer: Avise 2004). Such issues drive debate between advocates of biological, phylogenetic, evolutionary and other species concepts, with the choice of species concept influencing many aspects of biodiversity research (see for example Agapow *et al*. 2004). A full review is beyond the scope of this study, although there must be a decision on operational criteria for species assessments. The view followed here is that combined lines of genetic, morphological and biological evidence offer the best chance of identifying robust and diagnosable species (Adams *et al*. 1987; Sites and Marshall 2004; Page *et al*. 2005; Horner and Adams 2007).

Defining spatial scale and criteria for the identification of conservation units has proved controversial, in part due to contrasting regional socio-political contexts (e.g. threatened species legalisation: Wood and Gross 2008), but also reflecting differing attitudes on what biological and/or genetic criteria ought to be employed (see Crandall *et al*. 2000). Nevertheless, most researchers employ some variant of two basic concepts, namely Evolutionarily Significant Units (ESUs) and Management Units (MUs), with the most widely adopted genetic criteria being those proposed by Moritz (1994; 2002). These genetic criteria (ESUs = reciprocal monophyly of mitochondrial DNA (mtDNA) haplotypes plus statistically-significant differences at nuclear gene loci; MUs = differences in frequency of nuclear or MtDNA alleles) are among the most stringent proposed, and conservation units thus identified will also merit recognition under less demanding definitions. They therefore represent a convenient starting point for any initial conservation genetic assessment and platform for combination with any morphological and ecological data.

1.2. PAST AND PRESENT SOUTHERN AUSTRALIA

Southern Australia, the landmass and islands south of latitude 25°S (Figure 1-1), is a broad (*c*. 4 million km^2), naturally-divided landscape that has undergone considerable anthropogenic alteration. It is an ideal region for biodiversity assessment and applying conservation frameworks.

An excellent review of the history and formation of aquatic habitats in southern Australia is provided in a biogeographical analysis by Unmack (2001). The landscape has been remarkably stable, with most of the major landforms and drainages established by the Tertiary (e.g. the last uplift of the Great Dividing Range (GDR) was complete by *c*. 90 Mya). Landforms also have been relatively stable, due to limited glaciation and volcanism. Changes in climate have had pronounced effects, such as the effective division of southeastern and southwestern Australian due to increasing aridity 16-14 Mya. However, changes in sea level are possibly the most pervasive recent phenomena to affect habitat availability. The region has experienced major fluctuations in sea level, with prominent areas such as the lower Murray region being inundated during highs (*c*. 5 Mya), sea water barriers maintained during intervening periods (e.g. current separation of mainland Australia and Tasmania), and potential points of drainage coalescence exposed during lows (e.g. during glacial maxima every 100-150 Ky, the last occurring 16 Kya) (Figure 1-2). Finally, the localised evolution of drainage systems or flow paths is likely to have shaped patterns of between- and within-system connectivity.

The modern organisation of surface water systems follows a broad hierarchy of major drainage divisions which represent collections of river basins (AWRC 1976). River basins themselves either represent large and defined systems (e.g. Glenelg River, Basin 38) or groups of small proximate catchments grouped arbitrarily for simplification (e.g. Surrey, Fitzroy, Shaw and Moyne catchments, Basin 37). Eleven drainage divisions and 46 river basins occur wholly or partly in southern Australia (Figure 1-1). The Murray-Darling Basin (MDB) is the largest drainage division contained wholly in the region, covering more than 1 million km^2. It is bounded by the GDR and smaller features including the Mount Lofty Ranges (MLR) in the west (Figure 1-3). The MLR are a long-established topographic outlier (Twidale 1976) at the terminus of the MDB, drained by streams flowing towards the lower River Murray or Lake Alexandrina. Unmack (2001) proposes the grouping of river basins as freshwater fish biogeographical provinces (Figure 1-2) (see Discussion).

The current climate across southern Australia is mostly temperate, but can vary regionally, reflecting its area and latitudinal span. Spatial variability is matched by strong seasonal variability of a mostly Mediterranean climate, and interannual fluctuations due to broader climatic cycles from El Niño events, positioning of subtropical high pressure ridges and the episodic infeed of tropical moisture from the north. Seasonal, winter-dominated rainfall is concentrated along the GDR, MLR, Tasmania and in the southwest, with most of the Murray-Darling Basin lowlands becoming drier towards the north and centre (Figure 1-4). Median annual rainfall is up to 2500 mm in western Tasmania, but is mostly less than 800 mm across the region. Spatial and temporal variability in rainfall (or evaporation) dictate that patterns of stream flow also are highly variable (e.g. Walker *et al*. 1995).

The arrival of Europeans about 200 ya and subsequent human industry have had dramatic effects on the landscape and rivers of southern Australia. Few catchment areas are excluded from significant water use, infrastructure-related changes to habitat, and indirect effects from land use. An example is the River Murray, part of Australia's largest river system and with a highly-modified flow regime (Walker 1985; Walker and Thoms 1993; Maheshwari *et al*. 1995). Another is the Blackwood River in the southwest, which is affected by salinisation as a result of land clearance (Schofield and Ruprecht 1989). The introduction of alien fishes is also a widespread threat (Arthington 1991; Lintermans 2004; Morgan *et al*. 2004; Olden *et al*. 2008).

1.3. AUSTRALIA FISHES AND MOLECULAR STUDIES

The definition of what constitutes a 'freshwater' fish varies among authors. From 160 to 300 species have been recorded from Australian freshwater environments (cf. Unmack 2001; Allen *et al*. 2002). The lower of these figures represents species restricted entirely to life inland (*obligate freshwater fishes*), and the larger captures *euryhaline* species plus others with certain life stages that occur only in fresh water (*diadromous fishes*) (*sensu* Hammer and Walker 2004). In either case, the list of formally accepted species in Australia has remained relatively stable over the last 20 years. The greatest change has involved taxonomic revisions identifying 10 additional species in central Australia (*Mogurnda*, *Chlamydogobius*, plotosid catfishes: Larson 1995; Allen and Feinberg 1998; Allen and Jenkins 1999), plus a few discoveries in the tropical north (e.g. Bloomfield cod *Guyu wujalwujalensis* and cling-gobies *Stiphodon* and *Sicyopterus* spp.: Pusey and Kennard 2001; Allen *et al*. 2002). In southern Australia, the last additions occurred in the 1980s: the variegated pygmy perch *Nannoperca variegata* was discovered in Ewens Ponds and the Glenelg system (Kuiter and Allen 1986), and the two-spined blackfish *Gadopsis bispinosus* was described from MDB highlands (Sanger 1984). Taxonomic complexity has been confirmed in the eleotrids, partially resolved with the eventual description of the dwarf flathead gudgeon *Philypnodon macrostomus* (Hoese and Reader 2006), but remaining for *Hypseleotris* (Larson and Hoese 1996; Bertozzi *et al*. 2000).

Southern Australia has a small but unique complement of obligate freshwater fishes, dominated by a few families, namely Percichthyidae, Galaxiidae and Eleotridae (McDowall 1996a; Unmack 2001; Allen *et al*. 2002). Three primary groups occur:

(1) Southern endemic obligate freshwater species including the pygmy perches (genus *Nannoperca*), other larger percichthyids (*Gadopsis* and most species of *Maccullochella* and *Macquaria*), several galaxiids (notably *Galaxiella*) and the unique salamanderfish *Lepidogalaxias salamandroides*,

(2) Species with temperate to subtropical (e.g. *Retropinna*, *Maccullochella peelii mariensis*, *Macquaria ambigua* complex, *Philypnodon*) and sometimes tropical (e.g. *Nematalosa erebi*, *Tandanus tandanus*, *Mogurnda adspersa*) distributions, and

(3) Temperate diadromous species including lampreys, anguillid eels and numerous galaxiids.

With respect to conservation status, the regional fish fauna includes significant numbers of threatened species or regional populations (Koehn and Morison 1990; Pollard *et al*. 1990; Wager and Jackson 1993; Hammer *et al*. 2007a).

Molecular tools have yet to play a significant role in refining Australian freshwater fish taxonomy (cf. Allibone *et al*. 1996; Kocher and Stepien 1997; Johnson *et al*. 2004). While there is a growing literature of molecular studies, the identification of species boundaries has not been at the forefront of most assessments (see however, Crowley and Ivantsoff 1990; Musyl and Keenan 1992; Rowland 1993; Bertozzi *et al*. 2000). Many phylogeographic studies nevertheless have revealed deep divergences (Watts *et al*. 1995; Hurwood and Hughes 1998; Page *et al*. 2004; Wong *et al*. 2004; Thacker *et al*. 2007; Jerry 2008), demonstrating the likely presence of cryptic taxa and the need for broad-scale molecular systematic investigations. Similarly, population genetic studies have largely focused on narrow regional or theoretical issues, with only secondary consideration of conservation units. Nevertheless, high levels of sub-structure have been observed in most obligate freshwater species (e.g. Hughes *et al*. 1999; McGlashan and Hughes 2002; Faulks *et al*. 2008).

The existing regional coverage of molecular studies is biased toward the east coast of Australia, and the few studies on southern endemics are constrained, as above (e.g. Ovenden *et al*. 1993; Watts *et al*. 1995; Smith *et al*. 2002). Other applications of molecular studies in the region include identification of hybridization, confirmed by nuclear genetic markers (Douglas *et al*. 1995; Jerry and Woodland 1997; Bertozzi *et al*. 2000), fine-scale assessment of gene flow (Cook *et al*. 2007), and forensic-like investigations to identify native compared to translocated populations (Waters *et al*. 2002b).

1.4. STUDY OVERVIEW

As naturally-divided and often restricted environments, freshwater habitats are further segregated by the spatial and temporal variability created by complex flow regimes and physical and biological isolating mechanisms (see Chapter 2). Accordingly, opportunities for isolation and diversification are increased, and freshwater biota such as fishes often show high biodiversity (Nelson 1994; Ward *et al*. 1994) which can remain undocumented (e.g. Mulvey *et al*. 1997; Hanken 1999; Lundberg *et al*. 2000). In these circumstances, distinct units may be overlooked, and their survival prejudiced (e.g. Austin and Ryan 2002; Ferguson 2004; Johnson *et al*. 2004). Moreover, this richness of species and conservation units is disproportionately threatened by intensive human industry focused around or utilising fresh water (Allan and Flecker 1993; Poff *et al*. 1997; Cambray and Bianco 1998; Ricciardi and Rasmussen 1999). The combination of high levels of diversity and threats suggest that freshwater environments in southern Australia have a special need for genetic assessments aimed at identifying distinctive components. The virtual lack of regional molecular systematic studies of species boundaries, conservation units and general population genetic structure is another motivation for a dedicated study.

Studies to document species boundaries and conservation units ultimately rely on the local geopolitical framework to acknowledge and enact upon findings. In the Australian context, much activity already exists with regard to biodiversity conservation (i.e. Federal, State and community threatened species programs/legislation). Natural resource management has a focus on fishes as part of ecosystems and as indicators and icons for ecosystem function or health (e.g. Harris and Silveira 1999; Humphries and Lake 2000; Kennard *et al*. 2006), supported through a net of regional bodies and other programs (notably including the MDB Native Fish Strategy: MDBC 2003). Significant work has also occurred to improve ecological understanding of local fishes (e.g. McDowall 1996a; Morgan and Gill 2000; Pusey *et al*. 2004; Lintermans 2007; McNeil and Hammer 2007).

The objective of this study is to conduct inclusive and holistic molecular studies on a range of single species, as currently defined, to allow a more complete recognition of aquatic biodiversity, confident assignment and collection of ecological data, and frameworks for conservation and management. This approach will facilitate a second focus, the examination of extrinsic environmental and intrinsic biological factors affecting historic and contemporary gene flow and fragmentation, including a comparative element across different species.

The technique of allozyme electrophoresis is ideally suited to the characterisation of genetic variation above and below the species level. Allozymes have the advantage of providing rapid, multi-locus assessment of nuclear genetic characters, and have particular utility in detecting instances of hybridisation (Richardson *et al*. 1986). The general method is to source comprehensive collections across the range of each chosen species, genotype individuals for a large suite of nuclear loci (allozymes) and, where possible, cross reference the allozyme analyses with a complementary mtDNA dataset. Major genetic groups within allozyme data will be used to identify diagnosable taxa (cf. evolutionary species in allopatry and biological species in sympatry), as the platform for other evidence to assign species. Sub-groups (lineages) within taxa, and divergent sub-populations or population groups, then form the operational criteria for assessing the nuclear genetic component of ESUs and MUs, respectively.

The thesis includes an initial literature review that establishes a framework for the selection of study species (Chapter 2), a series of data chapters individually focused on species with contrasting intrinsic biological characteristics (Chapters 3-8), and a general discussion summarising the key themes of the study, namely taxonomy, genetic sub-structure, biogeography, ecology, and conservation (Chapter 9). The data chapters are presented in manuscript format, allowing for ready preparation and submission (e.g. Hammer *et al*. 2007b). The Appendices contain additional molecular data and includes two complementary papers contributing to an improved understanding of fish biodiversity in southern Australia (Hammer and Walker 2004; Hammer 2006b).

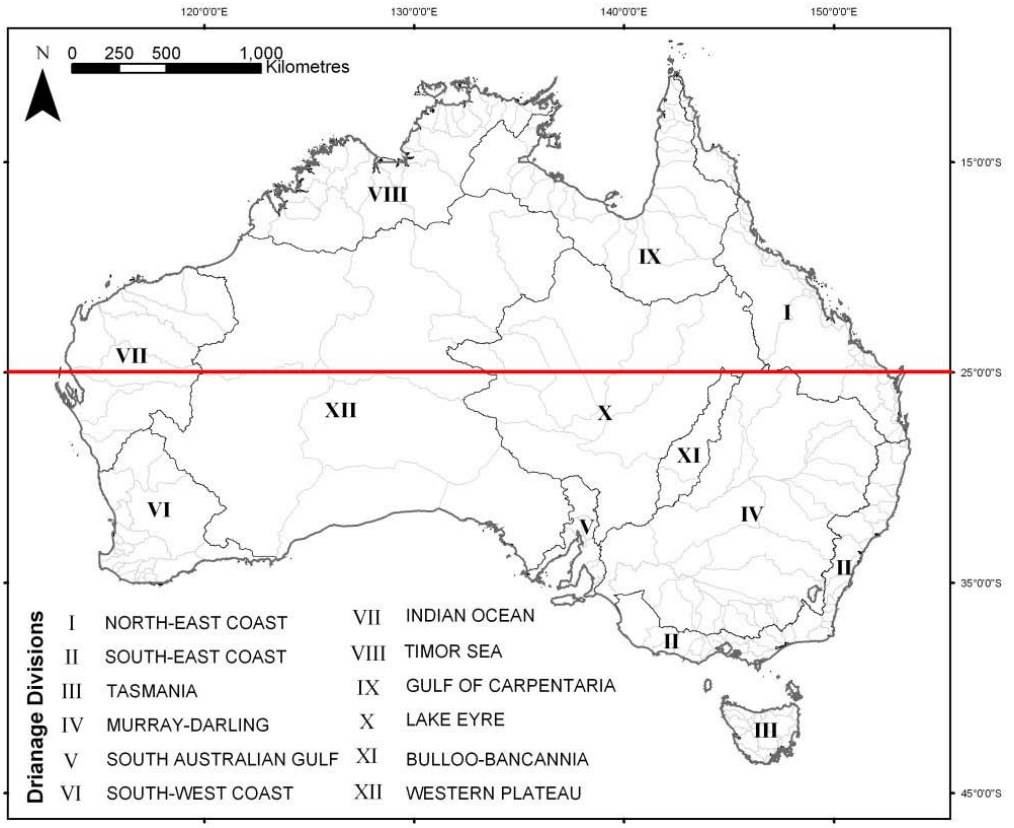

Figure 1-1. Organisation of aquatic habitat to major Australian drainage divisions (AWRC 1976), the horizontal line demarcates southern Australia as covered in this study.

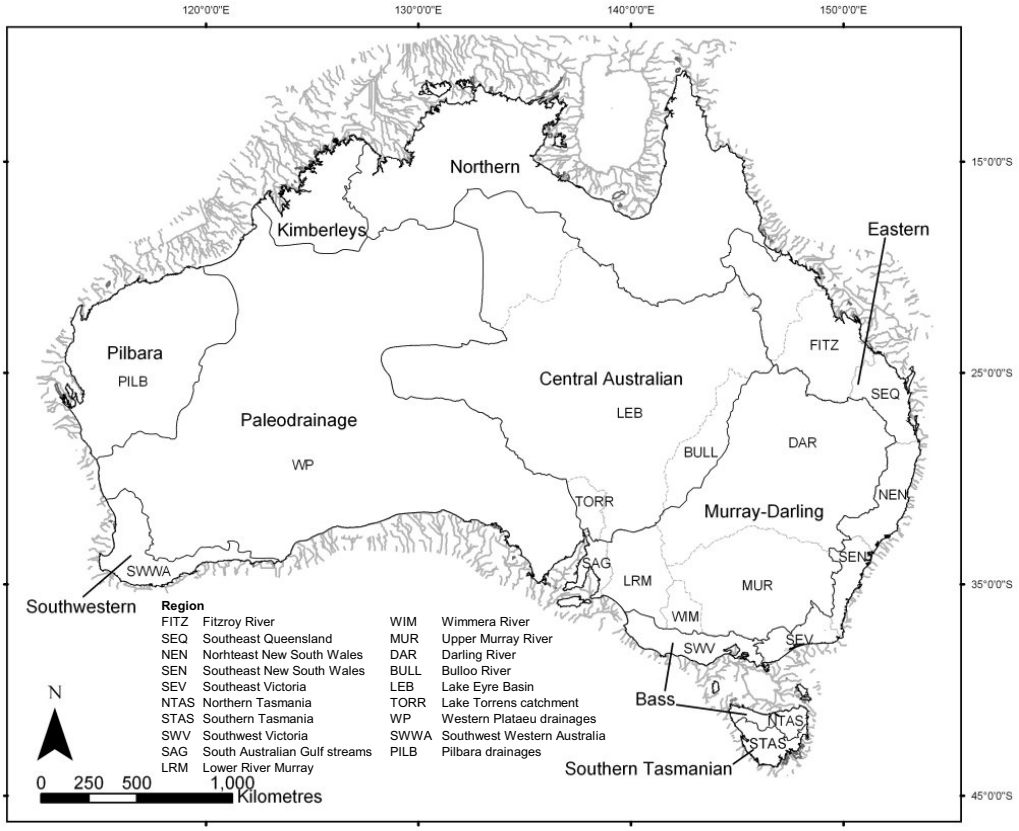

Figure 1-2. Australia freshwater fish biogeographical provinces and drainage patterns under low sea-levels interpreted from bathymetric data (adapted from Unmack 2001). Coded regions represent groups of river basins for southern Australia (e.g. LRM = Lower River Murray).

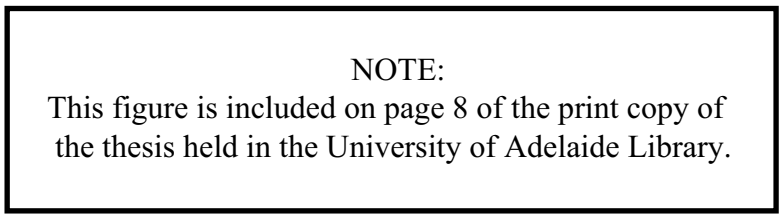

Figure 1-3. Topography of Australia including major features referred to in the text (base layer © Geoscience Australia 2004).

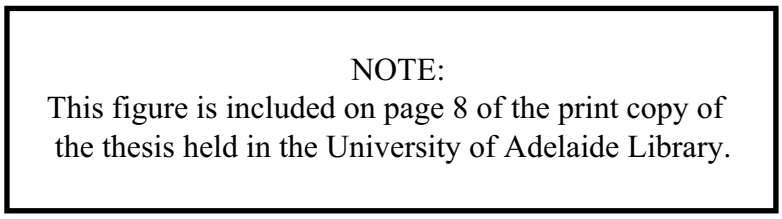

Figure 1-4. Indicative summer and winter rainfall in southern Australia, highlighting general aridity and pronounced seasonality (base layer © Geoscience Australia 2004).

2. LITERATURE REVIEW

Habitat fragmentation and the conservation of freshwater fishes

2.1. INTRODUCTION

Habitat fragmentation is universally recognized as a process threatening biodiversity, and there is a voluminous literature on the nature, extent and effects of the process in global terrestrial environments (e.g. Wilcox and Murphy 1985; Saunders *et al*. 1991; Young and Clarke 2000; Davies *et al*. 2001). A reader could be forgiven, however, for assuming that the phenomenon is of little or no consequence in marine and freshwater environments. This neglect is particularly evident in the conservation biology literature, which includes comparatively few references to aquatic systems (Irish and Norse 1996; Ricciardi and Rasmussen 1999; Hixon *et al*. 2001; Abell 2002). Such an environmental bias potentially limits holistic treatment of threats to biodiversity and the understanding of ecological patterns (Allan and Flecker 1993; Cambray and Bianco 1998; Fagan *et al*. 2002).

This review invokes the concepts of conservation biology to analyse declines in freshwater organisms, particularly of fishes. Freshwater fishes are of special interest in the current context because a comparatively large proportion of the world fauna is conservation listed, they respond to changes in aquatic habitats and are good indicator species, and they are iconic species for the conservation of freshwater systems (Angermeier 1995; Bruton 1995; Harris 1995; Ricciardi and Rasmussen 1999; Jackson *et al*. 2001). The review outlines the nature of fragmentation in the environment generally, and freshwater systems in particular, then applies this conceptual framework to freshwater fishes.

2.2. FRAGMENTATION OVERVIEW

Fragmentation is the process of separating one formerly continuous entity into discrete parcels. It has ramifications in social, economic and environmental terms. In an environmental context, fragments are remnants of a formerly continuous habitat, surrounded by a matrix of altered habitats, and are distinct from islands or new features of the landscape (Davies *et al*. 2001; Watson 2002). Fragmentation may be cyclic or largely irreversible, and may occur at any spatiotemporal scale. In undisturbed environments, it is implicated in the origins, history and form of landscapes, biota and ecosystems. Natural divisions can arise from global events such as glaciation, continental separation and climate change (e.g. Moritz *et al*. 1997) or localised features such as fire or rivers bisecting a landscape (e.g. Wright 1974). In recent evolutionary

time, the biological and cultural ascent of *Homo sapiens* has introduced a potent new force with the ability to divide habitats and populations, causing massive environmental changes within relatively short periods of time. The best-documented examples of anthropogenic fragmentation relate to land clearance, while other forms of agricultural and urban development have partitioned once-continuous terrestrial landscapes (e.g. Whitemore 1997; Kemper *et al*. 2000; Young and Jarvis 2001). The effects are progressive, cumulative and complex (Debinski and Holt 2000). Fragmentation isolates remnants and imposes external influences on fragments. In the following selective review, the ecological consequences of fragmentation are viewed in terms of these two key elements, leading to discussion of freshwater environments.

Isolation

Isolation is driven by the formation of hostile intervening habitats or other physical barriers preventing the dispersal of organisms between fragments (thus, habitat fragmentation can lead to population fragmentation). Accordingly, isolation promotes divergence between remnants, leaves fragmented biota vulnerable to insular effects, restricts distribution to particular areas (geographic fixation) with unavoidable exposure to deleterious conditions (e.g. diminishing resources, drought, climate change), and limits opportunities for recolonisation from other areas, particularly for small fragments and populations (MacArthur and Wilson 1967; Caugley 1994; Holsinger 2000).

In contrast to the natural rates of ecological, behavioural and genetic divergence which occur as a result of isolation, the rate and extent of recent habitat destruction and geographic fixation leaves species with little opportunity to adjust and adapt to new environmental conditions. Man-kind continues to divide landscapes into ever finer portions at rates measurable in decades or even years (e.g. Hobbs and Hopkins 1990; Ehrlich 1993; Skole and Tucker 1993).

The degree of isolation imposed by fragmentation, and the consequent exposure to deleterious conditions, is usually species specific, being influenced by factors such as their initial spatial distribution and abundance (Davies et al. 2000; Fagan et al. 2002), habitat needs (Boswell et al. 1998), mobility (determined by specific intrinsic characteristics such as size), trophic position and biological interactions, dispersal method and behaviour (e.g. Davies et al. 2000; Biedermann 2003; Hausner et al. 2003; Driscoll and Weir 2005). Idiosyncratic responses however, also imply a degree of interaction between species traits and environmental conditions (Henle et al. 2004), landscape spatial configuration (e.g. suitability of transport routes, threats in external areas, the degree of isolation and arrangement of fragments) and temporal dynamics, especially in variable environments (Fahrig and Merriam 1994).

External influences

External influences act to reduce connectivity, further isolating populations and interrupting physical processes. The degree of external influence may be dependent on landscape characteristics (e.g. connectivity and patch size) as well as the time since perturbation (McIntyre and Barrett 1992; Andrén 1994). As with isolation above, certain species are more vulnerable or resilient to external influences. Some generalists or 'weedy' species increase in abundance following fragmentation, taking advantage of resources in the matrix surrounding remnants or being favoured by the new combination of habitats (e.g. Marvier *et al*. 2004). External influences are best categorised as the result of habitat loss and the reduction and alteration in surrounding habitats.

Habitat loss is a significant landscape change in its own right, but it also disrupts environmental processes, genetic gradients and population dynamics. Physical processes such as nutrient cycling may be interrupted (Saunders *et al*. 1991), and removing proportions of a landscape may eliminate certain habitat types, hence species, components of demographic structure and ecological links (e.g. Purvis *et al*. 2000). Similarly, extirpating part of a species' range may selectively abolish unique or locally adapted genetic and ecological forms and reduce overall genetic variability, especially for rare or patchily-distributed species (Sherwin and Moritz 2000).

An overall reduction in habitat area can lead to a variety of interlinked problems such as the concentration and turnover of biota and breakdown of ecological relationships, with fragments serving as crowded focal points for intra and interspecific competition and predation (e.g. Crooks and Soulé 1999; Ford *et al*. 2001). Loss of intervening habitat facilitates edge exposure to physical conditions such as wind, fire, solar radiation and alternate microclimates (e.g. moisture levels), serving to modify the conditions and habitat within fragments (Saunders *et al*. 1991; Kapos *et al*. 1997). Such 'edge effects' exemplify the continual feedback faced by fragments and hence fragmentation has momentum; its effects can accumulate well beyond particular divisions and impacts.

2.3. FRESHWATER SYSTEMS

Two key elements emerge in the comparative lack of coverage given to aquatic fragmentation in the conservation biology literature. Firstly, aquatic systems may be overlooked because of the contrasting physiological properties of air and water (see Hixon *et al*. 2001), and certainly the physical form of water acts as a barrier to understanding of organisms and processes beneath its surface (water blindness: Cambray and Bianco 1998). Beyond this physical mask, the other fundamental distinction involves differences in connectivity, organization and system dynamics. Aquatic systems generally have restricted habitat continuity and are highly dynamic on small and large spatial and temporal scales, compared to the generally more continuous nature of terrestrial habitats. The distinction is especially pronounced for freshwater systems.

Connectivity and dynamics

Pathways for transmitting freshwater extend across and below terrestrial surfaces throughout drainage areas worldwide. However, freshwater systems constitute only a tiny fraction of the Earth's surface (> 0.01%) and occur as spatially restricted and patchy habitats (Pringle 2001; Turner *et al*. 2001). Different systems are often independent, being entrapped topographically and then further segregated by physicochemical barriers such as that imposed by the marine environment whereby waters of catchments with only minor spatial separation may never intermix. Connectivity can also be cyclic, influenced by temporal variation in climates and geological events like the advance and retreat of the sea, periods of increased aridity or humidity, and drainage rearrangement. For instance, many water features in the deserts of today represent the restricted extremities of more extensive and interconnected systems formed during wetter times (e.g. Minckley and Douglas 1991; Johnson 2002).

The nature of connectivity within systems comprises the combination of variable spatial and temporal characteristics. Essentially, a model for freshwater systems can be constructed as an interaction between, and a defined hierarchy of, linear, lateral and vertical connections (Frissell *et al*. 1986; Ward 1989). Linear or longitudinal connections in stream and riverine systems involve networks of smaller features (e.g. tributaries) that converge towards a simplified structure (e.g. lowland river) before terminating (e.g. sea, endorheic lake, wetland system). Lateral connectivity encompasses outward lying associations (e.g. floodplains, riparian areas), and vertical connections involve linkage to subterranean sources of water (aquifers).

This complex, multidimensional habitat templet is influenced by standard, terrestrially-focussed clines in climate and geology, and by variable abiotic characteristics such as flow regime and

local habitat and geomorphic characteristics (Ward and Stanford 1995b). The flow regime provides spatiotemporal variability to linear and lateral connections through different aspects of flows (e.g. amplitude, duration, frequency) and the nature and timing of water level change (e.g. Walker *et al*. 1995). This can shape the physical form and processes of ecosystems (e.g. Vannote *et al*. 1980; Junk *et al*. 1989; Puckridge *et al*. 1998) and provide an avenue for disturbance that shapes aquatic communities, like the displacement and subsequent recolonisation of biota from stream surfaces or refuges (i.e. the Patch Dynamics Concept: Pringle *et al*. 1988; Townsend 1989).

Drivers of fragmentation

Local habitat loss such as the removal and destruction of woody debris or physical disturbance to the benthos may create local habitat fragments (Kershner 1997; Goodsell and Connell 2002). However, isolation and external influences take on additional complexity in aquatic systems within a broader level of ecosystem change. The confined, multi-dimensional nature of freshwater systems, in particular lotic (running) and riverine habitats, ensures they are vulnerable to both natural and artificial fragmentation through physical disruptions to connections (isolation), especially along linear flow paths. Natural physicochemical barriers occur at the interface of salt and fresh water, as geohydrological features such as waterfalls and dry stream sections, or as physical arrest following freezing (e.g. Currens *et al*. 1990; Power *et al*. 1999). Other more selective barriers include high velocity, dense swamp, structurally sterile habitat (e.g. Warren and Pardew 1998) and biological drivers relating to competitive exclusion and predation (see below).

The extreme of natural temporal fragmentation relates to flow characteristics, with the extent of continuous habitat formed during the peak of a flood. Receding water levels sever linkages, and the aquatic landscape is segregated into a spatial and temporal mosaic of refuges that await cyclical reconnection. Contraction is an especially prominent feature, and integral component, of intermittent or dry land streams, and aquatic biota show adaptation to these natural patterns of flow and disturbance (Townsend 1989; Ward and Stanford 1989; Sheldon *et al*. 2002).

In contrast to natural variability, regulation *via* artificial barriers has become a common feature of aquatic habitats across the globe. Dynesius and Nilsson (1994) report of some 39,000 large dams in the northern hemisphere alone, up until 1986. This, combined with more recent figures for the southern hemisphere (e.g. Kingsford 2000; Arthington and Pusey 2003) and continuing developments (e.g. the massive Three Gorges Dam: Wu *et al*. 2003), demonstrates how extensive the fragmentation of the world's rivers has been, even before considering smaller barriers and

finer scale issues. Impoundments to secure water supply and navigational passage, barriers as the result of trans-aquatic crossings (e.g. road culverts), in stream structures such as flow gauging stations, and levees to separate and reclaim swamp and floodplains all serve to disrupt previously contiguous habitat, with related consequences to biota (e.g. Dodd 1990; Walker and Thoms 1993; Warren and Pardew 1998; Benstead *et al*. 1999; Ward *et al*. 1999; Andersson *et al*. 2000; Cumming 2004; Leyer 2004).

Obstructions caused by artificial barriers are accompanied by habitat alterations and associated changes in productivity pathways and biological communities (Poff *et al*. 1997). Thus, habitat types can be transformed (e.g. lotic to lentic), sediment transport is altered (entrapment and suspension), downstream habitats are often subject to unfamiliar physical conditions (e.g. high water velocities, lowered temperatures) and abstraction might cause unnatural drying or undermine natural thresholds for connectivity. A general alteration in flow regime, especially the timing, duration and rates of change for flow events, can either exacerbate or de-emphasise natural connections and interrupt biological responses (Ward and Stanford 1995a; Stanford and Ward 2001; Bunn and Arthington 2002).

External influences have an expanded scope in aquatic systems due to hydrological connections and the position of habitats within broader landscape configurations. Alterations from regulation and pollution (physical and biological) can be telegraphed considerable distances downstream, and in otherwise pristine habitat, and result in less obvious but equally influential problems (e.g. Zwick 1992; Knapp and Matthews 2000). As an ultimate destination for rainfall, aquatic habitats incur the additional burden of being affected by many of the environmental changes to interlinked terrestrial environments. Hence, issues associated with land clearance and land use, such as increased sediment and nutrient input, the altered nature of water run-off (delivery and quality), higher levels of physical disturbance from stock, and habitat removal (terrestrial sources of shade, physical and biological cover) fall within the larger picture of external influences in aquatic environments (Pringle 2001), particularly considering the corridor-like structure (i.e. dominant edge environments) of stream and riverine systems.

2.4. FRAGMENTATION AND FRESHWATER FISHES

Living underwater, freshwater fishes are dependent on the availability of continually submerged habitats for persistence and are physically locked into the freshwater habitat templet. They are also biologically attuned to natural flow regimes and connectivity as cues for reproduction and recruitment (cf. Bunn and Arthington 2002) and rely on connectivity to move within spatially restricted and inherently variable freshwater habitats (e.g. migration and recolonisation: Schlosser and Angermeier 1995; Dunham and Reiman 1999). Consequently, freshwater fishes are strongly disposed to react to the aforementioned changes brought about by natural and artificial fragmentation in freshwater systems.

A general vulnerability to fragmentation is enhanced by the often spatially restricted patterns of freshwater fish distribution, either permanently (e.g. isolated desert fishes, habitat specialists) or temporarily such as over summer in Mediterranean type climates (Closs and Lake 1994; Magalhães *et al*. 2002; Hammer 2004). In an evolutionary context, a propensity for isolation combined with heterogeneous aquatic habitat contributes to freshwater fishes as a group being speciose (around 21% of the world's vertebrates, despite the relative scarcity of their habitat) and displaying highly structured patterns of spatial genetic variation (Nelson 1994; Ward *et al*. 1994; Berra 1997). Strong isolation and geographic fixation expose them to natural short-term change and the vagaries of dynamic systems. However, they are also vulnerable to human mediated habitat divisions and other rapid changes which can alter within-species genetic variation and spatial structure (Neraas and Spruell 2001; Melgaard *et al*. 2003; Yamamoto *et al*. 2004) and cause local or global extirpations (e.g. Angermeier 1995; Dunham and Reiman 1999; Fagan *et al*. 2002; De La Vega-Salazar *et al*. 2003).

While migratory fishes may have flexibility to overcome alteration in affected habitats (e.g. recolonisation, selection of favourable habitats), they cannot avoid all negative aspects of losses of connectivity in aquatic environments. Diadromous species requiring passage between fresh and saltwater are vulnerable to the direct elimination or interference of lifecycle components (e.g. Harris 1984; Moyle and Williams 1990), while potamodromous species requiring spatial and temporal passage within systems face similar problems of exclusion from required or preferential habitat and spawning areas (lateral and linear movements) as part of large home ranges (e.g. Neraas and Spruell 2001). Species with a lesser reliance on migration, particularly small demersal taxa, are vulnerable at more localised scales. Indeed, comparison of pre- and post-impoundment fish communities as the most readily identifiably cause of aquatic fragmentation, often highlights two key groups of vulnerable species: migratory taxa and small-bodied, lotic

specialists, compared to a typical increase in the abundance of generalist or exotic taxa (Winston and Taylor 1991; Ruiz 1998; Taylor *et al*. 2001).

Clearly, as in terrestrial situations, differing intrinsic characteristics of freshwater fishes will dictate each species' response to altered conditions, with some species more vulnerable to fragmentation or conversely favoured by associated changes. Thus, linking a species response to fragmentation with particular intrinsic characters under a certain environmental context may allow for a greater understanding of fragmentation, guide the establishment of testable hypotheses for assessing patterns and impacts to regional faunas, and provide predictions concerning vulnerable taxa or groups and related conservation management (e.g. Angermeier 1995; Park *et al*. 2003). Table 2-1 presents a framework of intrinsic characters designed to assess the species-specific response of freshwater fishes to fragmentation, derived from a synthesis of literature studies to examine the effects of fragmentation on freshwater fishes and adapting the useful assessment categories employed by Tibbets and Dowling (1996) and Angermeier (1995). Three categories emerge as banners for groups of intrinsic characteristics: dispersal capabilities, reproductive behaviour and biological characteristics.

Dispersal capabilities

In addition to large-scale issues involving a species' life history strategy, adult mobility (looking at smaller-scale movements) might play a critical role in metapopulation dynamics and determine capability to overcome barriers. Vagility relates to differing aspects of locomotion such as burst speed, critical swimming ability and leaps (a reflection of size and morphology) with small size in general limiting dispersal due to issues of scale (Mallen-Cooper 1992; Angermeier 1995; Warren and Pardew 1998). Pelagic, free-swimming species represent the most suited vertical position for dispersal, although sedentary behaviour might be advantageous in certain conditions (e.g. for negotiating riffles: Tibbets and Dowling 1996). Species that frequent areas close to drainage divides such as headwaters or estuaries are in a better position or are more exposed to between system dispersal (e.g. Waters *et al*. 2002b). Various adaptations enhance abilities to overcome isolation, such as specific anatomical features for climbing (McDowall and Fulton 1996; Potter 1996). Conversely, survival strategies such as aestivation reduce the need to recolonise, instead providing the ability to persist in areas that undergo seasonal habitat desiccation (e.g. Beck 1985; Pusey 1989; McDowall 1990).

Reproductive behaviour

Most fishes show seasonal patterns of reproduction to focus on optimum conditions for offspring survival. A specific reproductive strategy reliant on particular components of a flow regime or synchrony of extrinsic cues and flows accordingly leaves a species vulnerable to external influences associated with regulation (e.g. Brown and Ford 2002). A long spawning duration increases chances to coincide with events favourable to dispersal and the general mode of reproductive strategy would also likely affect responses to perturbation. Generally short-lived (*r* selected) strategists would more likely be impacted by exacerbated patterns of drying, unnatural short-term isolation and environment-related reproductive failure than longer-lived, less-fecund (*K* selected) species (MacArthur and Wilson 1967). Various nesting behaviours could influence a species' vulnerability to fragmentation, particularly fish spawning near to the edge or even out of water (e.g. some galaxiids: McDowall and Fulton 1996). Fixing or scattering demersal eggs within a confined area (e.g. affixed to structure) provides exposure to the problems of geographic fixation at microhabitat scales (e.g. siltation: Berkman and Rabeni 1987; Soulsbya *et al*. 2001). Conversely, broadcasting semi-buoyant or negatively geotactic eggs facilitates dispersal, but possibly into areas unfavourable for recruitment (Winston and Taylor 1991). Larval characteristics should also be considered in parallel with adult mobility and habitat specificity where pelagic larvae would be less prone to fragmentation than sedentary larvae with strong habitat specificity (e.g. Vrijenhoek 1998).

Biological characteristics

Flowing through a variety of terrestrial biomes with pronounced spatiotemporal variability, freshwater systems display a remarkable degree of habitat heterogeneity. Particular types of habitat often represent rare spatial extremities or are distributed patchily in a system (e.g. Townsend 1989). Consequently, habitat specificity provides a pathway for isolation and geographic fixation (e.g. Rahel *et al*. 1996). Responses to environmental clines and variability in conditions relate directly to physicochemical tolerance and also to behaviour (i.e. avoidance of certain conditions). For example, upper and lower thermal limits can determine distribution (e.g. Closs and Lake 1994; Power *et al*. 1999) and migratory behaviour might be stimulated by flow events (e.g. Meffe 1984; Chapman and Kramer 1991) or be programmed in memory (e.g. natal homing in salmonids). Biotic interactions (competition or predation) can fragment populations (Fraser *et al*. 1995; Thuesen *et al*. 2008), especially following the introduction of larger growing and mobile species (e.g. Townsend and Crowl 1991; Kershner 1997).

2.5. REVIEW SUMMARY

Fragmentation is a natural process acting at a multitude of spatial and temporal scales. However, when coupled with significant anthropogenic change, it can be a pervasive and serious threat to the persistence of biota and the function of ecosystems. Some changes in aquatic landscapes are as stark and visually confronting as those witnessed in terrestrial situations (e.g. large dam construction *versus* land clearance) and the two biomes are generally interlinked (e.g. each are affected by altered water runoff, drainage, riparian habitat loss). A major difference in the nature of fragmentation between terrestrial and aquatic systems, however, concerns the additional level of complexity in freshwater habitats though a four-dimensional habitat templet, and the ease by which connectivity is broken: a relatively minor or local habitat alteration may lead to far-reaching changes.

Given a level of natural exposure to fragmentation, aquatic biota might be expected to have a level of resilience or resistance to anthropogenic fragmentation. This being the case, localised impacts could be countered by specific management solutions to restore dispersal (e.g. fishways). The response of a species to fragmentation (and restoration) might also be predicted in a general sense on the basis of intrinsic characteristics, and hence more vulnerable species could form targets of indicator species for broader ecosystem restoration and monitoring. However, the broader and potentially catchment wide scale of isolation and external influences, and the current precarious status of indicator organisms such as some freshwater fishes, suggests that the real challenge for management is to understand the dynamics of species within ecosystems and accommodate ecological complexity in the face of extensive and rapid alteration to aquatic systems. A key component is environmental flow regimes that cater for vulnerable or specialist species rather than strictly generalists.

Although fragmentation is entrenched in the natural structure and dynamics of freshwater systems, the topic is also integrated within theoretical and applied knowledge of these environments. Consequently, models for connectivity, serial discontinuity and flow regimes already provide a strong basis for the holistic understanding and management of artificial fragmentation in freshwater systems (Ward 1989; Ward and Stanford 1995a; Poff *et al*. 1997). Such integration is perhaps less pronounced in terrestrial realms, as fragmentation is treated more as a separate phenomenon and a discrete theoretical branch of conservation biology. Ultimately, to address the current neglect of aquatic systems in the literature, a synthesis of perspectives from wider disciplines such as freshwater ecology is required. This could in turn aid the development of largely terrestrially-based theory in conservation biology, and inject a constructive and objective basis for managing threats to freshwater biodiversity.

2.6. STUDY SPECIES

The selection of study species was designed to encompass a range of factors, namely species with broadly complementary distributions, including comparison between southern endemics and those of wider occurrence, a mix of common and threatened species, and diversity in intrinsic characteristics that may influence dispersal (Table 2-2, with further elaboration in Chapters 3-8). A group of obligate freshwater fishes was selected from the southeast due to greater accessibility and the existence of some tissue collections, but the fauna and environments of southwestern Australia remain as an ideal complementary study in future.

2.7. TABLES

Table 2-1. Intrinsic characteristics of freshwater fishes as a framework for assessing their vulnerability to fragmentation.

Intrinsic characteristics	Traits to examine
Dispersal capabilities	
Life history strategy	Larger scale movements: diadromy, potamodromy, home range
Adult mobility	Locomotion, size, vertical position (e.g. benthic, pelagic), morphology
Adaptations	Anatomical features, aestivation
Reproductive behaviour	
Strategy	Relationship to flow regime, timing, duration, *K*- or *r*-selected
Nesting behaviour	Spawning substrate and position
Larval characteristics	Dispersal potential (e.g. demersal or pelagic)
Biological characteristics	
Habitat specificity	Specialised or opportunistic, lotic- or lentic-adapted, flow requirements
Physicochemical tolerance	Tolerance of abiotic factors (e.g. flow, temperature)
Behaviour	Deterrents or response, memory (e.g. natal homing)
Biotic interactions	Potential exposure to competitors, vulnerability to predation

Table 2-2. Study species with indications of intrinsic traits, hypothesised genetic structure, distribution and conservation status (after Koehn and O'Connor 1990; McDowall 1996a; Humphries and Lake 2000; Allen *et al*. 2002; Growns 2004; Pusey *et al*. 2004).

Intrinsic characteristics	*Nannoperca australis*	*Nannoperca obscura*	*Gadopsis marmoratus*	*Mogurnda adspersa*	*Philypnodon macrostomus*	*Philypnodon grandiceps*	*Retropinna semoni*
Dispersal capabilities							
Life history strategy -Non-migratory: N -Diadromous: D -Small home range: H	N	N	H	N	N	N	D?
Adult mobility -Small bodied: S -Large bodied: L -Pelagic: P or Benthic: B	S, B	S, B	L, B	S, B	S, B	S, B-P	P
Adaptations -Climbing: C	?	?	?	C?	?	?	?
Reproductive behaviour							
Strategy -*K*- or *r*-selected -Low flow recruitment: LF -Flow cues: F, -Opportunistic: O	*r*, F	r, F	K, F	r, L	r, L	r, L	r, O
Nesting behaviour (eggs) -Scattered in vegetation: V -Affixed to substrate: A -Broadcast pelagic: B	V	V	A	A	A	A	B
Larval characteristics -Large and demersal: D -Semi-pelagic: S -Pelagic: P	D	D	D	S	S	S	P
Biological characteristics							
Habitat specificity -Specialists: S -Generalists: G	S	S	S	S	G	G	G
Physicochemical tolerance -Narrow: N -Moderate: M or High: H	N	N	N	M	H	H	H
Behaviour -Response to flow: R -Natal homing: N	R	R?	R?	R?	-	-	N?
Biotic interactions -Vulnerable to predators: P -Vulnerable competitors: C	P, C	P, C	C	P, C	P	P	P
Hypothesised genetic structure	Strong	Medium-strong	Medium-strong	Medium	Medium	Low-medium	Low
Distribution	Southern endemic	Southern endemic	Southern endemic	Southern-tropical	Southern-sub-tropical	Southern-sub-tropical	Southern-sub-tropical
Conservation status	Common	Threatened	Threatened	Threatened	Rare	Common	Common

3. A RE-THINK ON *RETROPINNA*

3.1. INTRODUCTION

Southern Hemisphere smelts and graylings (Retropinnidae) are small- to medium-sized fishes (< 320 mm) endemic to southeastern Australia and New Zealand, and related to the Northern Hemisphere Osmeridae (Johnson and Patterson 1996; Waters *et al*. 2002a; Lopez *et al*. 2004). Members of both families have a distinctive cucumber-like odour (McDowall *et al*. 1993). The Retropinnidae comprises two sub-families, Prototroctinae (southern graylings) and Retropinninae (southern smelts). The later contains two genera of small fishes (< 150 mm), *Retropinna* (three species) and *Stokellia* (one species), typical of lowland rivers, streams, lakes and estuaries (McDowall 1979, 1990, 1996b). *Retropinna* species are schooling, pelagic fishes, often found in very large numbers. They have attracted little attention in conservation (McDowall 1990, 1996b), although there has been concern over the loss of morphologically-distinct lacustrine populations in New Zealand (Ward *et al*. 2005). Australian species are regarded as 'forage' in natural ecosystems and in fisheries based on alien salmonids (Milton and Arthington 1985; McDowall 1996b), and may also be food for humans (Lake 1967, 1971). They are prominent in assessments of ecosystem function (Lieschke and Closs 1999; Puckridge *et al*. 2000; King *et al*. 2003), riverine health (Arthington *et al*. 1983; Harris and Silveira 1999; Humphries and Lake 2000), and biodiversity (Raadik 1992; Cashner *et al*. 1999; Wedderburn and Hammer 2003).

Retropinna in Australia includes *R. semoni* (Weber) from the mainland and *R. tasmanica* McCulloch from Tasmania (McDowall 1979). However, traditional taxonomic assessments are hindered by limited characters (retropinnids are morphologically conservative), and difficulty in examination for key morphological characters that are present (e.g. scales are thin, unpigmented and easily dislodged: McDowall 1979). Some geographic isolates have been suggested as separate taxa (Ogilby 1908; Stokell 1941; Lake 1971; Wager and Unmack 2000), but none are recognised in the current taxonomy. *Retropinna semoni* occurs in widely dispersed and naturally divided inland waters (McDowall 1979; Unmack 2001; Hammer and Walker 2004), where it breeds (Milward 1965; Legget and Merrick 1987), and is vulnerable to isolation and genetic divergence.

The apparent restriction of *R. tasmanica* to Tasmania is curious if, as suspected, the species is anadromous (McCulloch 1920; Lake 1971; Fulton 1990; McDowall 1996b), because migratory behaviour would facilitate wider dispersal. The integrity of these two taxa is also of interest because of opportunities for dispersal between Tasmania and mainland Australia during glacial maxima and sea-level regressions (Unmack 2001).

3.2. METHODS

Sampling and analyses

Samples were obtained from coastal systems between Baffle Creek, Queensland and the Glenelg River, Victoria; from the inland Murray-Darling and Lake Eyre drainage divisions (basins); and from Tasmania (Figure 3-1 and Table 3-1). Fish sampled were euthanased and snap frozen in liquid nitrogen, then stored at -70°C in the laboratory, pending analysis. Additional voucher specimens have been deposited at the Australian, South Australian and Victorian museums.

Allozyme analyses were conducted in two stages. The first involved an *overview study* to examine species boundaries and broad population structure, and thereby incorporated a large number of loci, small sample sizes per locality, and numerous localities spread across the geographic range (as recommended by Richardson *et al*. 1986). Geographic coverage included 52 localities from five Australian states, with two fish per location screened for allozyme variation at 50 loci in two gel batches. Where appropriate, sample sizes were increased (n = 3-6) in the second gel batch for those locations and areas which displayed genotypes inconsistent with the presence of a single panmictic population (i.e. multiple fixed differences between sympatric individuals). The second stage, a *population study*, involved more detailed screening to clarify patterns detected in the overview study, and to investigate population sub-structure. This involved increasing the sample sizes (final n = 5-12, mean 7.4) at informative polymorphic loci (i.e. frequency of most common allele < 95%) for 24 regional populations from among the 52 original localities. Ten populations were added (bringing the total number of sites examined to 62), including eight from the Lower Murray, one from the Glenelg River and a temporal (10 years) comparison at one site in Lake Alexandrina. Localities and sample sizes are indicated in Table 3-1 and Figure 3-1.

Allozyme electrophoresis

Homogenates comprised a small piece of caudal muscle sonicated in an equal volume of buffered lysing solution (0.02M Tris-HCl, pH 8.0, with 0.2% 2-mercaptoethanol and 0.02% NADP). After centrifugation for 10 min. at 10,000 g, supernatant fluids were stored at -20°C as 10-20 µL aliquants in glass capillary tubes. Allozyme electrophoresis was conducted on cellulose acetate gels (Cellogel™), following Richardson *et al*. (1986). Thirty one enzymes or non-enzymatic proteins produced zymograms of sufficient intensity and resolution for genetic interpretation in the overview study: ACON, ACYC, ADA, AK, CA, CK, ENOL, EST, FDP, FUM, GAPD, GLO, GOT, GP, G6PD, GPI, GSR, IDH, LDH, MDH, ME, MPI, PEPA, PEPB, PEPD, 6PGD, PGK, PGM, PK, TPI, and UGPP. Details of enzyme and locus abbreviations, enzyme commission numbers, electrophoretic conditions and stain recipes are in Richardson *et al*. (1986) and Bostock

et al. (2006). Allozymes were designated alphabetically and multiple loci, where present, were designated numerically, both in order of increasing electrophoretic mobility (i.e. *Mpi*^{a}, *Mpi*^{b}, *Gpi1*, *Gpi2*).

Data analysis

The initial unit for analysis was individual specimens. No *a priori* assignments to taxa were made because the allozyme data were intended to provide an independent assessment. The genetic affinities of individuals from the overview study were explored using principal co-ordinates analysis (PCO), as implemented on a pairwise matrix of Rogers' genetic distance (Rogers 1972) using PATN (Pattern Analysis Package, DOS version, Belbin 1994). Together with an examination of the raw data, these analyses revealed the presence of sympatric taxa at three sites. Otherwise, each site was treated as a distinct Operational Taxonomic Unit (OTU).

Two between-OTU estimates of genetic similarity were calculated, namely (1) percentage fixed differences (%FD, Richardson *et al*. 1986), allowing a 10% tolerance for shared alleles (i.e. the cumulative total of any alleles in common at a locus should not exceed 10%), and (2) Nei's unbiased Distance (Nei D, Nei 1978). As explained by Richardson *et al*. (1986), the number of fixed or diagnostic differences is biologically more relevant than any measure based on differences in allele frequency (such as Nei D) for the delineation of species boundaries, whereas the latter is a more appropriate measure of between-population divergence and relevant for assessing systematic hierarchies above and below the rank of species.

For the population study, the genetic affinities among individuals were explored using PCO on a pairwise Rogers' genetic distance matrix, as in the overview study. The genotypic data were examined for statistical evidence of any deviation from Hardy Weinberg expectations or linkage disequilibrium within populations and any heterogeneity of allele frequencies between populations. These tests involved estimating exact probabilities using GENEPOP version 3.4 (Raymond and Rousset 2003), with all probability values adjusted for multiple tests using the sequential Bonferroni correction factor (Rice 1989). F-statistics were used to assess the degree of population genetic sub-division within and among individual populations and regions. F_{ST} and F_{IS} values plus their 99% confidence intervals were obtained using the program FSTAT 2.9 (Goudet 2000). Finally, the data were examined for geographic patterns of genetic diversity, using observed heterozygosity levels (H_O, direct count method) as a measure of within-population diversity, and by mapping allele frequencies for the most informative loci at each site using ArcMap^{TM} 8.3 software.

For both overview and population studies, the genetic affinities of OTUs or populations were displayed visually as UPGMA (Unweighted Pair-Group Method of arithmetic Averages) dendrograms and Neighbor Joining (NJ) networks constructed from Nei D values using the NEIGHBOR routine in PHYLIP 3.5c (Felsenstein 1993) and drawn using TREEVIEW 1.6.0 (Page 1996). Allele frequencies, observed heterozygosity levels and genetic distances were calculated using BASIC programs written by Mark Adams, South Australian Museum.

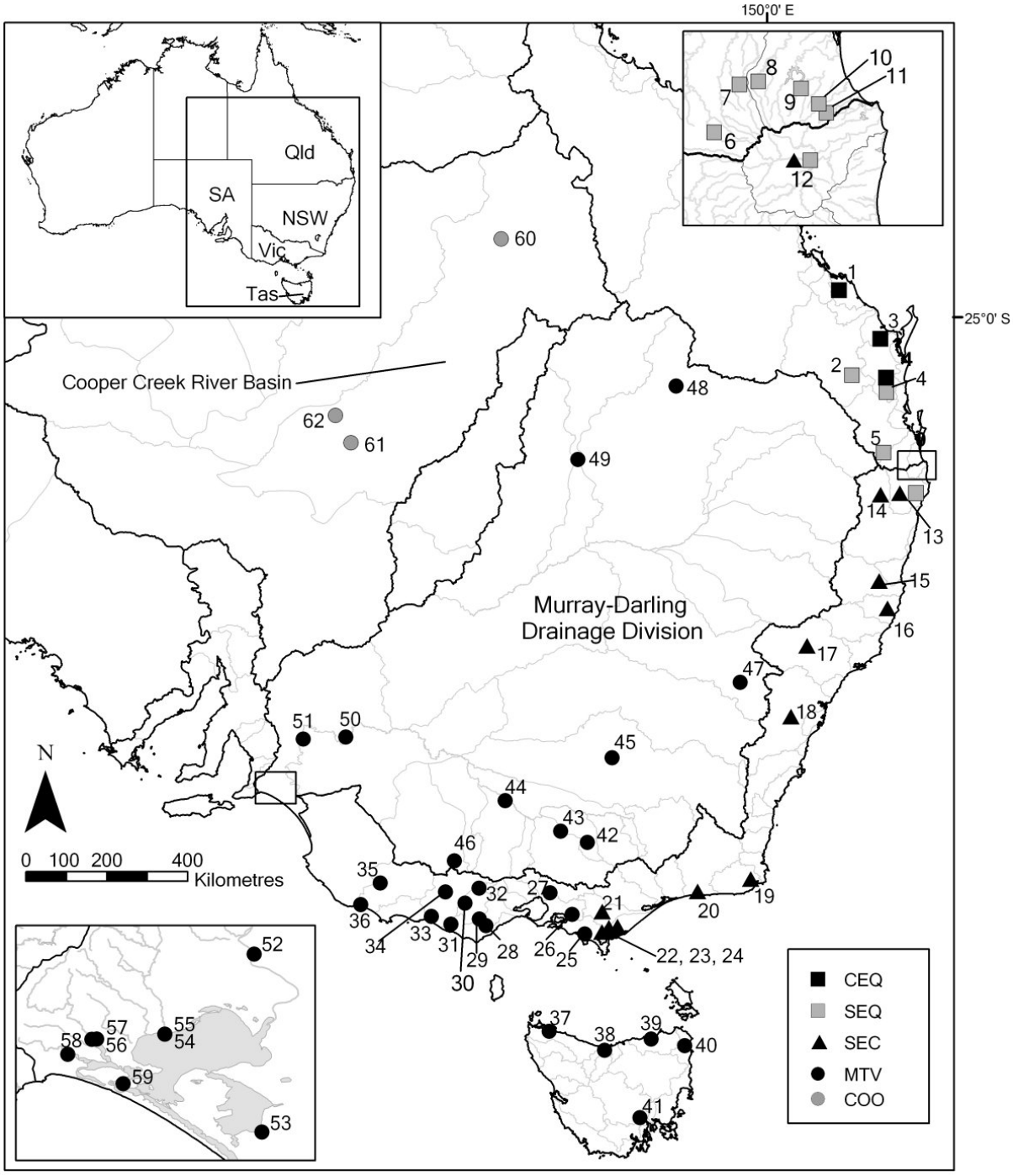

Figure 3-1. Geographic relationships of *Retropinna* samples subject to allozyme electrophoresis. Also shown are major drainage divisions and river basins (AWRC 1976). Sites codes as per Table 3-1 and taxon abbreviations as per the text.

3.3. RESULTS

Major genetic groupings

Forty three putative loci were interpretable in the overview study, six of which (*Gapd1*, *Gapd2*, *Gp*, *Idh2*, *Pk1*, and *Tpi1*) were invariant amongst all 122 individuals screened (allozyme profiles are given in Appendix 1). An initial PCO grouped individuals, in three dimensions, to one of five well-separated and discrete clusters (i.e. no intermediate or hybrid forms were detected) and identified sympatric individuals belonging to different major groups at three sites (Figure 3-2). This allowed confident allocation of 55 OTUs (49 sites with a single taxon; three sites each with two sympatric taxa).

The genetic affinities of the 55 OTUs identified are shown visually in the dendrogram of Figure 3-3. Five major genetic groups were obvious (see also Table 3-2 and Table 3-3) and these have largely abutting and distinctive geographic ranges. Based on geographic distribution (Figure 3-1), the groupings are hereafter referred to as CEQ (central-east Queensland), SEQ (southeast Queensland), SEC (southeast coast), MTV (Murray-Darling Basin + Tasmania + western coastal Victoria), and COO (Cooper Creek River Basin).

Three taxa occurred in drainages along the eastern coastline of mainland Australia. CEQ was diagnosable by fixed or near-fixed differences at seven loci from SEQ (*Acyc*, *Enol1*, *Got1*, *Got2*, *Gsr*, *Ldh1*, and *Mpi*), and SEC was diagnosable by fixed or near-fixed differences at 12 loci from CEQ (*Ak1*, *Ca*, *Enol1*, *Est1*, *Gpi1*, *Gpi2*, *Gsr*, *Me1*, *Me2*, *PepA1*, *PepB*, and *Pk2*;) and 14 loci from SEQ (*Ak1*, *Ca*, *Est1*, *Fum*, *Got1*, *Gpi1*, *Gpi2*, *Gsr*, *Ldh1*, *Me2*, *Mpi*, *PepA1*, *PepB*, and *Pk2*) (Table 3-2). The three instances of sympatry between individuals representing two different groups were: CEQ and SEQ at site 4 (Yabba Creek), and SEQ and SEC at site 12 (Oxley River) and site 13 (Richmond River). The persistence of these groups without evidence of genetic exchange at numerous loci (six loci for CEQ *v*. SEQ at site 4; 17-20 loci for SEQ *v*. SEC at sites 12/13: see Appendix 1) unambiguously demonstrates that these three groups are distinct species. Visual examination of the whole frozen specimens sub-sampled for genetic analysis indicated that the sympatric individuals also differed in external appearance (e.g. head bluntness, body depth, colouration of body and fins), sufficiently so that novel individuals were correctly assigned *a priori* to their major genetic groupings in the second batch of gels (Figure 3-4).

The fourth major genetic grouping, MTV, was by far the most widespread, occurring in three drainage divisions in five Australian states and one territory (i.e. coastal Victoria west of Wilsons Promontory, the Murray-Darling Basin, and Tasmania). Its range abuts that of SEC near Wilsons Promontory (*Retropinna* spp. are apparently absent from the Promontory), where there are

localities separated by only *c*. 75 coastal kilometres. Here, the two groups displayed fixed differences at 10 loci (23%FD: *Acon2*, *Ak1*, *Enol1*, *Est1*, *Gsr*, *Me1*, *Me2*, *PepD*, *6Pgd*, and *Ugpp*; Appendix 1) when sites were compared (sites 25-26 pooled to represent MTV and sites 21-24 pooled to represent SEC). Given that MTV also displayed numerous fixed differences in allopatry from both CEQ and SEQ (23%FD and 36%FD respectively: Table 3-3) the allozyme data strongly support the notion that MTV represents a distinctive fourth taxon.

The three OTUs from the Cooper Creek River Basin (Drainage Division X) were as divergent from those in drainage divisions II, III, and IV (0.32 Nei D, Figure 3-3; minimum 12%FD, Table 3-3) as the dichotomies between CEQ/SEQ and SEC/MTV (0.25 Nei D and 17%FD, 0.35 Nei D and 14%FD respectively; Figure 3-3 and Table 3-3). MTV and COO were fully diagnosable at four loci (*Acyc*, *Ak1*, *Gpi1*, and *PepD*), a result comparable to the yardstick of differentiation provided by the most closely-related sympatric species CEQ *versus* SEQ, and by near-fixed differences at three others (*Acon1*, *PepA1*, and *Ugpp*). Importantly, the two taxa exhibited pronounced differentiation between proximate allopatric populations, namely 14%FD between COO compared to Darling River populations of MTV (sites 48 and 49; see Appendix 1). Moreover, all COO individuals were homozygous at each of four loci for an allele not found elsewhere, namely $Acyc^{g}$ $Ak1^{c}$, $Gpi1^{i}$, and $PepD^{a}$ (apart from a single rare *PepD* heterozygote in SEC: Table 3-2). This indicates that the distinctiveness of COO and MTV is more than the stochastic variance typical of allopatric populations which recently shared an ancestral gene pool (other alleles detected only in COO included $Acon1^{e}$, $Got1^{d}$, Gsr^{f}, $PepB^{e}$ and Pgk^{a}), and supports the recognition of COO as a fifth taxon.

Intriguingly, the expectation of an endemic Tasmanian species, based on current taxonomy, was not supported by the overview study. Specimens from the five Tasmanian sites were scattered among the genetically-heterogeneous MTV cluster that also included 11 Victorian populations (Figure 3-3).

Genetic structure in MTV

Significant geographic and genetic diversity displayed in MTV was confirmed, and is addressed in the population study. The final dataset for the population study comprised genotypes for 250 individuals (202 newly-screened plus 48 from the overview study) at those variable loci which displayed strongly-staining, unambiguous electrophoretic phenotypes for all putative genotypes. Allele frequencies at 26 polymorphic loci are presented in Table 3-4 for the 34 populations surveyed.

The first analysis undertaken was a PCO on all 250 specimens (Figure 3-5). This analysis also revealed no indication of a simple '*tasmanica*' (Tasmanian sites) *versus* '*semoni*' (mainland sites) dichotomy in MTV (Figure 3-3). Instead, regional distinctiveness was evident for five groupings, namely (1) Upper Murray plus Darling rivers (i.e. upstream of river basin 26), (2) Lower River Murray, (3) Glenelg River, (4) western coastal Victoria, and (5) Tasmania. Only the Glenelg cluster (sites 35-36), however, is unequivocally different; this involves alleles that are absent (*Acon1*^{c}, *6Pgd*^{c}) or rare (*Me2*^{b}) elsewhere (Table 3-4). The Upper Murray/Darling, Lower Murray, and Tasmanian clusters occurred as discrete but adjacent groups. In sharp contrast, and despite individuals representing a single site invariably forming a relatively-cohesive cluster (Figure 3-5, not all sites shown), coastal western Victorian populations were spread out along the entire length of Axis 1 and most overlapped one or more of the three aforementioned clusters.

Figure 3-6 summarises the genetic affinities between the 34 MTV populations. The same general features displayed by PCO are evident here, namely discrete clusters for each of the Upper Murray/Darling, Lower Murray, Glenelg, and Tasmanian regions, with the coastal Victorian sites interspersed throughout. However, the dendrogram provides two additional insights. First, the primary dichotomy delineates what could be construed as '*tasmanica*-like' (all Tasmanian plus four coastal Victorian sites) and '*semoni*-like' (all other sites) groups (although these do not appear to be reciprocally monophyletic in a Neighbor-Joining tree, analysis not presented). Secondly, all but three coastal Victorian sites occupy basal or near-basal positions within their cluster, except (1) site 32, one of two isolated lakes within the Upper Murray/Darling genetic cluster, (2) site 25, closely aligned with site 41 in Tasmania, and (3) site 31, within the main cluster of Tasmanian sites. Together, Figure 3-5 and Figure 3-6 indicate that coastal western Victorian sites were the most genetically heterogeneous of all the geographic regions, and displayed a complex mosaic of affinities with the other regional groupings.

One possible explanation for the complexity observed in MTV is suggested by the observation that the Upper Murray/Darling, Lower Murray, and Tasmanian regional PCO clusters were arranged from left to right along Axis 1 (Figure 3-5). Such an outcome might be anticipated for the scenario of a 'pure' inland form, a 'pure' Tasmanian form, and an intermediate Lower Murray 'hybrid sink'. Under such a scenario, the heterogeneity of coastal Victorian populations could then reflect varying degrees of historical distinctiveness, tempered by differing levels of gene flow. A comparison of the allele frequencies for the Upper Murray/Darling *versus* Tasmania support this scenario, by revealing considerable divergence involving near-fixed differences at four loci (*Ca*, *Mdh*, *Ldh2*, *PepD*; Table 3-4) plus significant differences at numerous other loci (mean across all pairwise comparisons = 5.7).

Figure 3-7 displays the geographic distribution of alleles among all MTV populations at the four near-diagnostic loci. Each locus exhibits a geographic pattern entirely consistent with the 'hybrid sink' model, namely (1) all Tasmanian and all Upper Murray/Darling sites largely fixed for different alleles, and (2) all Lower Murray sites polymorphic for these alleles. Moreover, the coastal Victorian and Glenelg sites display a complicated pattern of relatedness which varies from locus to locus, consistent with them having been subjected to differing levels of gene flow involving '*tasmanica*' alleles. More support for this observation is evident for alleles at other polymorphic loci (*Ca*^{a}, *Glo*^{b}, *Got2*^{g}, *Gsr*^{c}, *Me2*^{b}, and *Pgm*^{d}; Table 3-4).

Heterozygosity estimates for the regional groups provide indirect corroboration for the idea that Lower Murray populations represent a 'hybrid sink' between pure Tasmanian and pure Upper Murray/Darling lineages. The H_O value for the pooled Lower Murray sites was 0.091 ± 0.025, which is significantly larger than values for the other two regions (Upper Murray/Darling = 0.051 ± 0.015; Tasmania = 0.060 ± 0.018). However, the overall H_O values for coastal Victoria (0.061 ± 0.015) and Glenelg (0.050 ± 0.018) did not differ significantly from those of the two 'pure' regions.

Statistical methods provided no evidence to reject the two null hypotheses assumed to apply in any analysis of population structure (i.e. individual populations are panmictic, and no two loci are in linkage disequilibrium). Further support for within-population panmixia was provided by F-statistics, with no F_{IS} value differing significantly from zero (Table 3-5). Neither set of replicate samples (sites 56 *v*. 57, spatially proximate in the Finniss River; sites 54 *v*. 55, collected 10 years apart at the same site in Lake Alexandrina) showed any significant differences in allele frequency, allowing each population to be represented by pooled sites.

Two measures were employed to quantify the extent of between-population divergence, namely (1) the number of statistically-significant differences in allele frequency among pairwise combinations of the 32 populations, and (2) F-statistics for various hierarchical levels (Table 3-5). The UPGMA dendrogram was used both to provide the population hierarchy and to summarize the number of significant differences present between populations (*via* branch thicknesses: Figure 3-6). Both analyses demonstrate that MTV displays considerable heterogeneity throughout most of its geographic range. Indeed, there were only five instances where groups of populations did not display any significant differences in allele frequency from one another (Table 3-5 and Figure 3-6). These were the Lower Murray sites (50-57, 59), a subset of the Upper Murray/Darling sites (42-46, 48), the two Glenelg sites (35, 36), three sites along the north coast of Tasmania (37-39), and the Derwent and Tarwin River sites (41 and 25).

All other pairwise comparisons revealed one or more significant differences among at least some of the sites grouped together in the dendrogram (Figure 3-6), while seven populations (sites 27, 34, 28, 35/36, 33, 26, 40; Figure 3-7) were statistically distinct from all others, generally at multiple loci (Table 3-5). F-statistics revealed an even greater level of differentiation. All F_{ST} values were significantly positive (mostly $p < 0.01$, Table 3-5), not just those for population clusters differentiated by pairwise comparisons of allele frequency (Figure 3-6). Thus, divergence is evident within each of the five groups identified earlier, indicating that genetic distinctiveness is the rule rather than the exception among populations of MTV, even at smaller geographic scales.

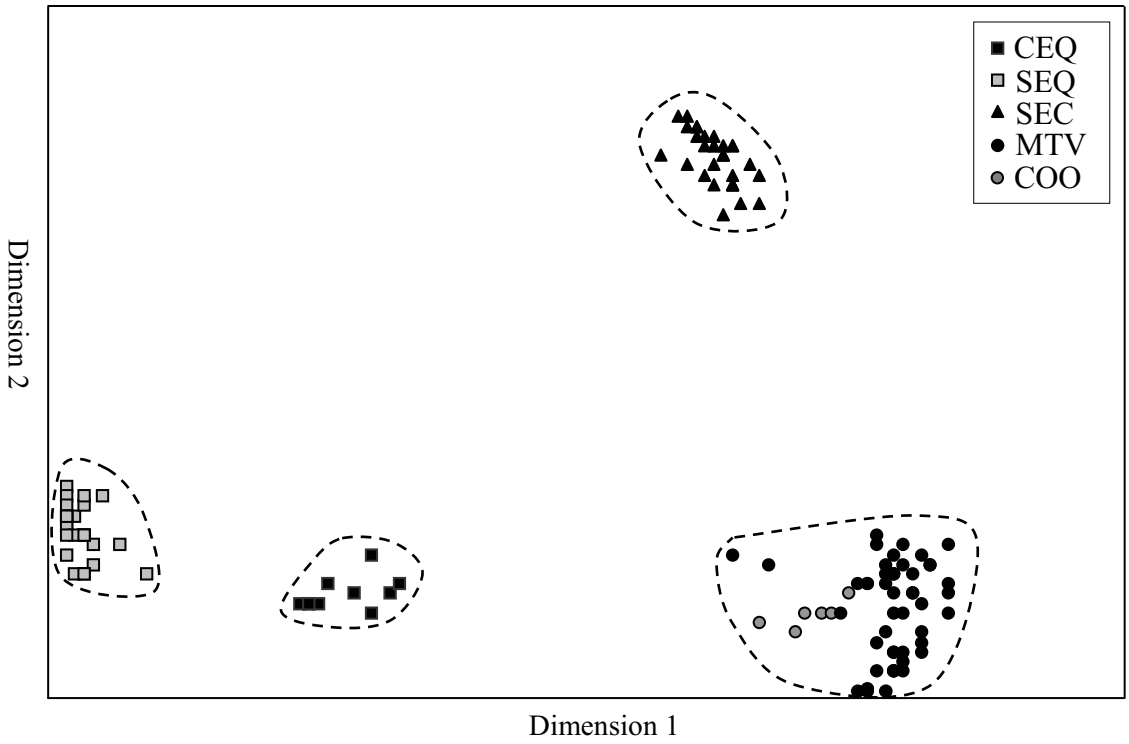

Figure 3-2. Principal Coordinates analysis of the 122 specimens in the allozyme overview study. The relative PCO scores have been plotted for the first and second dimensions, which explain 33% and 17% of the total variance, respectively. Envelopes highlight major genetic groups. Note that the COO taxon is distinctive in the third dimension of the ordination (not shown).

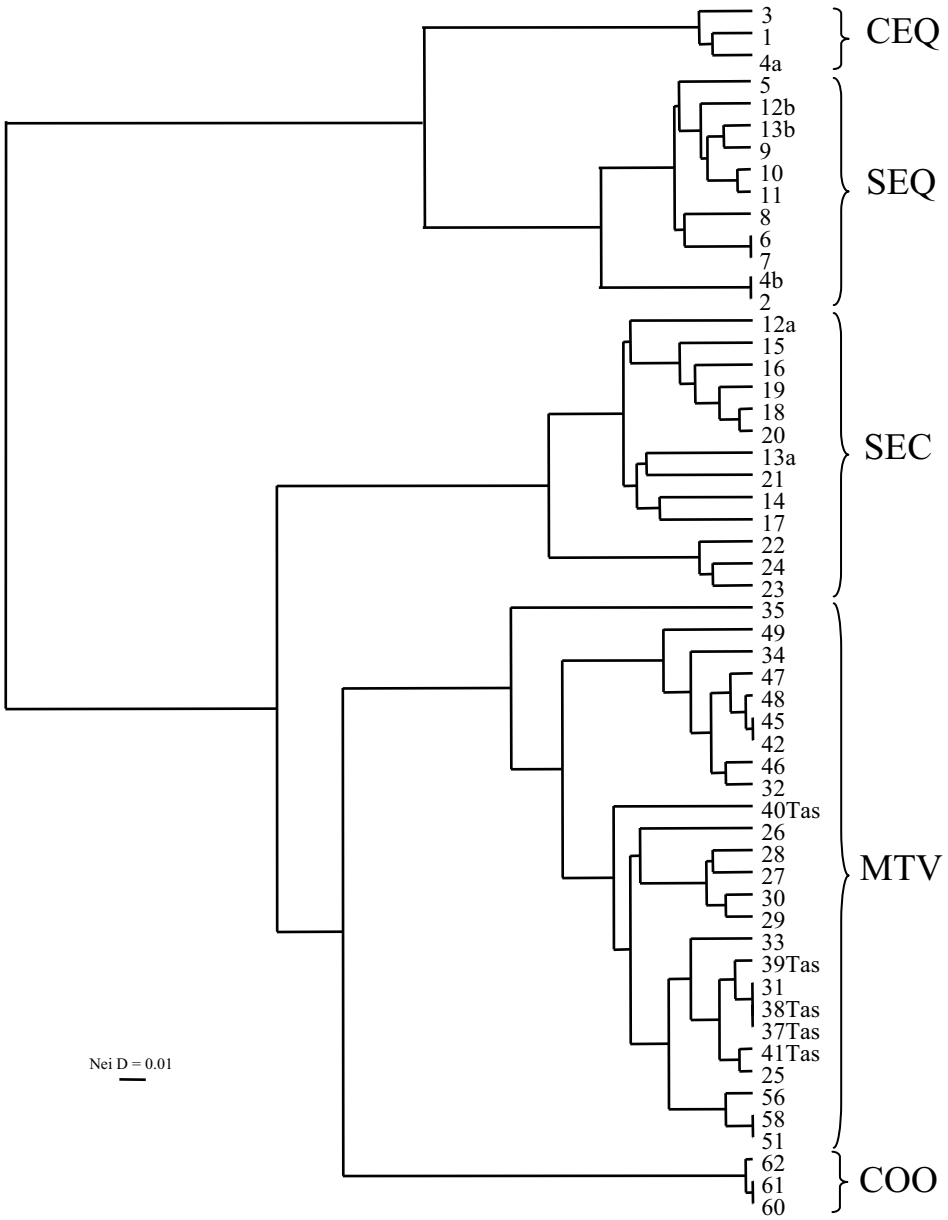

Figure 3-3. UPGMA dendrogram depicting genetic affinities among the 55 OTUs in the overview study, based on pairwise Nei Distance values. Sample locations from Tasmania (Tas) are shown within the broader genetic grouping of the MTV taxon.

Figure 3-4. Visual comparison of frozen specimens of sympatric *Retropinna* (Yabba Creek). Individuals on the left are the SEQ taxon; those on the right are CEQ.

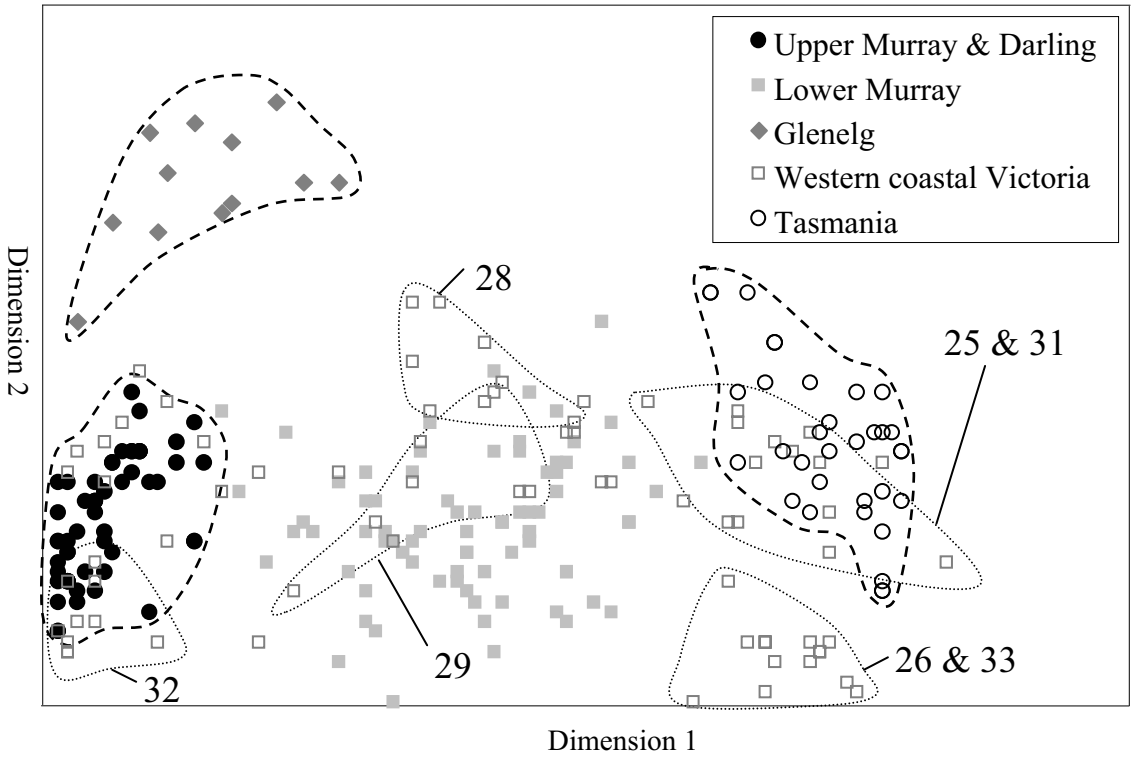

Figure 3-5. Principal Coordinates analysis of the 250 specimens involved in the examination of population structure in the MTV taxon. The relative PCO scores have been plotted for the first and second dimensions, which explain 28% and 7% of the total variance, respectively. Envelopes highlight three major genetic groups (dashed lines) and selected western coastal Victorian populations (dotted lines).

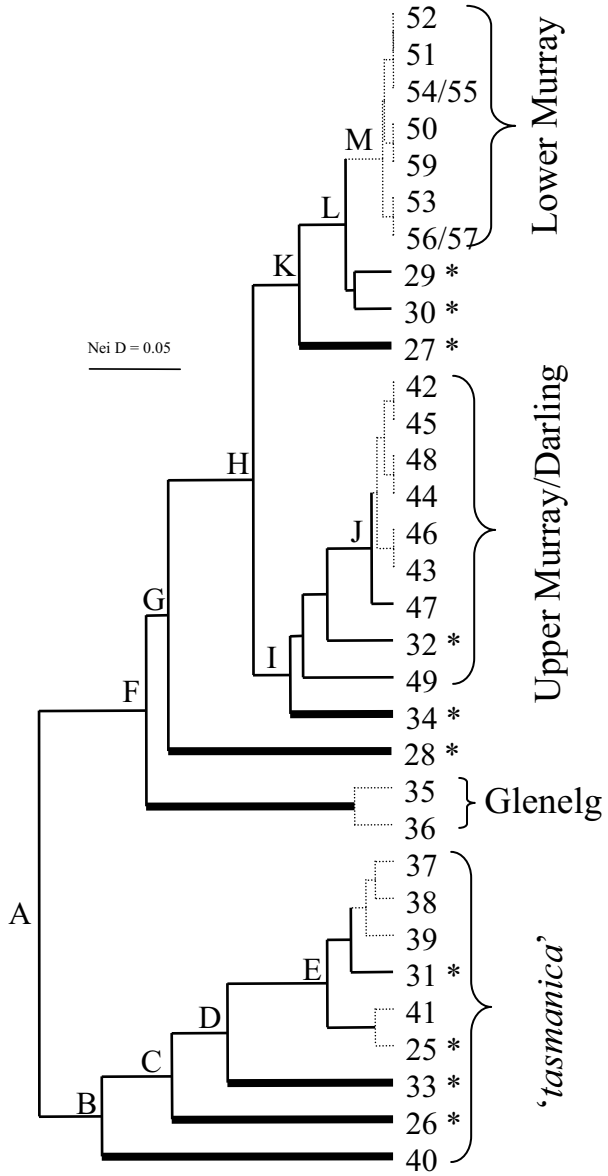

Figure 3-6. UPGMA dendrogram depicting genetic affinities among the 34 sites sampled during the population study, based on pairwise Nei D values. Replicate sites (54 and 55, 56 and 57) were pooled for this analysis. Branch thicknesses reflect the number of statistically-significant differences in allele frequency; thick = at least one difference between that site and all others, thin = some but not all sites show at least one difference, dashed = no differences. Letters represent hierarchical levels used in statistical evaluation (see Table 3-5). (*) denotes western coastal Victorian populations.

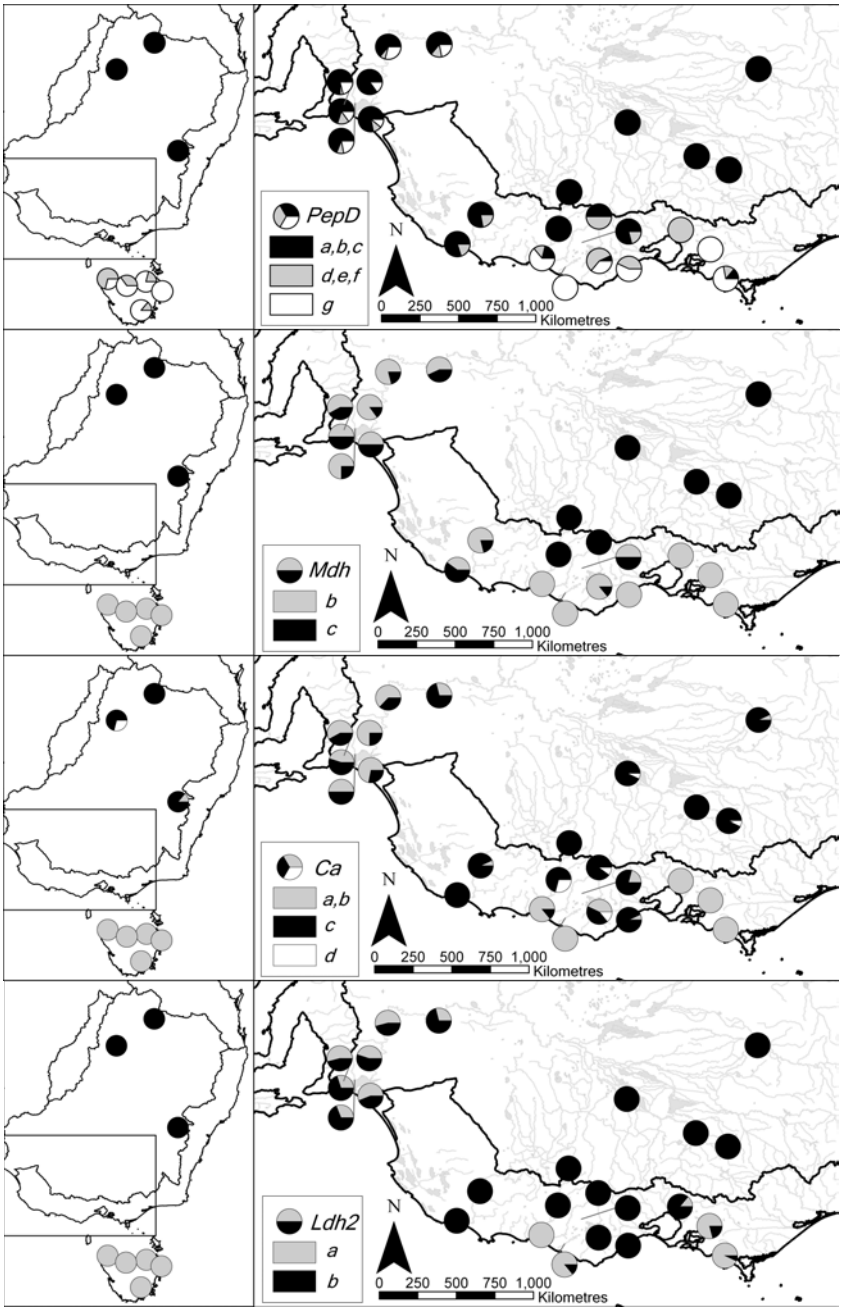

Figure 3-7. Maps of allele distribution for the four loci which best distinguish the Tasmanian and Upper Murray and Darling regions within MTV. The loci from top to bottom are *PepD*, *Mdh*, *Ca*, and *Ldh2*.

3.4. DISCUSSION

This study provides a compelling illustration of the need for systematic frameworks that incorporate molecular approaches. Previously, only two species of Australian *Retropinna* were recognised and no information was available on population genetic structure. In contrast, the allozyme data indicate the presence of at least five species in Australia, suggest that one of the previously-recognised species may not be valid, and reveal significant intra-specific substructuring, signalling the need for a major review of taxonomy, ecology and conservation within the group. Five species are strongly supported by the allozyme data, based on genetic divergence, discrete geographic ranges, and, most importantly, instances of sympatry among three species, CEQ, SEQ, and SEC, on the eastern seaboard.

Taxonomic considerations

Whilst morphological and molecular data often concur, it is not unusual to encounter situations where molecular markers reveal additional cryptic diversity, or where a single taxon includes multiple morphotypes (Avise 2004). Both scenarios were uncovered in this study. The type locality for *R. semoni s.s.* is the Burnett River, Queensland, within the range of both CEQ and SEQ, and hence this name may ultimately be applied to either species. The status of *R. tasmanica*, on the other hand, is unclear. The name *R. tasmanica* would apply to MTV, should MTV prove to be a single species. However, the presence of two distinct gene pools within MTV, with extensive hybridisation in their zone of contact, could reflect a predominantly Tasmanian species (*R. tasmanica*) and a mainland species.

Clearly, there is a need for review of morphological criteria for separating Tasmanian *Retropinna* from those in key Victorian rivers, and for additional molecular datasets to resolve the MTV complex. There is also a need for a morphological re-appraisal of all Australian *Retropinna*, including specimens identified using molecular markers in zones of potential overlap. Qualitative observations from this study on combinations of SEQ *versus* CEQ/SEC in sympatry suggest that external differences may exist. Morphological and ecological differences have previously been noted for COO (e.g. Lake Eyre Basin fish have larger eyes and dwarfed size at maturation: McDowall 1979; Wager and Unmack 2000), and Musyl and Keenan (1992) used a similar genetic rationale to that employed here to distinguish golden perch (genus *Macquaria*) from the Lake Eyre Basin as a distinct (but still undescribed) species from those in the Murray-Darling Basin (cf. multiple diagnostic loci and a Nei D of 0.32 for COO/MTV *v*. 0.23 in *Macquaria*).

High species richness and multiple instances of sympatry, detected using only 14 sites, argue strongly for the need to survey additional river basins in Queensland and northern New South Wales, where the distribution of the three species was assessed only at a broad scale. Additional material from river basins in New South Wales (Bellinger River, Karuah River, Macquarie-Tuggerah Lakes: tissues supplied by the Australian Museum), obtained after analyses were completed, displayed the diagnostic characters expected for SEC (data not presented). Genetic distance values and allozyme profiles also suggest divergent regional populations from one or more river basins in SEQ and SEC, and these warrant more in-depth examination to identify any distinctive evolutionary components.

Genetic sub-structure

Most populations within MTV were genetically distinct, involving both conspicuous sub-populations (e.g. Glenelg River) and including unexpectedly distinctive gene pools in the Lower and Upper Murray-Darling Basin. Observed structure within MTV shows a general coastal homogenisation between two broadly disparate mainland and Tasmanian genetic profiles, but with genetically distinctive populations (possibly reflecting potentially older evolutionary components) randomly interspersed in western coastal Victoria, particularly for inland locations buffered from the influence of coastal dispersal. This appears to reflect stochastic mixing and subsequent drift as a signature of intermittent historic gene flow. The present genetic data (i.e. large between-region F_{ST} values plus the numerous highly-significant differences in allele frequency) suggest no current exchange across Bass Strait, or between any populations separated by marine barriers. As a basis for future studies using DNA markers to probe phylogenetic relationships, it is hypothesized that fish from Tasmania spread northward during periods of connection between freshwater or more sheltered estuarine habitats.

Biogeographic patterns

The overall pattern of strong genetic sub-structuring within and deep divergence between species is characteristic of a group with endemic origins and a long association with Australian fresh water or inland habitats. The Great Dividing Range, separating coastal and inland river basins in eastern Australia, is a well-documented barrier to east-west dispersal (see Unmack 2001), and clearly has affected *Retropinna* along its length. In Queensland and northern New South Wales, the distributions of CEQ, SEQ and SEC mirror three lineages identified in the ornate rainbowfish *Rhadinocentrus ornatus* (Page *et al*. 2004), reaffirming that biogeographic relationships in this region are complex (e.g. Iredale and Whitley 1938; Georges and Adams 1992; Musyl and Keenan 1996; Chenoweth and Hughes 2003; Munasinghe *et al*. 2004). Species divisions roughly align to

the northern end of Fraser Island (CEQ *v*. SEQ) and to the McPherson Range (SEQ *v*. SEC), which also forms the state boundary between New South Wales and Queensland (a similar divide occurs in *Rhadinocentrus ornatus*: Page *et al*. 2004). Further south, the boundary between SEC and MTV at Wilsons Promontory is the distributional limit for many freshwater fishes (potentially a relict of ancient drainage patterns: Unmack 2001), reflected also in an essentially east-west genetic division in *Nannoperca australis* (Chapter 5). The single most distinctive population in MTV, that in the Glenelg River, mirrors biogeographical distinctions for other species including the restricted variegated pygmy perch *Nannoperca variegata*, spiny crayfish *Euastacus bispinosus* and freshwater mussel *Hyridella glenelgensis*. Lastly, the current and recent-historic isolation of the Lake Eyre Basin seems surmountable, through natural means, by only the most highly mobile taxa (e.g. spangled perch *Leiopotherapon unicolor*: Bostock *et al*. 2006).

Ecology

Researchers have often pooled data on *Retropinna* from different regions (e.g. Cadwallader and Backhouse 1983; McDowall 1996b; Allen *et al*. 2002; Pusey *et al*. 2004), and applied this to local areas (Humphries *et al*. 1999). However, such an approach is likely to mask ecological heterogeneity resulting from deep genetic separations between species that occur in contrasting and isolated environments, especially the Lake Eyre Basin (cf. Milward 1965; Milton and Arthington 1985; Puckridge *et al*. 2000; Humphries *et al*. 2002), and through competitive forces acting on sympatric species. Comparisons even within the same system may be problematic, given the genetic divergence apparent in the upper and lower Murray-Darling Basin. The number of cryptic species with discrete ranges plus the strong genetic sub-structuring in MTV together provide a contrast from traditional views on the ecology of Australian *Retropinna*, in that dispersal appears to be limited by certain ecological traits and/or environmental conditions. In particular, the presumption that *R. tasmanica s.l.* is diadromous (anadromous) is not supported by this study. Varying degrees of genetic heterogeneity are evident among Tasmanian coastal rivers, whereas near-panmixia would be anticipated for any species undergoing regular dispersal through marine environments in the absence of natal homing or strong ecological structuring (e.g. Ward *et al*. 1994). While it appears that *R. tasmanica* can routinely be sampled from estuarine areas (e.g. samples were collected from the Derwent River and Duck River estuaries as part of this study), their presence within whitebait runs could simply be incidental to normal habitat use, arising from being euryhaline rather than diadromous.

Conservation

Australian *Retropinna* have long been regarded as 'common' species, and thereby have attracted little conservation concern. Yet conventional taxonomy has obscured the diversity that exists within the genus, and this is significant considering the generally low species richness among freshwater fishes in southeastern Australia (Unmack 2001; Allen *et al*. 2002). There are implications also for state-based wildlife management: one species becomes four in Queensland, three in New South Wales, and two in South Australia and Victoria.

Identification of conservation units is hindered by the considerable taxonomic complexity encountered and the scope for even greater within species sub-structure. Intensive assessments of population sub-structure, incorporating nuclear and mtDNA markers, are warranted for all *Retropinna* species, especially those in coastal eastern Australia to help clarify taxonomy and define conservation units. Several observed sub-groups within species represent a first focus for the assessment of Evolutionarily Significant Units. The high degree of genetic sub-structuring suggests that individual river basins are the appropriate scale for management as the overall basis for protecting biodiversity and evolutionary potential (Moritz 1994). Little or no gene flow implies that once populations are extirpated, natural recolonisation is highly unlikely in the short to medium term.

Although *Retropinna* is abundant in some habitats, there is no certainty that populations are secure, or that some genetic components have not already been lost. For example, *Retropinna* is rare in the northern coastal part of its range (aligning with CEQ), and less common in small Queensland rivers than elsewhere (Pusey *et al*. 2004). Coastal southeastern Queensland is exposed to pressures from a fast-growing human population (see Arthington *et al*. 1983; Hughes *et al*. 1999), and genetically distinct populations in inland lakes of Victoria could be vulnerable to environmental change as in New Zealand (Ward *et al*. 2005). Issues of translocation and mixing of different populations are significant for sub-structured species like *Retropinna*, as they may eliminate local variants and change evolutionary trajectories (e.g. Esa *et al*. 2000; Austin and Ryan 2002; Hughes 2003; Utter 2004). Translocations of Australian *Retropinna* have occurred, for example, as attempts to establish forage species for introduced salmonids (Lake 1971; Frankenberg 1974; McDowall 1979).

3.5. TABLES

Table 3-1. *Retropinna* locality and sample size information for the overview (ov) and population (pop) studies. Site numbers match those in Figure 3-1. DD = Drainage Division; RB = River Basin (AWRC 1976).

Site	Field code	Locality	State	DD	RB	Latitude (S)	Longitude (E)	*n* (ov)	*n* (pop)
1	PU02-50	Baffle Ck	Qld	I	34	24º21'	151º36'	4	-
2	PU99-52	Barambah Ck	Qld	I	36	26º14'	151º53'	4	-
3	PU02-36	Lenthal Dam	Qld	I	37	25º26'	152º32'	3	-
4	PU99-54	Yabba Ck	Qld	I	38	26º27'	152º39'	6	-
5	PU97-44	Reynolds Ck	Qld	I	43	27º57'	152º35'	4	-
6	PU02-29	Christmas Ck	Qld	I	45	28º15'	153º00'	2	-
7	PU02-26	Canungra Ck	Qld	I	45	28º06'	153º07'	2	-
8	PU02-25	Coomera R.	Qld	I	46	28º05'	153º08'	2	-
9	PU97-142	Little Nerang R.	Qld	I	46	28º07'	153º18'	2	-
10	PU02-20	Tallebudgera Ck	Qld	I	46	28º10'	153º21'	2	-
11	PU02-21	Currumbin Ck	Qld	I	46	28º12'	153º23'	3	-
12	PU02-22	Oxley R.	NSW	II	1	28º21'	153º18	4	-
13	PU02-19	Richmond R.	NSW	II	3	28º52'	153º02'	6	-
14	PU99-44	Timbarra R.	NSW	II	4	28º54'	152º31'	2	-
15	PU99-41	Macleay R.	NSW	II	6	30º49'	152º30'	2	-
16	PU99-38	Mortons Ck	NSW	II	7	31º25'	152º41'	2	-
17	PU02-06	Hunter R.	NSW	II	10	32º15'	150º53'	2	-
18	F-FISHADD1-1	Nepean R.	NSW	II	12	33º50'	150º32'	2	-
19	PU99-84	Maramingo Ck	Vic.	II	21	37º26'	149º38'	2	-
20	PU99-86	Snowy R.	Vic.	II	22	37º43'	148º27'	2	-
21	PU99-78S	Morwell R.	Vic.	II	26	38º18'	146º19'	2	-
22	PU99-74	Macks Ck	Vic.	II	27	38º30'	146º40'	2	-
23	PU02-99	Albert R.	Vic.	II	27	38º31'	146º28'	2	-
24	PU02-101	Tin Mine Ck	Vic.	II	27	38º37'	146º19'	2	-
25	PU02-76	Tarwin R.	Vic.	II	27	38º39'	145º56'	2	7
26	PU02-104	Lang Lang R.	Vic.	II	28	38º14'	145º39'	2	7
27	F-FISHADD4-1	Yarra R.	Vic.	II	29	37º45'	145º10'	2	7
28	PU02-86	Barwon R. East Branch	Vic.	II	33	38º28'	143º44'	2	7
29	PU02-89	Lake Colac	Vic.	II	34	38º19'	143º35'	2	7
30	TR01-199	Lake Tolliorook	Vic.	II	34	37º59'	143º16'	2	7
31	PU02-91	Curdies R.	Vic.	II	35	38º26'	142º57'	2	7
32	PU01-59	Lake Burrumbeet	Vic.	II	36	37º30'	143º35'	2	10
33	PU02-111	Merri R.	Vic.	II	36	38º16'	142º31'	2	7
34	PU02-116	Lake Bolac	Vic.	II	36	37º43'	142º50'	2	7
35	F-FISH18-1	Glenelg R. (mid)	Vic.	II	38	37º32'	141º23'	2	7
36	F-FISHY2-1	Glenelg R. (estuary)	SA	II	38	38º01'	140º57'	-	5
37	F-FISH98-1	Duck R.	Tas.	III	14	40º50'	145º09'	2	7
38	F-FISH98-2	Mersey R.	Tas.	III	16	41º15'	146º23'	2	7
39	F-FISH90-1	Great Forrester R.	Tas.	III	19	41º00'	147º25'	2	7
40	F-FISH98-3	Last R.	Tas.	III	2	41º09'	148º10'	2	5
41	F-FISH98-4	Derwent R.	Tas.	III	4	42º45'	147º10'	2	7
42	PU94-44	Ovens R.	Vic.	IV	4	36º38'	146º00'	2	7
43	F-FISH18-2	Goulburn R.	Vic.	IV	5	36º23'	145º24'	-	7
44	PU94-32	Murray R. (Black Swamp)	Vic.	IV	9	35º42'	144º09'	-	7
45	F-FISH18-3	Murrumbidgee R.	NSW	IV	10	34º45'	146º33'	2	7
46	PU03-02	Wimmera R.	Vic.	IV	15	37º02'	143º01'	2	7
47	PU02-54	Turon R.	NSW	IV	21	33º04'	149º24'	2	7
48	PU99-60	Maranoa R.	Qld	IV	22	26º29'	147º58'	2	7
49	PU99-63	Warrego R.	Qld	IV	23	28º07'	145º41'	2	7
50	F-FISH99-1	R. Murray (Berri)	SA	IV	26	34º17'	140º36'	-	7

Site	Field code	Locality	State	DD	RB	Latitude (S)	Longitude (E)	*n* (ov)	*n* (pop)
51	F-FISH5-1	Bryants Ck	SA	IV	26	34º20'	139º40'	2	12
52	F-FISH99-2	R. Murray (Swanport)	SA	IV	26	35º09'	139º18'	-	10
53	F-FISHY2-2	Lake Albert	SA	IV	26	35º40'	139º20'	-	9
54	PU94-22	Bremer R. (1994)	SA	IV	26	35º23'	139º03'	-	7
55	F-FISHY2-3	Bremer R. (2004)	SA	IV	26	35º23'	139º03'	-	9
56	F-FISH84-1	Finniss R. (L. Alexandrina)	SA	IV	26	35º24'	138º50'	2	5
57	F-FISHY2-4	Finniss R. (main channel)	SA	IV	26	35º23'	138º49'	-	7
58	F-FISHADD2-1	Currency Ck	SA	IV	26	38º30'	146º40'	2	-
59	F-FISH98-5	Mundoo Channel	SA	IV	26	35º31'	138º54'	-	10
60	F-FISH40-1	Darr R.	Qld	X	3	23º12'	144º04'	2	-
61	F-FISH5-2	Cooper Ck	SA	X	3	27º45'	140º44'	2	-
62	F-FISH94-1	Coongie Lakes	SA	X	3	27º02'	140º17'	2	-

Table 3-2. Allele frequencies for the five taxa identified within *Retropinna* at those loci displaying fixed or near-fixed differences among taxa. For polymorphic loci, the frequencies of all but the rarer/rarest alleles are expressed as percentages and shown as superscripts (allowing the frequency of each rare allele to be calculated by subtraction from 100%). A dash indicates no genotype was assignable at this locus. Sample sizes in brackets.

Locus	CEQ (10)	SEQ (26)	SEC (30)	MTV (50)	COO (6)
Acon1	c	-	c^{95},a	c^{94},d^{4},b	d^{83},e^{9},b
Acon2	b	b	b^{97},a^{2},c	c	c
Acyc	f	e^{65},b^{27},a	e^{67},f^{21},b	f^{97},c^{1},d^{1},b	g
Ak1	b	b	a	b	c
Ca	c	c^{98},b	b	b^{57},c^{38},d^{4},a	c^{83},d
Enol1	d	c^{96},a^{2},d^{2}	c^{97},b	d^{90},e^{10}	d
Est1	c	c^{96},d	e^{62},b^{22},a^{12},d	e^{96},b	e^{83},c
Fum	a^{65},b^{35}	a	b^{98},c^{2}	b^{99},c^{1}	b
Glo	b	b^{75},d	b^{65},c^{32},a	c^{63},b	c
Got1	b^{95},c^{5}	c^{98},e	b	b^{99},a	d^{75},b
Got2	h^{30},g^{25},c^{20}, e^{15},d^{5},a	d	d^{70},g^{28},b	d^{80},f	d
Gpi1	b^{95},f	b^{85},a^{8},g^{6},f	e^{88},b^{7},c	e^{83},h^{11},d	i
Gpi2	e^{95},b	e^{94},f	b^{63},c^{32},a^{3},d	b^{95},e	b
Gsr	b	a^{92},b	d^{88},e	c^{81},d	c^{92},f
Ldh1	b	a^{98},c	b	b	b
Mdh	b	b^{98},a	b^{98},a	b^{61},c^{38},a	c
Me1	b	b^{79},c^{12},d^{7},f	e^{43},c^{38},g^{14}, b^{3},a	b^{79},a	a
Me2	d^{85},e^{10},b	d^{56},f^{27},g	b^{55},c^{23},a	c^{90},b	c^{92},d
Mpi	c	d^{98},f	c^{93},e	c^{93},b^{6},a	c
PepA1	d^{85},c	d	b	b^{95},c^{3},a	c^{83},b
PepB	b	b^{98},a	d^{83},f^{13},g	b^{49},d^{48},c	b^{67},e^{25},g
PepD	d^{85},b	d^{92},e^{6},a	d^{72},i^{26},h	d^{45},j^{36},g^{5},f^{4}, h^{3},c^{3},e^{2},i^{1},b	a
6Pgd	c^{75},d	c	c^{97},d	b^{90},a^{5},c	b
Pk2	a	a	b	b	b
Ugpp	c^{85},b	c^{92},b^{4},a	b^{95},a	c^{89},a^{6},b	b^{92},a

Table 3-3. Genetic distance estimates among taxa identified in the overview study. Lower left triangle = %FD; upper right triangle = unbiased Nei D.

Taxon	CEQ	SEQ	SEC	MTV	COO
CEQ	-	0.20	0.41	0.33	0.59
SEQ	17	-	0.47	0.52	0.68
SEC	28	36	-	0.24	0.46
MTV	23	36	14	-	0.25
COO	35	48	30	12	-

Table 3-4. Allele frequencies for the population study. The presentation of frequencies matches that of Table 3-2. As the overview and population studies were each run as stand-alone projects, they do not share the same allelic nomenclature; for example, *Acon1*^{b} in the overview is not the same allele as *Acon1*^{b} in the population study, etc. To avoid bias due to only including polymorphic loci, observed heterozygosity estimates (H_O) were calculated for all 43 loci run in the overview study by assuming that the 17 loci not run in the population study were invariant.

	----Upper Murray-Darling -------								---------- Lower Murray ----------									Glenelg ----------		western coastal Victoria ----------								------ Tasmania ------				
Locus	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**59**	**35**	**36**	**25**	**26**	**27**	**28**	**29**	**31**	**30**	**32**	**33**	**34**	**37**	**38**	**39**	**40**	**41**
Acon1	b	b	b	b	b	b^{86},a	b	b	b	b	b	b	b	b^{94},e	b	b	b	c	c^{60},b	b	b	b	b	b	b	b	b	b	b^{79},e	b	b^{75},a	b^{93}, d	b	b
Acon2	a	a	a	a	a	a	a	a	a	a	a	a^{89},b	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Acyc	d^{93}, b	d	d	d^{93},a	d	d	d^{93},c	d	d^{93},a	d^{96},a	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d^{95}, b	d	d	d	d	d	d	d
Ada	c^{43}, e^{43}, b	c^{93},e	c^{86},e	c^{71}, e^{22}, a	c	c^{75},e	c^{60},e	c^{75},e	c^{86},e	c^{94},e	c^{90}, b^{5}, e	c	c	c^{89},e	c	c	c	c	c	c	c	c	c	c^{57}, b^{36}, d	c^{93},b	b^{33}, c^{33}, d^{17},e	c^{70},e	c	c	c	c	c^{93},a	c	c
Ca	c^{93},d	c	c^{93},d	c^{93},b	c	c^{86},b	c	c^{71},d	c^{71},b	b^{63},c	b^{75},c	b^{72},c	c^{64},b	b^{61},c	b^{50},c	b^{64},c	c^{55},b	c^{93},b	c	b	b	b^{93},a	c^{93},b	b^{43}, c^{43},d	b	c^{79},b	c^{90},d	b^{86},c	c^{71},d	b	b	b	b	b
Ck	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b^{93},a	b	b	b	b^{93},a	b	b	b	a^{64},b	b	b^{93},a	b	b	a^{80},b	b^{86},a
Enol1	c	c	c	c	c	c^{93},d	c	c	c	c	c^{95},d	c	c	c	c	c^{93},d	c	c	c	c	c^{57},d	c^{57}, d^{36}, b	d	c^{93},d	c^{71},d	c^{93},d	c	c^{93},a	c	c	c	c	c	c
Fdp	b	b	b	b	b	b^{93},a	b^{86},a	a^{57},b	b	b	b	b	b	b	b	b^{93}, d	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b^{86},c	b	b	b
Glo	b^{93},a	b	b	b	b	b	b	b	a^{79},b	b^{62},a	a^{55},b	b^{56},a	a^{79},b	a^{67},b	b^{80},a	b^{64},a	a^{65},b	b	b	a^{50},b	a	a^{57},b	b^{71},a	a^{50},b	b^{71},a	b^{57},a	b	a	b	a^{71},b	a^{64},b	b^{57},a	b^{80},a	a^{86},b
Got1	c	c	c	c	c	c^{86},b	c	c	c	c^{96},a	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c	c
Got2	f^{86}, e^{7},c	f^{93},d	f	f	f	f^{93},a	f	f	f^{93}, b	f^{83}, g	f^{80}, g^{15},c	f^{83}, g^{11}, d	f^{93}, g	f^{83}, g^{11}, d	f	f	f^{90}, g	f	f	f^{64}, g	f	f^{93}, g	f	f	g^{86},f	f	f	f^{79}, g	f^{93},c	g^{57},f	g^{86},f	f^{57}, g^{36},c	f	g^{64},f
Gpi1	d	d	d^{79}, c^{14}, b	d^{86},c	d^{93},c	d^{86},c	d^{79},c	c^{57},d	d^{64}, e^{21}, f^{8},c	d^{75}, e^{21}, c	d^{85}, e^{10}, c	d^{83}, c^{6}, e^{6},f	d^{64}, e^{29}, c	d^{78}, e^{16}, c	d^{90},e	d^{79},e	d^{60}, c^{20}, e	d	d	d^{71},e	e	d	d	d^{93},c	d^{71},e	d^{93},c	d^{65}, c^{30}, a	e^{71},d	d	d	d	d	d	e^{72}, d^{21}, c
Gpi2	a	a	a	a	a	a^{93},b	a	a	a	a	a	a	a	a	a	a	a	a^{50},c	a^{90},c	a	a	a	a	a	a	a	a	a^{93},c	a^{93},d	a	a	a	a	a
Gsr	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b^{93},c	b	b	a^{57},b	b	c^{57},b	b^{93},a	b	c^{64},b	b	c^{64},b	c^{79},b	c	b^{90},c	b^{79},c

	----Upper Murray-Darling -------								---------- Lower Murray ----------									Glenelg	---------- western coastal Victoria ----------									------ Tasmania ------				
Locus	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**59**	**35**	**36**	**25**	**26**	**27**	**28**	**29**	**31**	**30**	**32**	**33**	**34**	**37**	**38**	**39**	**40**	**41**
Ldh2	b	b	b	b	b	b	b	b	b^{71},a	a^{54},b	b^{55},a	a^{56},b	b^{71},a	b^{67},a	a^{70},b	b^{57},a	b^{70},a	b	b	a^{93},b	a^{79},b	b^{86},a	b	b	a^{86},b	b	b	a	b	a	a	a	a	a
Mdh	c	c	c	c	c	c	c	c	b^{57},c	b^{79},c	b^{85},c	b^{50},c	b^{79},c	b^{72},c	b^{60},c	c^{71},b	b^{50},c	b^{79},c	c^{60},b	b	b	b	b	b^{86},c	b	b^{50},c	c	b	c	b	b	b	b	b
Me1	b^{57}, c^{36},a	c^{72}, b^{21},a	c^{71},b	c^{50}, b^{43},a	b^{50},c	c^{64},b	c^{57},b	c^{93},b	c^{93},b	c^{87},b	c^{85},b	c^{56},b	c^{93},b	c^{83},b	c^{80},b	c^{86},b	c^{80},b	c^{93},b	c^{60},b	c	c	c	c	c^{71},b	c	c^{79},b	b	b	b^{50},c	c	c	c	c	c
Me2	c	c	c	c^{93},d	c^{93},b	c	c	c	c	c	c	c	c	c	c	c^{93},b	c	b^{93},c	b^{90},c	c	c	c	c^{79},b	c	c	c	c	c	c^{93},a	c	c	c^{93},d	b	c^{79}, b^{14}, d
Mpi	c	c	c	c	c	c	c	c	c^{93},d	c	c	c	c	c	c^{90},a	c	c	c	c	c	c	c	c	c	c	c	c	c^{93},b	c	c	c^{86},b	c	b	c
PepA 1	b	b^{86},c	b	b	b^{57},c	a^{50},b	b	b	b^{93},c	b^{92},c	b	b^{94},c	b	b	b	b	b	b	b	b	b	b^{93},a	b	b	b	b	b	b	b	b	b	b	b	b
PepA 2	b	b^{93},a	b	b^{93},c	b	b	b	b	b	b	b	b	b	b	b	b^{93},c	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b^{93},a	b^{93},c	b	b
PepD	c^{93},b	c^{86},b	c	c	c	c^{93},b	c	c	c^{64}, g^{22}, f^{7}, e	c^{64}, g^{29}, e	c^{85}, g	c^{78}, e^{11}, g	c^{64}, g^{29}, e	c^{72}, g^{17}, e	c^{70},g	c^{71}, g^{14}, e^{8}, a	c^{70}, g^{15}, e^{10}, f	c^{71}, e^{22}, b	c^{80}, e^{10}, f	g^{72}, c^{14}, f^{7}, e	g	f^{86}, d	g^{57}, e^{36}, f	e^{36}, g^{36}, f^{14}, d^{7},c	g	c^{79}, e^{14}, d	c^{50},e	g^{64}, c^{21}, f^{8}, e	c	e^{57}, g^{29}, f	g^{65}, e^{21}, f	g^{79}, e^{14}, f	g	g^{86},f
6Pgd	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b^{90},a	b	b	c^{86},b	c^{90},b	b	b	b	b	b	b	b	b	b	a	b	b	b	b	b
Pgm	c	c	c	c	c	c	c	c	c	c^{96},d	c^{95},a	c^{94},b	c	c	c	c	c	c	c	c^{93},d	c	c	c	c	c^{93},d	c	c	c	c	c^{79},d	c^{64},d	c^{57},d	c	c^{86},d
Ugpp	b	b^{93},a	b^{93},a	b^{93},a	b	b^{93},a	b^{79},a	a^{71},b	b	b^{92},a	b^{95},a	b^{94},a	b^{71},a	b^{89},a	b	b	b^{90},a	b^{93},a	b	b	b	b	b	b	b^{79},a	b	b	b	b	a^{50},b	b^{93},c	b	b	b
Tpi2	c^{50},d	c^{93},d	c^{79},d	c^{79},d	c^{71},d	c	c	c	c	c	c^{90},d	c^{89},d	c	c	c	c^{86},d	c^{90},d	c^{93},a	c	c	b	c	c	c	c	c^{71},d	d^{67},c	c	c	c	c	c	c	c
Ho	0.065	0.043	0.030	0.063	0.037	0.085	0.046	0.040	0.093	0.102	0.063	0.106	0.083	0.148	0.123	0.153	0.095	0.056	0.042	0.060	0.023	0.043	0.056	0.086	0.070	0.089	0.067	0.076	0.043	0.070	0.085	0.043	0.023	0.070
S.E.	0.027	0.017	0.012	0.024	0.018	0.025	0.020	0.020	0.033	0.029	0.019	0.030	0.025	0.040	0.039	0.042	0.029	0.020	0.017	0.024	0.016	0.017	0.026	0.031	0.023	0.031	0.029	0.027	0.019	0.027	0.027	0.015	0.014	0.023

Table 3-5. Summary of quantitative analyses of populations structure in MTV. [1]Uppercase letters in the hierarchical level refer to clusters identified in Figure 3-6. [2]Mean and range (bracketed) for the number of statistically-significant differences in allele frequency detected among pairwise comparisons of populations (** $p < 0.01$; * $p < 0.05$). [3]F_{ST} confidence intervals calculated by bootstrapping (99% for $p < 0.01$ and 95% for $p < 0.05$). n/a = not applicable.

Hierarchical Level[1]	Sig. Diffs[2]	F_{IS}	F_{ST}	Confidence Intervals[3]
A (all 32 populations)	2.93 (0-9)	-0.006	0.472**	0.378 to 0.558
sites within B (all '*tasmanica*')	1.83 (0-6)	0.001	0.443**	0.292 to 0.626
site 40 *versus* sites within C	3.75 (2-6)	n/a	n/a	n/a
site 26 *versus* sites within D	2.57 (1-4)	n/a	n/a	n/a
site 33 *versus* sites within E	1.83 (1-3)	n/a	n/a	n/a
sites within E	0.47 (0-2)	0.024	0.198**	0.087 to 0.315
sites 37 - 39	0	0.073	0.108**	0.011 to 0.222
sites 41 *versus* 25	0	-0.054	0.116*	0.011 to 0.200
Glenelg *versus* all other sites	4.28 (2-9)	n/a	n/a	n/a
sites 35 *versus* 36 (within Glenelg)	0	0.133	0.128*	0.013 to 0.201
sites within F (all 'non-*tasmanica*')	1.68 (0-6)	-0.008	0.346**	0.243 to 0.465
Glenelg *versus* sites within G	3.21 (2-5)	n/a	n/a	n/a
sites within G	1.37 (0-6)	-0.015	0.306**	0.205 to 0.412
site 28 *versus* sites within H	3.00 (1-6)	n/a	n/a	n/a
sites within H	1.20 (0-5)	-0.011	0.281**	0.180 to 0.380
sites within I	0.53 (0-3)	-0.014	0.244**	0.138 to 0.394
site 34 *versus* other sites within I	1.22 (1-2)	n/a	n/a	n/a
sites within J	0.05 (0-1)	-0.002	0.075*	0.005 to 0.198
sites 42 - 46, 48	0	0.023	0.065**	0.001 to 0.148
sites within K	0.44 (0-2)	-0.012	0.100**	0.048 to 0.156
site 27 *versus* sites within L	1.22 (1-2)	n/a	n/a	n/a
sites within L	0.25 (0-1)	-0.018	0.066**	0.030 to 0.108
sites within M	0	-0.020	0.026*	0.002 to 0.050

4. CONSERVATION UNITS IN THE YARRA PYGMY PERCH

4.1. INTRODUCTION

The Yarra pygmy perch, *Nannoperca obscura* (Klunzinger), is a diminutive (< 100 mm total length) freshwater percichthyid endemic to a small section of southern coastal mainland Australia. It occurs in association with submerged and emergent macrophytes, at low elevations, in slow flowing and sheltered areas of streams, lakes and some larger rivers (Kuiter *et al*. 1996). Although biological information is limited, the species appears to be sedentary and has large, demersal larvae (Legget and Merrick 1987; Briggs 1999). There would be little scope for dispersal between river systems, especially those separated by marine barriers, suggesting the likelihood of strong-within species genetic structure (e.g. Hughes *et al*. 1999). *Nannoperca obscura* co-occurs across its range with the southern pygmy perch *N. australis*. Sympatry appears to be maintained by habitat segregation and behavioural characteristics (Sanger 1978; Woodward and Malone 2002), although occasional hybrids are reported (Kuiter *et al*. 1996). While the two pygmy perches appear superficially similar, *N. obscura* has a blunt compared to rounded head profile, a small mouth not reaching below the eye, olive to black compared to red fins and an irregularly-shaped eye pupil. Nevertheless, as demonstrated in Chapter 3 for *Retropinna*, the genetic integrity of previously described species should not be taken for granted in any molecular genetic assessment.

Decline since European settlement is evident, with *N. obscura* considered 'vulnerable' under IUCN criteria (IUCN 2006). Its remaining distribution is narrow and patchy (Unmack 1992; Saddlier 1993; Kuiter *et al*. 1996). Key threats include water abstraction, wetland drainage, loss of stream-edge habitat, and alien fishes, especially the predatory redfin perch *Perca fluviatilis* and the aggressive eastern gambusia *Gambusia holbrooki* (Wager and Jackson 1993). *Nannoperca obscura* is presumed extinct in its type locality, the Yarra River, and other eastern range edge sites (Yarra and Bunyip river basins: Saddlier 1993). Recent sampling and reviews of historic museum specimens have extended the known range *c*. 500km westward to include the whole Millicent Coast River Basin and a new, geographically-distinct population in Lake Alexandrina, part of the Murray-Darling Drainage Division (Hammer 2002; Hammer and Walker 2004). Several sites in this region are subject to critical habitat/hydrological threats, exacerbated by recent severe drought. At least one population has been extirpated (Henry Creek), and urgent *ex situ* conservation measures are underway for fish in Lake Alexandrina, where water-levels have reached unprecedented lows, eliminating macrophyte habitats (Hammer 2007c, 2008). It is likely, therefore, that genetic data will have a pivotal role to underpin current measures and future conservation.

In addition to the stated goals of comparative species assessments, this chapter (1) identifies diagnostic markers for *N. obscura* and *N. australis* and assesses the extent of hybridisation between these co-occurring pygmy perches, and (2) documents within-population genetic diversity, providing a suite of information to guide *ex situ* management of *N. obscura* (e.g. Williams and Osentoski 2007).

4.2. METHODS

Specimen collection

Samples of *N. obscura* were collected from across its present range, targeting representatives from different Australian river basins (AWRC 1976) and major rivers within basins (Figure 4-1). Its *threatened* status, nationally and locally, meant that collections were constrained by permits and sample sizes were necessarily small. In these circumstances, diagnosis of ESUs should focus on spatial replication (Moritz *et al*. 1995), and over splitting (Type I error) or failed recognition of distinct units (Type II error) were countered by screening large numbers of nuclear loci and long nucleotide sequences. Where possible, samples up to $n = 10$ per site were taken to limit impact on populations yet provide for meaningful statistical analysis (e.g. ensuring that major differences in allele frequency can be detected: Richardson *et al*. 1986). Field sampling utilised seine and dip nets and collections of *N. australis* were made concurrently.

Field samples were euthanased in an aqueous solution of clove oil, and muscle or whole fish samples were snap frozen in liquid nitrogen and returned to the Australian Biological Tissues Collection, Adelaide (ABTC) and stored at -70°C (most samples). Two collections could be stored only in 100% ethanol and so were limited to DNA analysis, although adjacent sites were available for allozyme analysis in both instances (Figure 4-1 and Table 4-1). Voucher specimens from most populations were lodged with either the South Australian or Victorian museums.

Allozyme electrophoresis

An overview study was conducted on individuals from four *N. obscura* populations across its geographic range. This included *N. australis* from five sites where the two species cohabited ($n = 2$ per population). The aims were to (1) assess the genetic distinctiveness between the species, (2) reveal genetic markers suitable for the molecular identification of each species and their F_1 hybrids, and any instances of localized introgression, and (3) identify genetic markers for an intensive study of population structure in *N. obscura* (herein called the 'population study'). The details of the 27 individuals used in the overview are presented in Table 4-1.

Muscle homogenates were screened for allozyme variation on cellulose acetate gels (Cellogel™), following Richardson *et al*. (1986). Thirty six enzymes or non-enzymatic proteins displayed sufficient activity and resolution after staining to permit allozymic interpretation: ACON, ADA, ADH, AK, ALD, AP, CA, CK, ENOL, EST, FDP, FUM, G6PD, GAPD, GDA, GDH, GLO, GOT, GPI, GSR, IDH, LDH, MDH, ME, MPI, NDPK, PEPA, PEPB, PEPD, PGAM, 6PGD,

PGK, PGM, PK, SORDH, and TPI. Details of enzyme and locus abbreviations, enzyme commission numbers, electrophoretic conditions and stain recipes are given in Richardson *et al*. (1986) and Bostock *et al*. (2006). Allozymes were designated alphabetically and multiple loci, where present, were designated numerically, in order of increasing electrophoretic mobility (e.g. *Ada*^{a}, *Ada*^{b}, *Ca1*, *Ca2*).

Having identified a suite of genetic markers to explore population structure and detect hybridization or introgression involving *N. australis*, all remaining 139 *N. obscura* were genotyped at these markers in the population study. Details of localities and sample sizes are presented in Table 4-1, and the geographic arrangement of localities is displayed in Figure 4-1. Data from the overview and population studies were independently subjected to principal co-ordinates analysis (PCO) to reveal genetic affinities of individuals from first principles (see Horner and Adams 2007). Thereafter, additional methods were used to analyse the allozyme data from the population study, each providing a differing perspective.

The raw genotypic data for individual sample sets were examined for statistical evidence of departures from Hardy Weinberg expectations or of linkage disequilibrium. Where no evidence was found to refute the null hypothesis that panmictic sample sets comprised unlinked loci, pairwise comparisons for statistically-significant differences in allele frequency were undertaken. All statistical tests of Hardy Weinberg Equilibrium, linkage disequilibrium and heterogeneity in allele frequencies were undertaken using GENEPOP 3.4 (Raymond and Rousset 2003). Probabilities were adjusted for multiple tests using the sequential Bonferroni correction factor (Rice 1989). F-statistics were used to compare divergence within and between sites at various hierarchical levels of population structure. F_{IS} and F_{ST} values and associated 99% confidence intervals were calculated using the program FSTAT version 2.9 (Goudet 2000). Observed heterozygosity values (H_O) were calculated for sites to indicate within site allozyme diversity. To ensure that values were comparable across studies (chapters), H_O values were calculated using all 52 loci surveyed in the overview study, under the assumption that the monomorphic loci therein were invariant in all sample sets.

Having identified operational taxonomic units from first principles, genetic divergence was assessed using Nei's unbiased genetic distance (Nei D: Nei 1978). PHYLIP (Felsenstein 1993) was used to construct UPGMA (unweighted pair-group method of arithmetic averages) dendrograms and NJ (neighbor joining) networks. The program TREEVIEW (Page 1996) was then used to visualise the tree structure. Allele frequencies and genetic distance measures were generated using unpublished BASIC computer programs written by M. Adams.

Mitochondrial DNA

The mtDNA data presented herein reflect a collaboration with Peter Unmack, Brigham Young University, who sequenced tissues, analysed data and assisted in the preparation of the methods and results sections. DNA was obtained from *c*. 0.25cm^3 of caudal fin or muscle *via* standard phenol/chloroform extraction. The entire cytochrome *b* gene (cyt*b*) was amplified by standard Polymerase Chain Reaction (PCR) techniques using primers Glu31 - PPThr41. When this failed to produce sufficient PCR product, the gene was amplified in two halves using Glu31 - HDALT602 and ppL505 - PPThr41. Final concentrations for PCR components per 25 μL reaction were: 25 ng template DNA, 0.25 μM of each primer, 0.625 units of Taq DNA polymerase, 0.2 mM of each dNTP, 5 μL of reaction buffer and 2.5 mM $MgCl_2$. Amplification parameters were: 95°C for 2 min followed by 35 cycles of 95°C for 30 s, 48°C for 30 s, and 72°C for 90 or 60 s, and 72°C for 7 min. PCR products were examined on a 1% agarose gel using cybrstain. The PCR products were purified using PrepEase PCR Purification 96-well Plates. DNA fragments were cycle-sequenced with Big Dye 3.0 dye terminator-ready reaction kits using 1/16th reaction size (Applied Biosystems, Foster City, CA). Sequencing reactions were run at 52^{o}C and products purified by passing reactions through sephadex columns. Sequences were obtained with an Applied Biosystems 3730 XL automated sequencer.

DNA sequences were edited using Chromas Lite 2.0 (Technelysium, Tewantin, Queensland, Australia) and imported and aligned by eye in BioEdit 7.0.5.2 (Hall 1999). Sequences were checked *via* amino acid coding in Mega 4.0 (Tamura *et al*. 2007) to test for unexpected frame shift errors or unexpected stop codons. Phylogenetic analyses were performed using both parsimony and likelihood approaches using PAUP (Swofford 2003). Maximum parsimony (MP) was conducted *via* a heuristic search with 1,000 random additions and TBR branch-swapping. Maximum likelihood (ML) models were estimated *via* hLRT in Modeltest 3.7 (Posada and Crandall 1998). ML was performed under the TrN+G model of evolution: Lset Base=(0.2429 0.3199 0.1461) Nst=6 Rmat=(1.0000 25.4495 1.0000 1.0000 10.4299) Rates=gamma Shape=0.0922 Pinvar=0. Robustness of nodes was estimated with PAUP by bootstrap with 1,000 replicates for MP using a heuristic search with 10 random additions of taxa and TBR branch-swapping, and 1000 replicates for ML *via* a heuristic search with 10 random additions of taxa and TBR branch-swapping. All tree lengths reported for MP include both informative and uninformative characters. Within- and among-taxon variation was calculated using Maximum Composite Likelihood Method in MEGA.

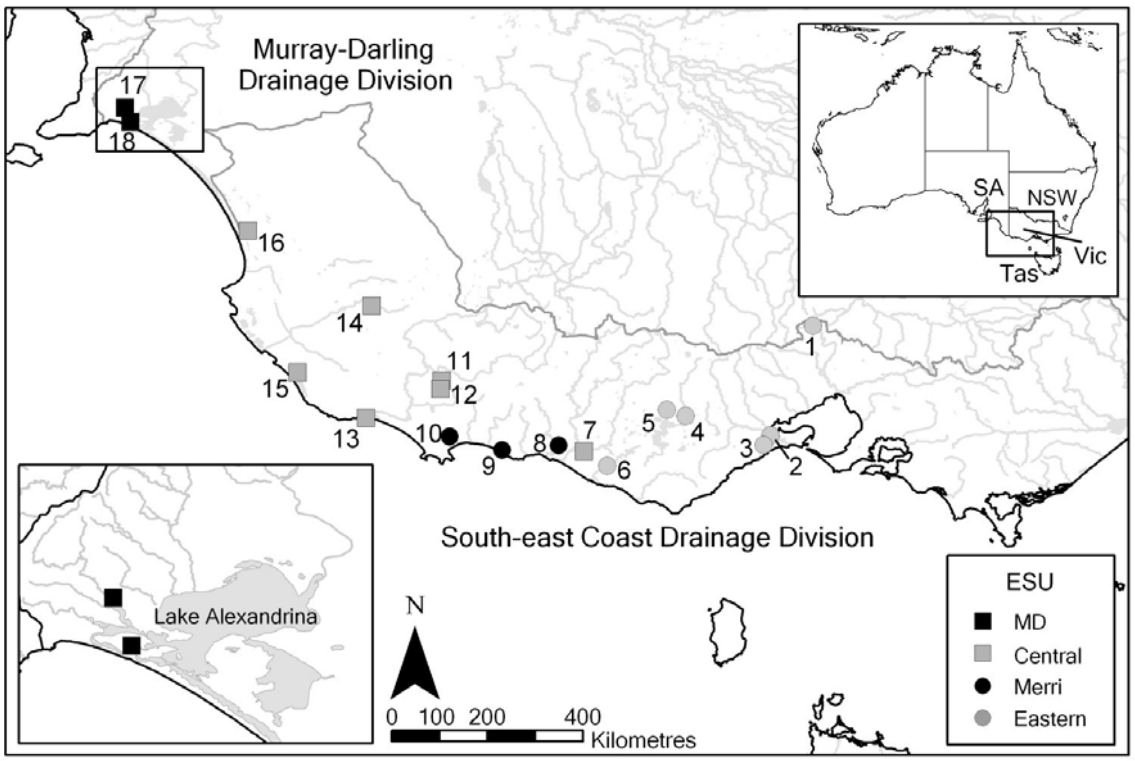

Figure 4-1. Geographic relationships and Evolutionarily Significant Units (ESUs) in *Nannoperca obscura* samples subjected to molecular analyses. Sites codes as per Table 4-1 and ESU codes as per the text.

4.3. RESULTS

Allozyme analyses

Seventeen *N. obscura* and ten *N. australis* individuals from representative sites were successfully screened for 52 putative allozyme loci during the overview study (see Appendix 2 for allozyme profiles). The two species displayed fixed differences at 24 loci (46%FD), demonstrating unequivocally that they maintain genetic and therefore taxonomic integrity in sympatry. This was supported by PCO (not shown), which revealed no indications of introgression between the two species. The existence of a large number of diagnostic allozyme markers ensured detection of sporadic hybridization or introgression in the population study.

The following 12 loci displayed variation in *N. obscura* in the overview study: *Ada*, *Adh*, *Ald2*, *Enol1*, *Est2*, *Gapd2*, *Gsr*, *Me2*, *PepA1*, *PepB*, *6Pgd*, and *Pgm1*. Of these, only the weakly-staining *Ald2* was not employed in the follow-up population study. An additional 12 loci were also screened, including some chosen for their ability to diagnose hybrids and those which routinely scoreable on the same zymograms as variable loci. Thus, 24 loci in all were screened in the population study, 11 of which were diagnostic for *N. obscura versus N. australis* (Table 4-2). The final dataset comprised the allozyme genotypes of 157 fish from 17 sites, at these 24 loci. A single *N. obscura* × *N. australis* F_1 hybrid was identified at site 13 (Crescent Pond), by virtue of being heterozygous at all 11 of the selected diagnostic loci; this individual was excluded from all further analyses. Incidentally, it was labelled as a suspected hybrid upon capture by the author, reflecting its intermediate physical features.

Neither an initial PCO of all 156 *N. obscura* (Figure 4-2) nor any follow-up PCO undertaken to explore within-cluster diversity (not shown) revealed any evidence that individual sites reflect an admixture of sub-groups. The most striking feature of the PCO was a strong phylogeographic signal. Figure 4-2 displays an obvious east *versus* west dichotomy in the first dimension (accounting for 46% of all variation), with the one anomaly being a single eastern site (site 7, Mt Emu Creek) which unequivocally is within the western cluster. The second PCO dimension further splits both major genetic groups into geographically-based sub-groups, namely distinct Murray-Darling Basin (MDB) *versus* Central/Mt Emu sub-groups for the western cluster, and marginally-overlapping Merri and Eastern sub-groups within the eastern cluster (Figure 4-1 and Figure 4-2). Thus, PCO identified four primary genetic lineages or sub-groups within *N. obscura*, hereafter referred to as 'MDB', 'Central/Mt Emu', 'Merri' and 'Eastern'. These lineages are also evident in the UPGMA dendrogram (Figure 4-3) and NJ network (not shown). Table 4-2 presents allele frequencies at all variable loci for the 17 sites examined. Statistical tests provided no evidence that individual sample sets violated Hardy Weinberg expectations or harboured loci in

linkage disequilibrium, thus disequilibrium, sanctioning further comparisons of allele frequency among samples sets. Given its small sample size (n = 2), site 4 was pooled with its near geographic neighbour (site 5, n = 10) to provide increased power in all statistical analyses.

Pairwise comparisons revealed the presence of numerous statistically-significant differences. Indeed, 95 of 105 comparisons identified at least one statistically-significant difference after Bonferroni correction, and 81% of all differences were highly significant ($p < 0.001$; Table 4-3). Importantly, all 10 cases where pairwise comparisons failed to confirm heterogeneity involved sites within the same genetic lineage, demonstrating the genetic distinctiveness of all four primary lineages. Moreover, within-lineage comparisons revealed that population sub-structuring was evident within all lineages except MD, either partially (Central and Eastern) or globally (Merri). A summary of the extent of genetic heterogeneity (mean numbers of pairwise significant differences and range of values) displayed at different levels in the population hierarchy is contained in Table 4-3, based on the hierarchy displayed in the UPGMA dendrogram (Figure 4-3).

Another perspective on between-site divergence was obtained by calculating F-statistics for the various hierarchical levels of population structure in *N. obscura* (Table 4-4). Large and significantly-positive F_{ST} values were obtained for all basal levels of the population hierarchy (nodes A, B and E; Figure 4-3), indicating strong support for the presence of four primary genetic lineages. Further population sub-structuring was also indicated within the Merri and Eastern lineages, but not the Central nor MD lineages. Table 4-5 also reveals that a single F_{IS} value was significant ($p < 0.01$) for an overall excess of heterozygotes, but such an outcome would be expected by chance as 14 statistical tests were undertaken.

A surprising feature of the UPGMA analysis was the close genetic similarity of the Mt Emu and Crescent Pond sample sets. Table 4-2 shows that both populations were monomorphic at every locus for the most common allele present in the MD plus Central/Mt Emu lineages, ensuring they displayed a pairwise Nei D of zero. Indeed, comparatively low levels of heterozygosity were evident at all sites, with H_O values ranging from 0.00 (the two previously-mentioned sites plus the MD site 17) to a maximum of 0.040 (overall population mean 0.017 ± 0.012), with no clear between-lineage trends apparent.

MtDNA analyses

A total of 24 cyt*b* haplotypes (*a*-*x*) were detected among the 128 individuals examined. Of 1,140 base pairs sequenced, 1,070 were constant, 34 variable characters were parsimony uninformative, and 36 characters were parsimony informative. Table 4-5 summarizes the distribution of all

haplotypes among the 17 sites surveyed in the allozyme population study plus an additional site (site 12, alcohol-preserved tissues only), referable to the Central lineage by geographic location (Figure 4-1).

A heuristic search with all characters weighted equally recovered three most parsimonious trees of 79 steps (CI 0.911, RI 0.967). ML recovered one tree with a -ln score of -2105.58502 (Figure 4-4). Both MP and ML analyses provided similar levels of bootstrap support, and displayed a well-supported primary dichotomy within *N. obscura*. This corresponded to that revealed by the allozyme data (Figure 4-2): clade I was found only at Merri or Eastern sites, whereas clade II was restricted to MD or Central/Mt Emu sites (Table 4-6). Together the two major clades were further resolvable into five geographically-restricted sub-clades, all supported by bootstrap values > 62%. These sub-clades have been labelled IA (Eastern sites), IB (Merri sites), IIA (Mt Emu site), IIB (Central sites), and IIC (MD sites). Thus, the mtDNA data demonstrated the same primary genetic structure within *N. obscura* as revealed by the nuclear genetic data, and in addition identified an obvious phylogeographic separation between Mt Emu and all Central sites. Importantly, the sample sizes involved for all five sub-clades (Table 4-6) are sufficient to demonstrate that each pairwise comparison is statistically heterogeneous ($p < 0.001$ in all cases).

Although strong phylogeographic structure was evident in the mtDNA phylogram, overall levels of haplotype diversity were low in *N. obscura*. Thus, mean maximum composite likelihood divergences between haplotypes within sub-clades ranged from 0.2-0.3%, with between sub-clades values being only two to five-fold higher (0.5- 0.7% between sub-clades of the same major clade and 1.3-1.7% between sub-clades from different major clades; Table 4-7). In contrast, the five *N. australis* haplotypes, representing only a small proportion of its geographic range, differed on average by 1.2%.

Additional sub-structuring was evident within several sub-clades, but did not correspond to any simple dichotomous geographic pattern (Table 4-5). Nevertheless, of the four geographic regions represented by multiple sites (all except Mt Emu), only the MD region did not display statistically-significant pairwise differences in haplotype frequency (Table 4-6). This pattern is in agreement with that displayed by the allozyme analyses (Table 4-3).

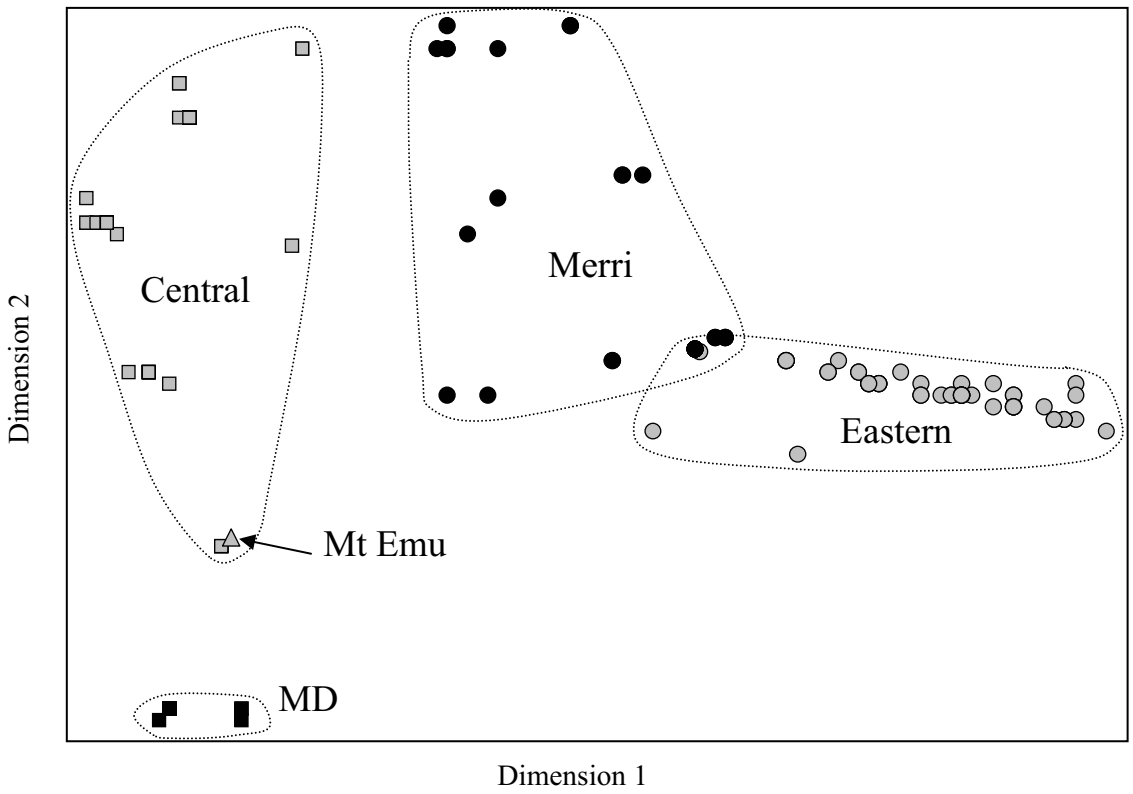

Figure 4-2. Principal Coordinates Analysis of the 156 specimens in the allozyme population study. The relative PCO scores have been plotted for the first and second dimensions, which explain 46% and 13% of the total variance, respectively. Envelopes highlight major genetic groups.

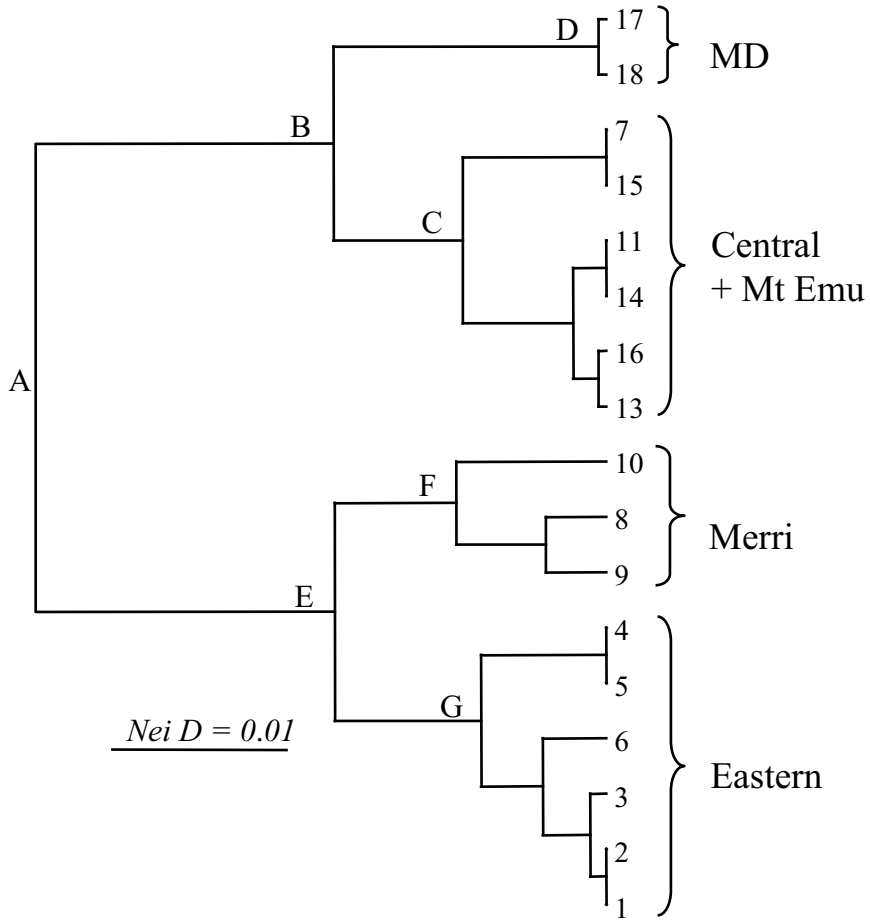

Figure 4-3. UPGMA dendrogram depicting genetic affinities among 17 sites sampled for allozyme analysis, based on pairwise Nei Distance values. Letters represent hierarchical levels used in statistical evaluation (see Table 4-4).

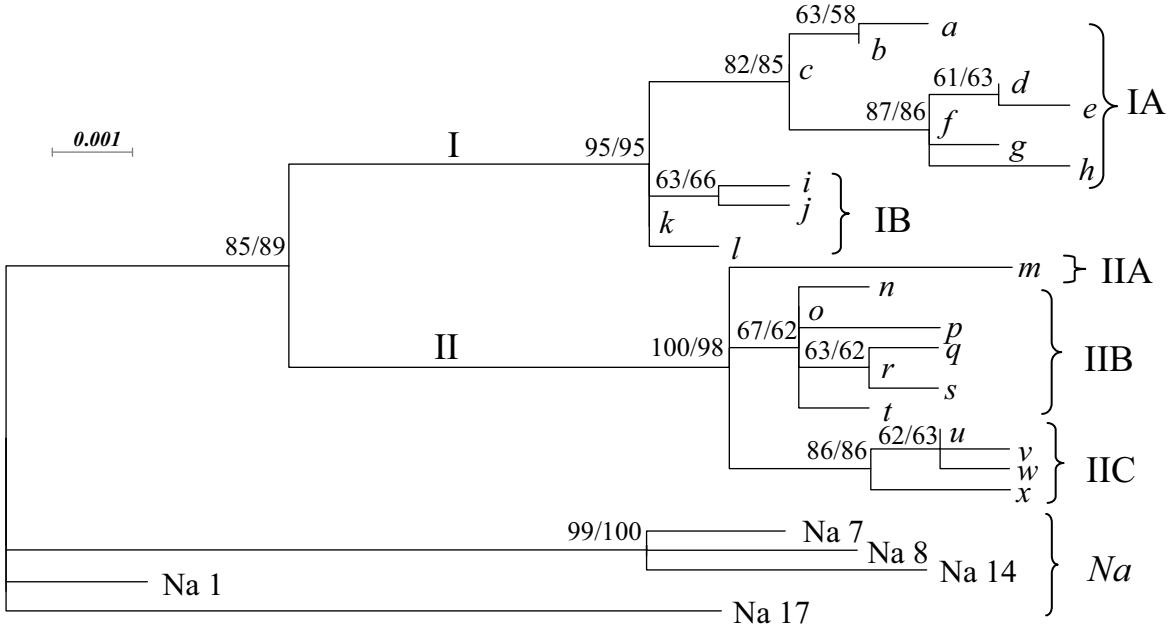

Figure 4-4. Maximum likelihood phylogram for the 24 Cyt*b* haplotypes detected among 128 *Nannoperca obscura*. The phylogram was rooted using the five *N. australis* sequences (*Na*). Haplotypes are labelled by letter (*a-x*) for *N. obscura* and according to site code for *N. australis*. Major clades (I and II) and sub-clades (IA, IB, IIA, IIB, and IIC), are labelled as per the text.

4.4. DISCUSSION

This study represents a comprehensive assessment, spatially and in relation to the breadth of its molecular coverage, of genetic sub-divisions within the present range of *N. obscura*. This 'threatened' species displays a major phylogeographic dichotomy and other obvious sub-structuring which is largely congruent across both nuclear and matrilineal data. The data provide a new perspective on recent and historic gene flow in *N. obscura* and an insight into how this might best be catered for in conservation and management.

Taxonomic considerations

Nannoperca obscura and *N. australis* are highly-divergent species occurring sympatrically without evidence of recent or historic genetic introgression. A very low incidence of inter-specific hybridisation was detected, with individuals easily diagnosed by molecular markers and a trained eye. A suite of nuclear markers has now been identified for the molecular diagnosis of hybrids, to ensure the integrity of broodstock in any captive maintenance or breeding program.

Subtle morphological differences at the level normally attributable to different sub-species have previously been noted between populations representing the eastern and western genetic lineages of *N. obscura* (Kuiter and Allen 1986; Kuiter *et al*. 1996). While the genetic data are broadly compatible with such a scenario, the placement of the Mt Emu Creek population within the western lineage creates a geographic picture which is inconsistent with a strict definition of sub-species (i.e. that they have disjunct distributions). If a detailed morphological review of populations across the species' range reveals concordant morphological differences, the lineages may qualify as distinct phylogenetic species. Although the shallow levels of genetic divergence observed for both allozyme and mtDNA markers suggest this is unlikely, similar or lower levels of divergence occur between sibling species in other freshwater fishes (e.g. cichlids, Barluenga *et al*. 2006).

Genetic sub-structure

Nannoperca obscura displays marked phylogeographic sub-structure, largely concordant with a number of geographically definable boundaries. The primary dichotomy between eastern *versus* western range sites aligns to the eastern boundary of the Glenelg River catchment. Two distinct lineages are also evident with each major group, namely the Murray-Darling Basin *versus* the combined Glenelg River and Millicent Coast river basins in the west, and the rivers including and immediately surrounding the Merri River catchment *versus* the remaining eastern range

populations (Deep Creek to Curdies River) in the east. A fifth divergent DNA lineage occurs in Mt Emu Creek, a site which is curiously tied to the western genetic grouping despite its location, nested among eastern populations.

The lack of nuclear divergence between the Mt Emu population and some others along the Millicent coast presumably reflects population genetic phenomena (e.g. founder effects) associated with initial and continuing small population size, as revealed by extremely low estimates of allele heterozygosity and haplotype variability (H_O = 0 and no within-site cyt*b* haplotype diversity). Indeed, overall estimates of heterozygosity values in *N. obscura* were relatively low, as was haplotype divergence. The distribution of *N. obscura* is generally reported as patchy with low abundance rather than as extensive and large populations (Unmack 1992; Saddlier 1993; Kuiter *et al*. 1996; Hammer 2002). Thus, low gene diversity may be the result of intermittently or permanently small effective population sizes (Nevo *et al*. 1984).

While within-population diversity was low, populations of *N. obscura* were characterised by moderate levels of genetic differentiation between sites, with most populations showing statistical differences in allele frequency and haplotype frequency, and high F_{ST} values. The general ecological assumptions of low dispersal ability in *N. obscura* are thus supported by the molecular data. Populations are clearly isolated in drainages separated by marine barriers or sub-divided by features such as waterfalls (Mt Emu Creek) and natural isolation (Crescent Pond). Dispersal and gene flow within systems appear to be minimal, although greater spatial replication and dedicated studies are required. Some insight is provided for three sites (14-16) with similar allele frequency and mtDNA profiles in the Millicent Coast River Basin, suggesting contemporary gene-flow across a naturally continuous landscape of intermittent, longitudinal wetlands. The recent and ongoing influence of drainage infrastructure which now heavily dissects the Millicent Coast River Basin (South Eastern Drainage Board 1980) has fragmented dispersal routes and populations and may ultimately cause reductions in genetic diversity, or extinction, due to local deterministic or stochastic influences (e.g. Henry Creek).

The Murray-Darling Basin represents a recently discovered and genetically distinct lineage in the western range. This finding supports other observations that peripheral populations are often quite distinct genetically (Lesica and Allendorf 1995; Eckert *et al*. 2008), and that it is important to be inclusive with sample coverage across a species' range in assessments of conservation units. Further, detailed sampling throughout a species' range is essential to ensure that interesting and perhaps pivotal anomalies such as Mount Emu Creek are detected (Moritz *et al*. 1995).

Biogeographic patterns

The major phylogenetic break in *N. obscura*, the eastern edge of the Glenelg River Basin, coincides with the extent of the Newer Volcanics in western Victoria (Johnson 1989; Joyce *et al*. 2003). This broad expanse of basalt (*c*. 15,000 km^2) was erupted during the period 4.5-0.5 Mya (Johnson 1989) and would correspond to the order of magnitude of within species genetic divergence witnessed. In some way the exclusion of the Glenelg system from volcanic activity appears to have provided an historic barrier to gene flow or refuge for the western genetic grouping. Interestingly, the eastern limit of *N. obscura* and the Newer Volcanics also align at Melbourne, and both are absent from northern Tasmania (despite relatively recent drainage connectivity with respect to fish movement), suggesting an association.

The alignment of Mt Emu Creek, a tributary of the Hopkins River, to the western genetic grouping indicates a physical connection to the west and isolation from local 'eastern group' gene flow. The Hopkins and Glenelg catchment abut in their upper reaches and dispersal may have occurred across low divides or swampy connections. Connections may have also existed due to the considerable volcanism and sedimentation that has disrupted many drainages and reduced topography in the area (Joyce *et al*. 2003). The presence of a divergent mtDNA lineage in Mt Emu Creek suggests that the timing of colonisation was historic rather than recent. The substantial Hopkins Falls isolate *N. obscura* in Mt Emu Creek (and potentially other parts of the upper Hopkins River, although there have been no recent records). Fine-scale genetic sampling around this barrier is required to confirm the restricted distribution of the Mt Emu lineage.

The genetic distinctiveness of the Murray-Darling population indicates a long-term barrier to fish movement in this region. This barrier corresponds to a major drainage divide created by topography (Great Dividing Range) and a sea-water barrier (Southern Ocean), and there appears to have been limited drainage connectivity even at low sea levels due to opposing flow directions as viewed from bathymetric contours (Unmack 2001). Any potential connectivity between the Lower Murray *via* the Coorong, an extensive bar-built marine to hypersaline lagoon abutting freshwater environments of the Millicent Coast River Basin, appears not to have been utilised by *N. obscura*. The genetic distinctiveness of Lower Murray *N. obscura* and other species (Keenan *et al*. 1995, Chapters 3, 5, 6 and 8) suggests that the occupied habitat in Lake Alexandrina have been predominantly fresh for a long time (i.e. thousands of years), an inference supported both by investigation of early post-European conditions (Sim and Muller 2004) and palaeolimnological studies (Fluin *et al*. 2007).

Ecology

The presumed low dispersal ability in *N. obscura* is supported by the observed strong genetic structure. The reproductive isolating mechanisms of sympatric *Nannoperca* are clearly well-evolved, but may come under threat with habitat alterations that disrupt ecological boundaries such as behaviour and habitat partitioning. Indeed, recent monitoring at Crescent Pond has revealed a high incidence of hybrid *N. obscura* × *N. australis*, perhaps due to prolonged drought and/or climate change eroding reproductive isolating barriers within a small habitat (Hammer 2007d). Degraded or altered habitats have been known to foster hybridization and introgression between species in other freshwater genera (e.g. Seehausen *et al*. 1997; Fisher *et al*. 2006).

Conservation

Numerous conservation units are identifiable on genetic criteria in *N. obscura*. Four diagnosable lineages were identifiable as ESUs (Moritz 1994), namely (1) the Murray-Darling Basin, (2) the Glenelg River Basin, Millicent Coast and Mt Emu Creek, (3) rivers including and immediately surrounding the Merri Catchment, and (4) eastern range populations. Mt Emu Creek represents a fifth mtDNA lineage, but it did not show significant differences at nuclear markers and does not strictly qualify as an ESU as defined herein. Nevertheless, its phylogeographic distinctiveness and outlying geographic distribution identify this population as a conservation unit of note. Mt Emu Creek qualifies as an MU (Moritz *et al*. 1995), and a logical extension of this perspective would see all independent drainage areas or catchments (i.e. those with marine barriers) regarded as distinct MUs, given that most populations screened showed significant difference at nuclear loci and/or unique haplotypes.

Several of the newly recognised conservation units are currently under significant threat of extinction. Moreover, given that one population has already become extinct (Henry Creek) and that most knowledge on species distribution has come *via* recent investigations (Saddlier 1993; Hammer 2002; Hammer and Walker 2004), it is conceivable that other components have already been lost. The long-cited western distribution limit at the Bool Lagoon/Mosquito Creek system (Kuiter and Allen 1986; Kuiter *et al*. 1996; Allen *et al*. 2002) overlooked a major part of the species' range, an area found by this study to contain significant within-species genetic variation and evolutionary potential. This has particular relevance to the Murray-Darling Basin ESU, which is on the verge of extinction due to the recent dramatic water-level decline in the unique ecosystem in Lake Alexandrina from combined climatic and human influences (cf. Maheshwari *et al*. 1995).

4.5. TABLES

Table 4-1. Locality and sample size information for the allozyme overview (ov) and population (pop) studies and mtDNA analyses. Site numbers match those in Figure 4-1. DD = Drainage Division, RB = River Basin (AWRC 1976).

Site	Field code	Locality	State	DD	RB	Latitude (S)	Long. (E)	*n* (ov)	*n* (pop)	*Cytb*
1*	PU00-03, PU02-106	Deep Ck, Lancefield	Vic.	II	30	37º16'	144º43'	-	10	8
2	PU00-29, PU02-84	Waurn Ponds Ck, Geelong	Vic.	II	33	38º11'	144º21'	-	10	9
3	PU02-107	Thompson Ck	Vic.	II	35	38º16'	144º17'	-	10	5
4	PU00-28#, PU03-06	Woady Yaloak R., Cressy	Vic.	II	34	38º01'	143º38'	-	2	8
5	PU00-27	Gnarkeet Ck, Lismore	Vic.	II	34	37º58'	143º28'	3	10	5
6	PU00-24, PU02-91	Curdies R, Curdie	Vic.	II	35	38º27'	142º57'	-	10	9
7*	PU00-23, PU02-112	Mount Emu Ck, Panmure	Vic.	II	36	38º20'	142º46'	-	10	7
8*	PU00-22, PU02-111	Merri R., Grassmere	Vic.	II	36	38º16'	142º32'	-	10	5
9	PU00-21, PU02-113	Shaw R., Yambuk	Vic.	II	37	38º02'	142º04'	-	10	5
10	PU00-20	Surrey R., Heathmere	Vic.	II	37	38º00'	141º37'	3	10	5
11	PU00-18, PU02-119	Palmer Ck, Merino	Vic.	II	38	37º43'	141º33'	-	10	9
12	PU00-19#	Stokes R., Digby	Vic.	II	38	37º48'	141º32'	-	-	9
13	F-FISHY2	Crescent Pond, Picks Swamp	SA	II	38	38º00'	140º54'	-	10	9
14*	PU00-16, F-FISH83	Mosquito Ck	Vic.	II	39	37º05'	140º57'	6	13	4
15	F-FISHY2	Drain 88, Lake Bonney	SA	II	39	37º39'	140º19'	-	10	10
16	F-FISH90	Henry Ck	SA	II	39	36º00'	139º53'	-	7	8
17*	F-FISH84 & 90	Finniss R., L. Alexandrina	SA	IV	26	35º02'	138º51'	5	7	5
18	F-FISH98 & FISHY2	Hindmarsh Is., L. Alexandrina	SA	IV	26	35º32'	138º53'	-	7	8

* Sites supplying two *N. australis* for the allozyme overview study.

Tissues preserved in ethanol

Table 4-2. Allele frequencies at 17 variable loci for the 17 sites surveyed in *N. obscura*. For polymorphic loci, the frequencies of all but the rarest alleles are expressed as percentages and shown as superscripts (allowing the frequency of each rare allele to be calculated by subtraction from 100%). Loci found to be diagnostic for *N. obscura versus N. australis* in the overview study are asterisked (*). Invariant loci: *Enol2*, *Est1**, *Gapd1*, *Got1**, *Got2**, *Gpi2**, and *PepD1**. Observed heterozygosity estimates (H_O) were calculated for all 52 loci run in the overview study, as discussed in the text.

Locus	**1 (10)**	**2 (10)**	**3 (10)**	**4 (2)**	**5 (10)**	**6 (10)**	**7 (10)**	**8 (10)**	**9 (10)**	**10 (10)**	**11 (10)**	**13 (10)**	**14 (13)**	**15 (10)**	**16 (7)**	**17 (7)**	**18 (7)**
Ada	c	c	c	c	c^{95},a	c	c	c	c	c	c^{80},d	c	c^{92},d	c	c	c	c
Adh	b	b	b	b	b	b	b	b	b	b	b	b	b^{96},a	b	b	b	b
Enol1	b	b	b	b	b	b	b	b	b	b	b	b	b^{96},a	b^{95},a	b	b	b^{86},a
*Est2**	c^{75},d	c^{55},d	c^{55},d	c^{50},d	c^{55},d	d^{60},c	d	d	d	d^{95},c	d	d	d	d	d	d	d
Gapd2	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b
*Gpi1**	e	e	e	e	e	e	e	e	e^{95},b	e	e	e	e	e	e	e	e
Gsr	b	b	b	b	b	b	b	b^{95},a	b^{95},a	a^{85},b	a	b	a^{85},b	a^{70},b	a^{93},b	b	b
Mdh1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a^{93},b
Me1	b	b	b	b	b	b	b	b	b	b^{95},a	b	b	b	b	b	b	b
Me2	c	c	c	c	c	c	a	c	c^{90},a	c^{85},a	a^{55},c	a	a^{65},c	a	a	a	a
*PepA1**	b	b^{95},c	b^{65},c	b^{50},c	c^{75},b	b^{70},c	b	b	b	b	b	b	b^{96},c	b	b	b	b
*PepA2**	b	b	b	b	b	b	b	b	b	b	b	b	b^{96},a	b^{95},a	b^{79},a	b	b
*PepB**	d^{60},c	d^{60},c	c^{45},d^{40},b	c	c	c	d	d	d	d^{95},b	d	d	d	d	d	d	d
PepD2	b	b	b	b	b^{80},c	b	b	b	b	b^{80},c	b	b	b	b	b	b	b
6Pgd	d	d	d	d	d	d	b	d	d^{80},e^{15},b	d^{75},b	b	b	b	b	b	b	b^{79},a
Pgm1	a	a^{85},b	a	b^{75},a	a^{50},b	a	a	a	a	a	a	a	a	a^{90},b	a^{93},b	a	a
*Pgm2**	b	b	b	b	b	b	b	a^{60},b	b	b	b	b	b	b	b	b	b
H_O	0.008	0.021	0.028	0.028	0.040	0.023	0.000	0.009	0.015	0.034	0.017	0.000	0.025	0.019	0.013	0.000	0.016
S.E.	0.006	0.011	0.017	0.021	0.021	0.016	0.000	0.008	0.009	0.014	0.012	0.000	0.014	0.012	0.009	0.000	0.010

Table 4-3. Summary of pairwise comparisons of allele frequency between samples sets. Lower triangle = total number of significant differences for $p < 0.05$; upper triangle = number of significant differences where $0.001 < p < 0.01$ (left-hand value), number of significant differences where $p < 0.001$ (right-hand value). A blank cell indicates no significant differences were found. All significance values were Bonferroni-adjusted to correct for multiple tests. Sites are boxed according to their genetic lineage, as displayed in Figure 4-1.

	----------Eastern ----------					**------ Merri -----**			**-------- Central + Mt Emu -------**						**--- MD ---**	
Site	**1**	**2**	**3**	**4/5**	**6**	**8**	**9**	**10**	**7**	**11**	**13**	**14**	**15**	**16**	**17**	**18**
1	-			0,3	1,0	1,1	0,1	0,2	0,3	0,3	0,3	0,4	0,4	0,4	0,4	0,4
2		-		0,2	1,0	1,1	1,0	0,1	1,2	2,2	1,2	0,4	1,3	0,3	0,3	0,3
3			-	2,0	1,0	2,1	2,0	1,1	1,3	2,3	2,2	1,4	2,3	0,3	0,3	0,3
4/5	3	2	2	-	1,0	1,4	0,4	1,3	0,6	2,5	2,4	1,6	1,5	0,5	0,5	0,5
6	1	1	1	1	-	0,2	0,1	0,2	0,3	1,3	0,3	0,4	0,4	0,4	0,4	0,4
8	2	2	3	5	2	-	1,0	0,2	0,3	1,3	0,3	0,4	0,4	0,4	0,4	0,4
9	1	1	2	4	1	1	-	0,1	0,2	0,2	0,2	1,2	0,3	0,3	0,3	0,3
10	2	2	3	5	2	2	1	-	0,3	0,1	0,3	0,1	0,2	0,2	0,4	0,4
7	3	3	4	6	3	3	2	3	-	0,1		0,1	0,1	0,1	0,1	0,1
11	4	4	5	7	4	4	2	1	2	-	0,1				0,2	0,2
13	3	3	4	6	3	3	2	3		2	-	0,1	0,1	0,1	0,1	0,1
14	5	5	5	7	5	4	3	2	1		1	-			0,2	0,2
15	4	4	5	6	4	4	3	2	1	1	1		-		0,2	0,2
16	4	4	5	6	4	4	3	2	1		1			-	0,2	0,2
17	4	4	5	7	4	4	3	4	1	2	1	2	2	2	-	
18	4	4	5	7	4	4	3	4	1	2	1	2	2	2		-

Table 4-4. Summary of quantitative analyses of populations structure in *N. obscura*, based on the allozyme data. [1]Nodes in the hierarchical level refer to those identified in Figure 4-3. [2]Mean and range (bracketed) for the number of statistically-significant differences in allele frequency detected among pairwise comparisons of populations. [3]Confidence intervals (99% CI) shown in brackets for F_{IS} and F_{ST} (** $p < 0.01$).

Hierarchical level[1]	**Sites**	**Sig. diffs[2]**	**F_{IS} (99% CI)[3]**	**F_{ST} (99% CI)**
species [node A]	all	2.8 (0-7)	-0.014 (-0.156 to 0.144)	0.687** (0.429 to 0.808)
MD/Central/Mt Emu [node B]	7,11,13-18	1.1 (0-2)	-0.112** (-0.205 to -0.011)	0.607** (0.049 to 0.857)
Central/Mt Emu [node C]	7,11,13-16	0.7 (0-2)	-0.112 (-0.213 to 0.001)	0.454 (-0.001 to 0.692)
MD [node D]	17,18	0	-0.125 (-0.200 to 0.000)	0.111 (0.000 to 0.167)
Merri/Eastern [node E]	1-3,4/5,6,8-10	2.1 (0-6)	0.027 (-0.178 to 0.212)	0.449** (0.241 to 0.599)
Merri [node F]	8-10	1.3 (1-2)	-0.067 (-0.192 to 0.137)	0.423** (0.011 to 0.655)
Eastern [node G]	1-3,4/5,6	1.0 (0-3)	0.063 (-0.347 to 0.375)	0.252** (0.004 to 0.394)

Table 4-5. MtDNA profiles of each *N. obscura* population, including a summary of which pairwise comparisons of cyt*b* haplotype frequencies between sample sets were statistically significant ($p < 0.05$) within each ESU or sub-clade, after correcting for multiple tests.

		IA								IB				IIA	IIB								IIC				
Popn	***(N)***	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**	**j**	**k**	**l**	**m**	**n**	**o**	**p**	**q**	**r**	**s**	**t**	**u**	**v**	**w**	**x**	**Significant differences**
1	8	6	2																							all
2	9			4	5																					all
3	5						4	1																		all except 4 & 5
4	8					2	5		1																	all except 3 & 5
5	5						5																			all
6	9					9																				all
8	5									5																10
9	5									2	1	2														10
10	5												5													8 & 9
7	7													7												all Central sites
11	9														9											all
12	9															9										all
13	9																			9						all
14	4																4									all except 15 & 16
15	10															1	5	2	2							all except 14 & 16
16	8															3	2				3					all except 14 & 15
17	5																					3	2			ns
18	8																					1	4	2	1	ns

Table 4-6. Distribution of cyt*b* sub-clades among the five geographic regions identified by allozyme analysis. Shown are the number of individuals included in each sub-clade; the number in brackets is the percentage of pairwise comparisons between sample sets within that sub-clade that were statistically-significant ($p < 0.05$, after correcting for multiple tests within a sub-clade) when the individual cyt*b* haplotype frequencies were compared.

	Sub-clade				
Lineage	**IA**	**IB**	**IIA**	**IIB**	**IIC**
Eastern	44 (87%)				
Merri		15 (67%)			
Central				49 (86%)	
Mt Emu			7 (n/a)		
MD					13 (0%)

Table 4-7. Mean genetic divergences within (± standard errors) and between sub-clades of *N. obscura* for cyt*b*.

ESU	**Eastern**	**Merri**	**Mt Emu**	**Central**	**MD**
Eastern	(0.003 ± 0.001)				
Merri	0.005	(0.002 ± 0.001)			
Mt Emu	0.017	0.014	n/a		
Central	0.016	0.013	0.006	(0.002 ± 0.001)	
MD	0.016	0.014	0.007	0.005	(0.002 ± 0.001)

5. HIGHLY SUB-STRUCTURED SOUTHERN PYGMY PERCH

5.1. INTRODUCTION

The southern pygmy perch *Nannoperca australis* Günther is the most widespread species in its genus, occurring across much of southeastern Australia. Its range comprises northern Tasmania (including King and Flinders islands), coastal Victoria, and the southern Murray-Darling Basin (MDB) (Merrick and Schmida 1984; Kuiter and Allen 1986; Kuiter *et al*. 1996). A small population in the Inman River, to the west of the Murray Mouth, represents a recently discovered western range limit and population isolate (Hammer and Walker 2004). *Nannoperca australis* is also known from a diversity of aquatic environments, ranging from small streams, large rivers, lakes and wetlands, generally at low to moderate elevations (Llewellyn 1974; Cadwallader and Backhouse 1983; Kuiter *et al*. 1996). It is distinguished from its congeners *N. obscura* and *N. variegata* in various parts of its range, by red fins, a more robust body, and several visible morphological characters (Kuiter *et al*. 1996, Chapter 4).

Aspects of *N. australis* biology are known from a seminal study in Tasmania (Macquarie River: Humphries 1995), breeding studies in the MDB (Llewellyn 1974) and other local assessments (e.g. Jackson and Davies 1983; Hammer 2001; Bond and Lake 2003), and summarised across the range (Koehn and O'Connor 1990; Kuiter *et al*. 1996). *Nannoperca australis* is associated with cover in sheltered micro-habitats, including shallow areas with dense aquatic macrophytes. They are relatively short-lived, with moderate fecundity, large eggs and demersal larvae, and display limited localised movement. Life-history traits and wide distribution across isolated drainages suggest high levels of broad genetic sub-structure should be expected (e.g. Cook *et al*. 2007).

Although considered widespread and 'common' across most of its range (Kuiter *et al*. 1996; Allen *et al*. 2002), *N. australis* is now patchily distributed in the Murray-Darling Basin, experiencing serious and continuing declines, and accordingly is listed as 'threatened' in New South Wales (Murray, Murrumbidgee and Lachlan river catchments) and South Australia (lower River Murray, Lower Lakes and Eastern Mount Lofty Ranges). Massive alteration of river flow regimes and habitat conditions, and the introduction of predatory or competitive alien fishes are presumed as key threats (Lloyd and Walker 1986; Unmack 1992; Morris *et al*. 2001; Hammer *et al*. 2007a). More broadly, extensive landscape change in coastal southern Australia has reduced available habitat, including extensive drainage of surface waters and wetlands in the Millicent Coast River Basin (Hammer 2002). A sound systematic framework will allow the identification of key conservation units.

Previous work on broader pygmy perch relationships (Hammer 2001; Unmack *et al*. in review) examined populations of *N. australis* and found two major groupings that likely represent separate species: one in eastern Victoria and northeastern Tasmania and the other in the remainder of the range. The eastern taxon has tentatively been referred to as *N.* 'flindersi', based on resurrecting a previously described sub-species (Scott 1970) and awaits a formal morphological re-analysis. This chapter presents an update and expansion of previous work by the author (Hammer 2001) but involving considerably more detailed analysis for comparison with other species (chapters), especially *N. obscura*, and significant additional molecular screening to increase spatial coverage in key areas. This includes two populations of conservation significance, namely (1) a newly discovered population from the Lachlan River NSW, the northernmost known MDB population, and (2) a site from Hindmarsh Island SA, where the species is likely to be extirpated due to severe water reductions from over-allocation of water and prolonged drought.

5.2. METHODS

Sampling and analyses

Samples were obtained from coastal systems between Genoa, Victoria and Inman River, South Australia; from northern Tasmania and Flinders Island, and from the MDB (Figure 5-1 and Table 5-1). A sample from the Lachlan River, New South Wales was supplied by D. Gilligan. Fish were euthanased, and samples taken of whole fish or a small lateral section of tail muscle. Samples were snap frozen in liquid nitrogen, then stored at -70^{o}C. Voucher specimens and have been deposited at the Australian, South Australian and Victorian museums.

An *overview study* to examine species boundaries and broad population structure was previously undertaken in Hammer (2001) and duplicated, in part, in Chapter 4. Diagnostic and polymorphic loci were selected for a detailed *population study*. This expands on previous work (Hammer 2001) by the addition of several new populations from key areas (n = 11 sites and 97 individuals). Between two and six individuals from eight populations (n = 27 fish) at putative biogeographic breaks were also 'typed' to major genetic groups or hybrid origins at a limited number of loci. Localities and sample sizes are indicated in Table 5-1 and Figure 5-1.

Allozyme electrophoresis

Homogenates comprised a small piece of caudal muscle sonicated in an equal volume of buffered lysing solution (0.02M Tris-HCl, pH 8.0, with 0.2% 2-mercaptoethanol and 0.02% NADP). After centrifugation for 10 min at 10,000 g, supernatant fluids were stored at -20°C as 10-20 μL aliquants in glass capillary tubes. Allozyme electrophoresis was conducted on cellulose acetate gels (Cellogel^{TM}), following Richardson *et al*. (1986). Of the 31 enzymes or non-enzymatic proteins which produced zymograms of sufficient intensity and resolution for genetic interpretation in the overview study, the following were employed in the population study presented herein: ACON, ADA, AK, CA, CK, FUM, GOT, G6PD, GPI, GSR, IDH, MDH, ME, MPI, PEPA, PEPB, PEPD, PGK, and PGM. Details of enzyme and locus abbreviations, enzyme commission numbers, electrophoretic conditions, and stain recipes are in Richardson *et al*. (1986) and Bostock *et al*. (2006). Allozymes were designated alphabetically and multiple loci, where present, were designated numerically, both in order of increasing electrophoretic mobility (i.e. *Ca*^{a}, *Ca*^{b}, *Gpi1*, *Gpi2*).

Data analysis

The genetic affinities of individuals were first explored using stepwise principal co-ordinates analysis (PCO), as implemented on a pairwise matrix of Rogers' genetic distance (Rogers 1972)

using PATN (Pattern Analysis Package, DOS version, Belbin 1994). The genotypic data were examined for statistical evidence of deviation from Hardy Weinberg expectations or linkage disequilibrium within populations. Quantitative statistical comparisons to assess any heterogeneity of allele frequencies between populations involved pairwise comparisons and global test across polymorphic loci based on Fisher's method (Fisher 1948). All tests involved estimating exact probabilities using GENEPOP version 3.4 (Raymond and Rousset 2003), with probability values adjusted for multiple tests using the sequential Bonferroni correction factor (Rice 1989). Homogenous genetic groupings from a site were treated as populations and between-population estimates of genetic similarity were calculated, using Nei's unbiased Distance (Nei D, Nei 1978). F-statistics were used to assess the degree of population genetic sub-division within and among individual populations and regions. F_{ST} and F_{IS} values plus their 99% confidence intervals were obtained using FSTAT 2.9 (Goudet 2000). Observed heterozygosity values (H_O) were calculated using all loci surveyed in the overview study (n = 52), under the assumption that loci found to be monomorphic were invariant in all sample sets. The genetic affinities of populations were displayed visually as UPGMA (unweighted pair-group method of arithmetic averages) dendrograms and NJ (neighbor joining) networks constructed from Nei D values using the NEIGHBOR routine in PHYLIP 3.5c (Felsenstein 1993) and drawn using TREEVIEW 1.6.0 (Page 1996).

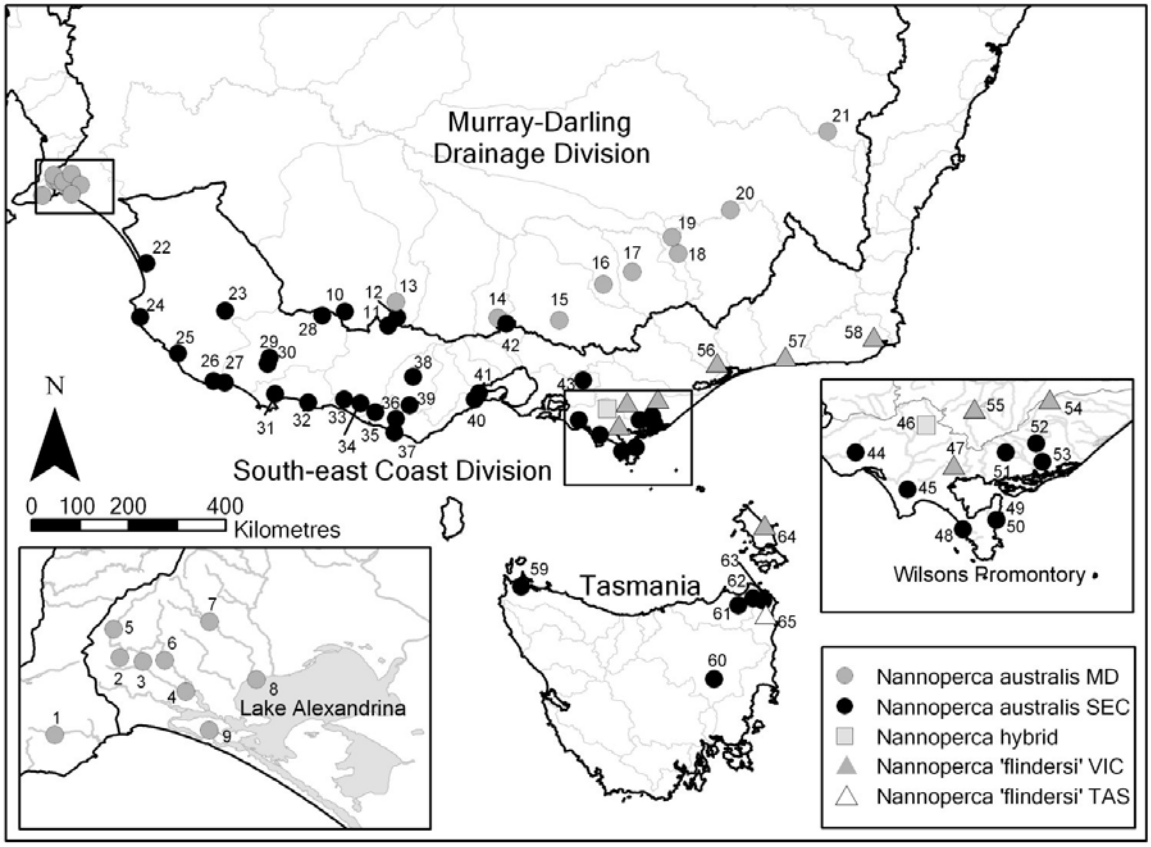

Figure 5-1. Distribution of sampling sites and provisional Evolutionarily Significant Units (ESUs) in *Nannoperca australis s.l.* Also shown are major drainage divisions and river basins (AWRC 1976). Sites codes as per Table 5-1, and taxon and sub-group codes as per the text.

5.3. RESULTS

Major genetic groupings

The final dataset comprised 57 populations and 532 fish genotyped at 23 putative allozyme loci (Table 5-1 and Appendix 3). An initial PCO based on all specimens confirmed the presence of two distinctive taxa (Figure 5-2), and demonstrated that fish collected at site 46 (Berrys Creek) have a hybrid origin involving these taxa. Both conclusions are supported by an examination of allele frequency data (Appendix 3), which indicates that (1) the two taxa are fully (i.e. no shared alleles) or effectively (i.e. cumulative percentage of shared allele < 10%) diagnosable at seven loci (*Ada*, *Ck*, *Fum*, *Mdh1*, *Me2*, *Mpi*, and *Pgk*), and (2) Berrys Creek possesses alleles representative of both taxa at these loci. Given its hybrid nature, Berrys Creek was excluded from subsequent analyses. Herein the eastern taxon, which occurs from Wilson Promontory to Genoa in Victoria, on Flinders Island, and in the Ansons River catchment of Tasmania (Figure 5-1), is referred to informally as *N.* 'flindersi', as foreshadowed in the Introduction.

A second level of PCO was undertaken on all populations of the western taxon (Figure 5-3). This revealed a geographically defined split along the first dimension, identifying MDB compared to coastal populations in Victoria and Tasmania. Four sites were notable in where they aligned within this dichotomy. Fish from the Wimmera (site 12) aligned with the coastal grouping (hereafter the SEC/Tas lineage), despite being collected from a distributary of the Murray (River Basin 15). In addition, three outlying populations were distinctive in the second PCO dimension when compared to other members of their lineage, namely site 34 (Mt Emu Creek) for the SEC/Tas lineage, plus sites 15 (Yea River) and site 21 (Lachlan River) for the MDB lineage. Nevertheless, there was minimal overlap between the two sub-groups, with further support for the presence of two lineages provided by the genetic divergence estimates (Nei D). For consistency, these are presented visually in the UPGMA dendrogram of Figure 5-4, although the dichotomy is more evident in a NJ Network (not shown). Raw profiles also distinguish the lineages by major differences in allele frequency or the presence of unique alleles in a suite of loci (notably *Acon1*, *Ca*, *Got2*, *Gpi1* and *Idh2*: Appendix 3).

The presence of sub-groupings within *N.* 'flindersi' was also examined *via* stepwise PCO (not shown) and genetic divergence estimates (Figure 5-5). In both analyses there is a primary split between five western sites (sites 47, 54-57) *versus* the two most eastern sites in Victoria and Tasmania, site 58 (Maramingo Creek) and site 65 (Ansons River), which in turn are both distinctive.

Genetic structure

Statistical tests of the genotypic data across the 56 non-hybrid populations revealed no evidence to reject the standard null hypotheses that individual populations are panmictic, and no loci are in linkage disequilibrium with one another. Hence between-population comparisons were undertaken at various hierarchical levels, covering each major genetic group (taxon), sub-groups (lineages), and any obvious regional groupings (using the nodes identified in Figure 5-5).

Comparisons revealed numerous statistically-significant differences in allele frequency between populations, usually at multiple loci. Due to the number of populations employed, subsets of these comparisons were made separately for the SEC/Tas lineage (average for all pairwise comparisons = 1.19, range 0-6: Table 5-2), the MDB lineage (average for all pairwise comparisons = 2.97, range = 0-7: Table 5-3), and *N.* 'flindersi' (average for all pairwise comparisons = 2.14, range = 0-4: Table 5-4), with a summary in Table 5-5. These analyses indicate that both taxa are genetically heterogeneous across their geographic range, often strongly so. Indeed, as shown in Figure 5-4, there are only six small clusters of sites where constituent sites did not display any statistically-significant differences in allele frequency from other sites in that cluster. Given the relatively small sample sizes involved (range 4-16, average = 9.3) and the consequent expectation that Type I errors would be common (see Richardson *et al*. 1986), it appears likely that population sub-structuring is present at virtually all hierarchical levels in both taxa. An examination of the F-statistics (Table 5-5) lends further support, as F_{ST} values for each taxon are overall very large and significantly positive at all of the hierarchical levels examined (i.e. nodes A-G in *N. australis s.l.* and nodes H-J in *N.* 'flindersi'). As expected, none of the F_{IS} values differed significantly from zero.

Examining patterns in the SEC/Tas lineage, a group of five of the six most divergent populations were recorded from central Victoria, namely Mt Emu Creek (site 34) and sites 36-39 (Corangamite River Basin and Gellibrand River: Figure 5-1). These sites were nested geographically within an otherwise widespread and relatively homogenous grouping, where many populations did not display significant differences in allele frequency at any locus (Table 5-2). The other divergent site in this lineage was site 60 on the upper Macquarie River in Tasmania.

Within the MDB lineage, considerable sub-structure was evident between most sites. The Lachlan River (site 21) and Yea River were most distinctive (Figure 5-5). Within the remaining 16 sites, a remarkable pattern emerged concerning populations from the Lower Murray region. Five proximate catchment areas (Inman River, Tookayerta Creek, Finniss River, Angas River and

Lake Alexandrina) were not necessarily each others closest relative but instead represented divergent groupings intermixed within populations 100s of kilometres away (Figure 5-1 and Figure 5-5). Each between catchment comparison in the Lower Murray region identified one to five significant differences in allele frequency and included unique alleles (especially at *Acon1*, *Gpi1* and *PepA2*: Appendix 3 and Table 5-3). A supplementary PCO helped to reveal a subtle geographic pattern evident once populations were coded to one of three groups: (1) Mount Lofty Ranges (MLR), (2) Lake Alexandrina, and (3) all upstream sites (Figure 5-5). A relatively neat divide separated upstream MDB from lower (MLR) sites, with Lake Alexandrina fish overlying and intermediate to both groups. Interestingly, site 16 (Swanpool Creek) was the only discordant upstream site, and displayed a similar pattern of overlay as did the Lake Alexandrina sites.

Support for Lake Alexandrina representing a zone of mixing is revealed by examination of patterns in the raw allele profiles plus a relatively high level of observed heterozygosity (H_O = 0.061-0.080 for sites 4, 8 and 9: Appendix 3). Overall, moderate to high levels of heterozygosity were evident in *N. australis s.l.*, being higher in the two lineages of the western taxon (mean 0.041 ± 0.020 and 0.037 ± 0.016 for MDB and SEC/Tas respectively) and lower in *N.* 'flindersi' (mean 0.014 ± 0.008).

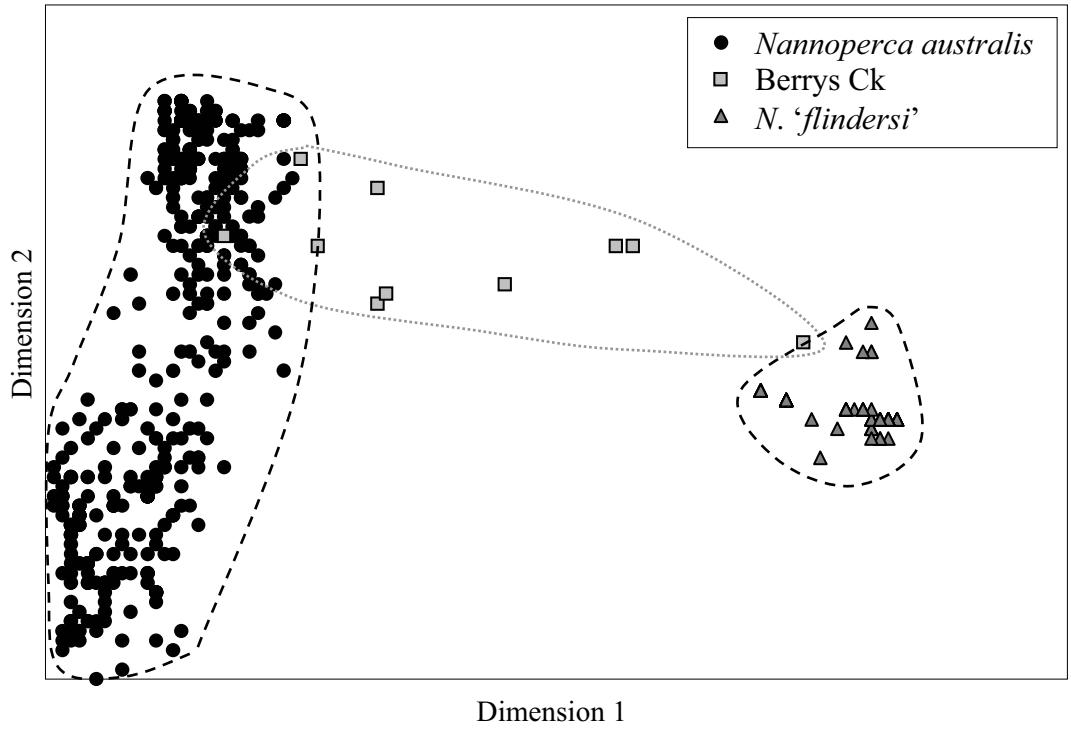

Figure 5-2. Principal Coordinates Analysis of the 532 specimens involved in the examination of population structure within *Nannoperca australis s.l.* The relative PCO scores have been plotted for the first and second dimensions, which explain 32% and 17%, of the total variance, respectively.

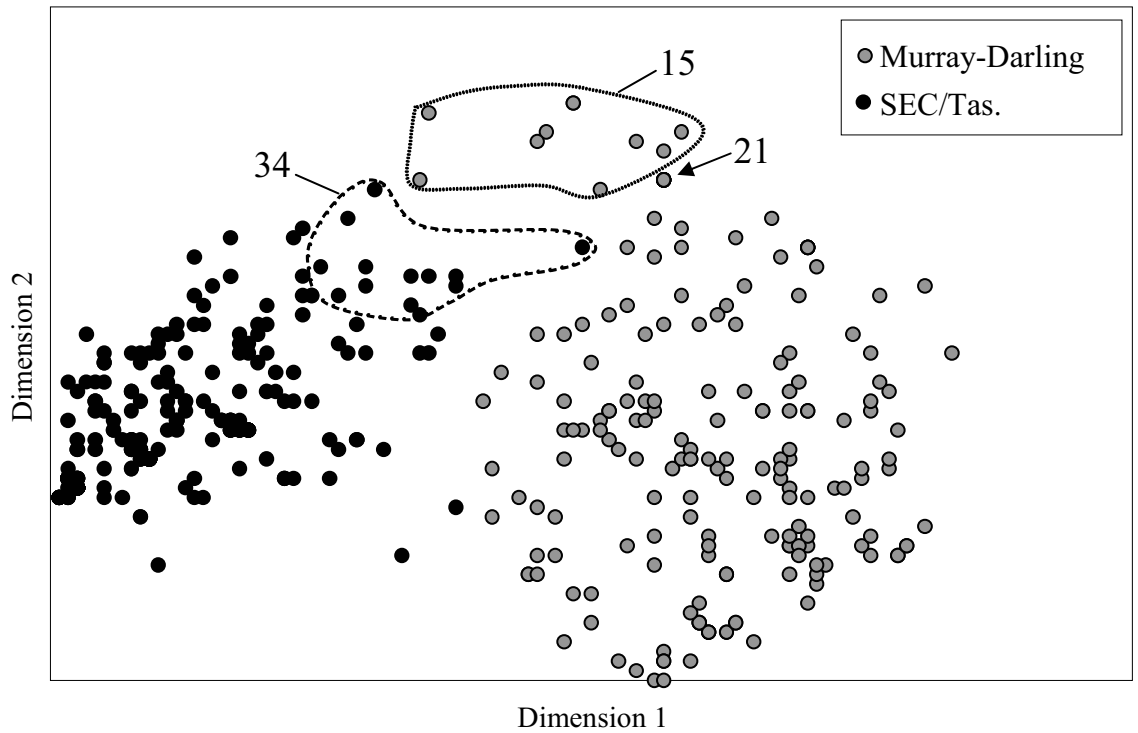

Figure 5-3. Principal Coordinates Analysis exploring sub-structure within *N. australis s.s.* (western taxon). The relative PCO scores have been plotted for the first and second dimensions, which explain 27% and 8% of the total variance, respectively. Murray-Darling and SEC/Tas lineages separate in the first dimension, with distinctive populations highlighted.

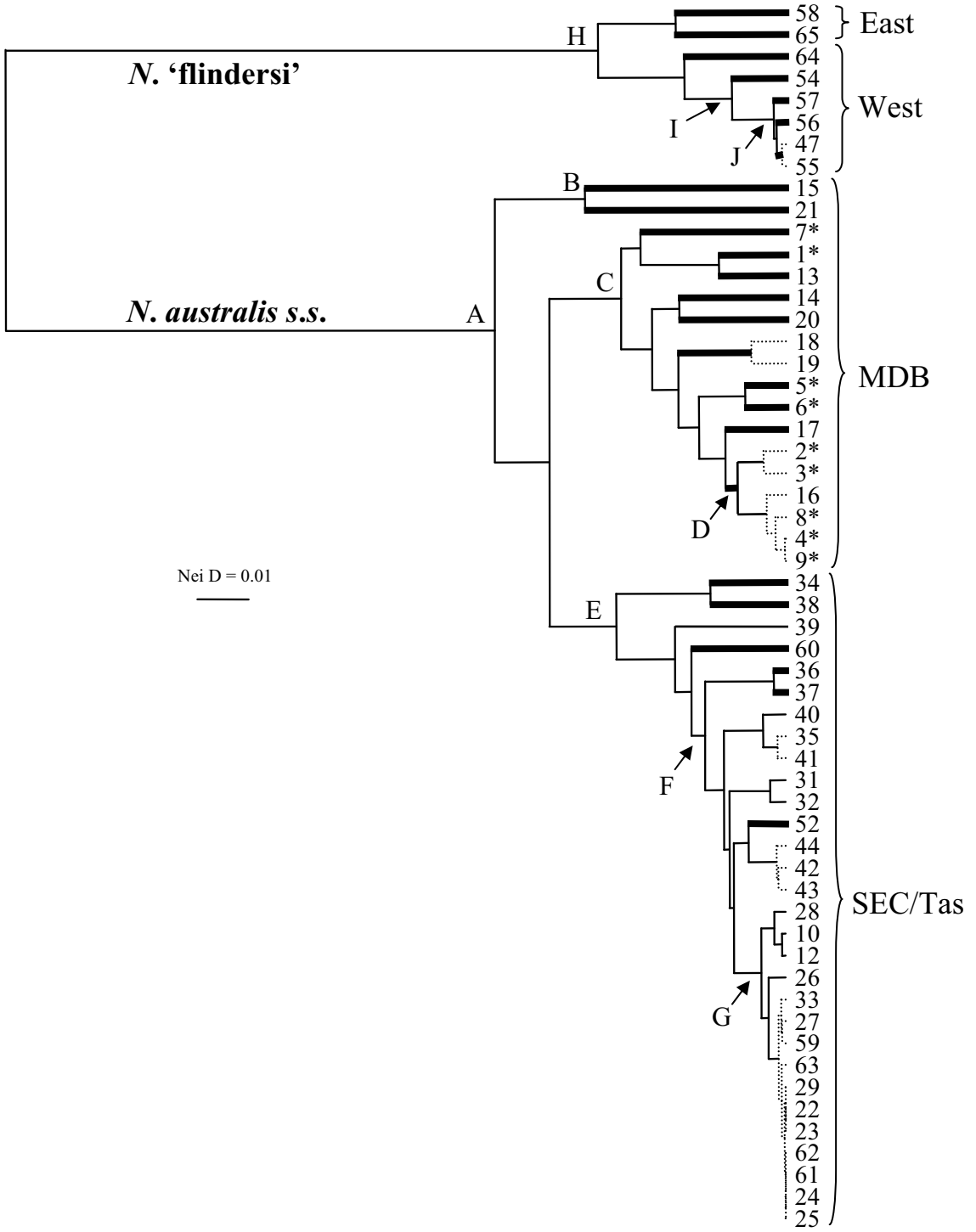

Figure 5-4. UPGMA dendrogram depicting taxa and lineages within *N. australis s.l.* and the genetic affinities among the 56 non-hybrid sites sampled during the population study, based on pairwise Nei Distance values. Branch thicknesses reflect the number of statistically-significant differences in allele frequency; thick = at least one difference between that site and all others, thin = some but not all sites show at least one difference, dashed = no differences. Letters represent hierarchical levels used in statistical evaluation (see Table 5-5). (*) denotes populations from the lower River Murray region.

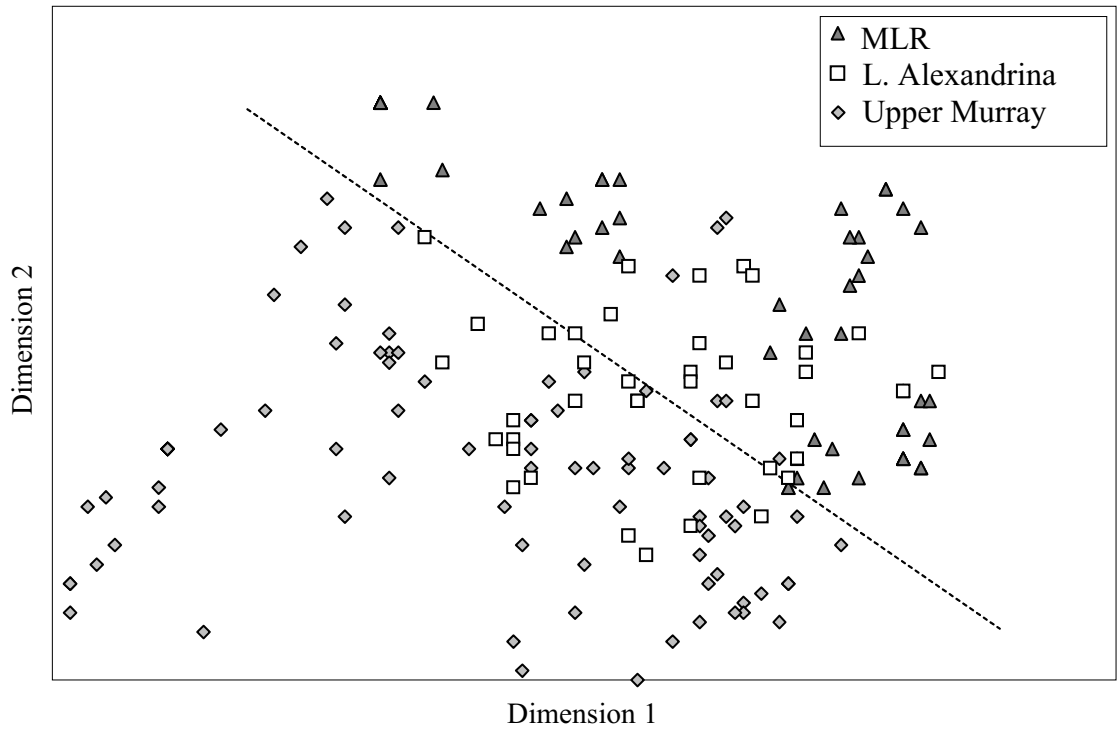

Figure 5-5. Principal Coordinates Analysis of the MDB lineage *Nannoperca australis s.l.* The relative PCO scores have been plotted for the first and second dimensions, which explain 18% and 11% of the total variance, respectively. A subtle division between upper Murray and lower Murray (Mount Lofty Ranges, MLR) sites is indicated with the dashed line, with fish from Lake Alexandrina overlain and intermediate to both groups.

5.4. DISCUSSION

The genetic complexity observed within *Nannoperca australis s.l.* further highlights major inadequacies in the systematic frameworks for southern Australian freshwater fishes. Previously, a single, widespread species was presumed, but the allozyme data indicate the presence of a major species-level divide, other lineages, and significant overall sub-structuring relevant to conservation management.

Taxonomic considerations

The allozyme data confirm the presence of two distinct taxa in southern pygmy perch, as previously found by Hammer (2001) and Unmack *et al*. (in review). Comparing the seven fixed difference found in this chapter against the full complement of 52 loci screened in the overview study of Hammer (2001), which included two additional diagnostic loci not screened, the effective divergence between the taxa is 17%FD. Thus, these taxa are readily diagnosable at a numerous genetic markers, are characterized by distinct mtDNA clades (Unmack *et al*. in review), and clearly merit recognition as distinct evolutionary species pending morphological confirmation (cf. Scott 1970). The species have largely parapatric distributions, with populations co-occurring around Wilson Promontory (Figure 5-1). They are capable of hybridising and backcrossing (as found at Berrys Creek), but this is not unusual among freshwater fishes under natural conditions (Hubbs 1955; Verspoor and Hammar 1991) or forced through translocation and altered environmental conditions (Esa *et al*. 2000; Fisher *et al*. 2006). Unmack (2005) discusses several reasons why the Berrys Creek result is likely to reflect the human-mediated translocation of *N. australis s.s.* into a region normally occupied only by *N.* 'flindersi'. Finally, the abutting distribution of the MDB and SEC/Tas lineages is compatible with sub-specific recognition, again depending on the availability of concordant morphological differences.

Genetic sub-structure

Both species of 'southern pygmy perch' are highly sub-structured, with divergent populations most pronounced in the MDB, central coastal Victoria and the eastern range distribution. In the SEC/Tas lineage, Mt Emu Creek was quite divergent, although only as a conspicuously distinctive population rather than as an anomalous geographic outlier as per *N. obscura*. There was also no apparent genetic divide in *N. australis* east and west of the Glenelg catchment as was detected in its congener. Instead there was grouping of sites from the Corangamite River Basin and Gellibrand River, nested within an otherwise broad genetic group. However, despite a lack of well defined population groups, current isolation was inferred from significantly different allelic profiles across isolated drainages.

The MDB lineage showed unexpectedly high levels of genetic divergence within a single interconnected drainage division. Population genetic data suggested a suite of isolated sub-populations (i.e. little to no gene flow). This sub-structure was apparent even at fine scales, as demonstrated by comparison of proximate populations in stream catchments of the MLR. Some of these MLR sites had closer genetic ties to populations hundreds of kilometres upstream than to their neighbouring catchments, and this may represent historic relatedness overlain by contemporary isolation or simply chance convergence from an ancestral gene pool. While certain populations were similar, overall upper and lower Murray gene pools were apparent which may signal regional isolation, tempered with more recent, perhaps sporadic, gene flow *via* dispersal along the Murray. Additional matrilineal data would help to further explore this pattern. Lake Alexandrina appears to have acted as a mixing point between upper and lower sites, as it displayed intermediate genetic similarities with both upper and lower Murray regions plus comparatively high heterozygosity estimates. The scale and frequency of this mixing are difficult to ascertain, and certainly would appear very limited in opportunity under the currently highly regulated and altered Murray corridor having much reduced frequency and duration of floods for maintaining suitable off channel habitats and dispersal. A near-continuous distribution associated with the main channel (e.g. billabongs and wetlands) can be partially inferred from limited historic data from the 100-40 ya (Llewellyn 1974; Rutherford 1991), with the more recent extirpation of most lowland wetland/floodplain populations.

Biogeographic patterns

The primary divergence within *N. australis s.l.* occurs in an area where historic drainages are in opposing directions. Gippsland, eastern Flinders Island and the northern section of east coast Tasmania all drained to the east, creating an isolating barrier and/or limiting genetic interchange among eastern and western populations (Figure 1-2). The distributions of the two species meet near Wilson Promontory, although there is no neat divide as with *Retropinna* (SEC and MTV: Chapter 3). Instead, the two *Nannoperca* species display a semi-chaotic pattern of occurrence plus one instance of hybridisation and introgression (which may reflect a natural phenomenon or human-mediated dispersal). Within *N.* 'flindersi', the two most genetically-distinct populations occur at the eastern range edge, which likely reflects remoteness and isolation, especially the Tasmanian site (Ansons River). Broad patterns in the western taxon reflect a simple north-south divide, consistent with the biogeographic isolation provided by the Great Dividing Range, although the genetic divergence observed is much younger than the formation of the barrier itself (Unmack 2001). The Wimmera region represents a possible exception, as its genetic similarly to the adjacent Glenelg River Catchment and is suggestive of more recent natural dispersal (e.g. across low divides) or human-mediated translocation (e.g. as bait fish or existing pipelines). As in

N. obscura, the potential of a coastal pathway for gene flow between the MDB and adjoining Millicent Coast River Basin appears to have had little influence on contemporary genetic structure. Such patterns provide biological support for limited and opposing drainage direction under lower sea levels (Unmack 2001) and geomorphic and palaeolimnological evidence for a stranded, last-interglacial shoreline that acted as a sill, limiting the exchange of flows (and potentially gene flow) between Lake Alexandrina and the Coorong (Fluin *et al*. 2007).

The lower levels of allozyme divergence between independent drainage areas along coastal southern mainland Australia and Tasmania are somewhat surprising and suggest recent admixture of many populations prior to the most recent reinstatement of the saltwater barrier that now isolates drainages. The likely opportunity for such dispersal occurred during lower sea levels in the last glacial maximum, where streams joined and a large lake existed between Victoria and Tasmania (Unmack 2001). In contrast, a group of central Victorian and a Tasmanian site, appear to have been buffered from this trans-Tasman gene flow, perhaps due to isolation by: distance inland (e.g. the Macquarie River site is *c*. 150km from the coast); land barriers (e.g. Corangamite River Basin is landlocked under current climatic conditions), and/or other instream barriers (e.g. Mt Emu Creek occurs upstream of Hopkins Falls).

Within the MDB, populations are widespread across southern tributaries of the system. Some recent range contraction and fragmentation due to anthropogenic change may have eliminated intermediate populations or enhanced genetic divergence, thus exacerbating observed patterns. However, sampling sites were concentrated enough to suggest that some level of population divergence occurred prior to anthropogenic change. Hence it is postulated that historic factors allowed widespread colonisation of the drainage, followed by apparent contraction and isolation (divergence) of many populations. Certainly the climate in the southern Basin was much wetter either side of the last glacial maxima *c*. 18 Kya, especially 6-5 Kya (see White 2006). The subtle divergence of lower Murray stream sites suggests a period of general isolation from upstream, perhaps due to prolonged aridity in the western MDB that occurred during the glacial maxima, or more likely due to fragmentation *via* river blockages or changes in channel direction (but certainly well short of the divergence excepted from isolation during the formation and departure of Lake Bungunnia *c*. 2-0.7 Mya).

Modern unidirectional mixing into Lake Alexandrina occurs from both MLR streams and upstream Murray areas. Conversely, several small scale biogeographical barriers appear to have minimised gene flow into systems, leading to a series of divergent populations in the MLR. Plausible biogeographic scenarios are available for each distinctive population: the Inman River is isolated by a marine barrier but probably was connected to the Murray under lower sea-level,

the Tookayerta Catchment has contrasting habitat with extensive dense swamp in the lower catchment, the Finniss has a small waterfall on its lower reaches, and the Angas River is connected to Lake Alexandrina only episodically with a mainly dry lowland channel (Hammer 2004).

Ecology

As with the *Retropinna* species complex, existing knowledge of 'southern pygmy perch' ecology in southeastern Australia must now be reassessed, given the potential for species-level differences in ecology. The seminal biological study of Humphries (1995) was undertaken in the upper Macquarie River, and hence applies to the western species, and perhaps only to the SEC/Tas lineage within that species. Until more is known, caution should be exercised before extrapolating beyond this initial study, because of the potential for genetically influenced differences in ecology in addition to other, already acknowledged environmental contrasts (i.e. Macquarie River is atypical of most regional populations, being the most southerly population and from a large riverine habitat). Indeed, Humphries (1995) noted several differences in reproductive biology from a previous study of MDB fish from the Murrumbidgee River (Llewellyn 1974). Overall however, all species and lineages within *N. australis s.l.* do appear to have relatively demersal adults and larvae, based on high levels of genetic sub-structure.

Conservation

While there has been some concern for the status of MDB *N. australis*, little attention has been paid to the remainder of the range of what is considered a widespread and 'common' single species. Certainly, the presence of a distinctive and relatively restricted second species highlights an immediate conservation priority, particularly in mainland Tasmania where intensive collecting has identified it in only one catchment.

A concurrent study using the cyt*b* section of the mtDNA genome (Unmack *et al*. in review) provides the second component to assess ESUs within the eastern and western species (Moritz 1994). The matrilineal data indicate reciprocal monophyly of an MDB clade compared to all coastal populations (plus the Wimmera fish) of the western species (the latter further being allocated to one of three geographically-based sub-clades, none of which correspond to any population sub-division evident in the allozyme analyses), and reciprocally monophyletic split in *N.* 'flindersi' between Ansons River and the other populations in Gippsland and Flinders Island. Thus, two ESUs can be assigned within each species, namely MDB and SEC/Tas ESUs in *N. australis s.s.*, and separate Victorian (including Flinders Island) and Tasmanian (Ansons

River) ESUs in *N.* 'flindersi'. The distributions of these four ESUs are displayed visually in Figure 5-1.

Considerable nuclear variation within most ESUs provides genetic criteria for identifying numerous Management Units (MUs) (Moritz 1994; Moritz *et al*. 1995). These involve both singular and groups or river basins, but also multiple conservation units within some river basins. For example most populations within the *N.* 'flindersi' Victorian ESU qualify as MUs. The Macquarie River, Mt Emu Creek and combined Corangamite populations also qualify, and fine scale analysis of both the nuclear, mtDNA and ecological data sets will likely reveal other MUs within the SEC/Tas ESU. The considerable genetic divergence within the MDB ESU demands more intensive spatial investigation to fully identify distinct genetic components. Certainly divergent outliers such as the Lachlan River and Yea River should get immediate attention, and five MUs should be recognised in the Lower Murray region as an area harbouring significant evolutionary potential in this species. In some instances (e.g. Murrumbidgee River: Morris *et al*. 2001), local extirpation is already presumed and pervasive flow and habitat change exacerbated by current drought conditions seriously threaten the viability of most Lower Murray populations (Hammer 2007a). Such populations will not easily be replaced or recolonised given the presumed limited dispersal ability of this species in the short term plus the highly modified nature of this regulated aquatic system.

5.5. TABLES

Table 5-1. Locality and sample size information for *Nannoperca australis*. Site numbers match those in Figure 5-1. DD = Drainage Division; RB = River Basin (AWRC 1976). Some sites were typed to major genetic group at selected loci only (*).

Site	Field code	Locality	State	DD	RB	Latitude (S)	Longitude (E)	*n*
1	F-FISH84	Inman R., Victor Harbor	SA	V	1	35º32'	138º30'	9
2	F-FISH84	Nangkita Ck, Mount Compass	SA	IV	26	35º21'	138º40'	9
3	F-FISH84	Tookayerta Ck trib	SA	IV	26	35º21'	138º43'	6
4	F-FISH84	Tookayerta Ck (Black Swamp)	SA	IV	26	35º26'	138º50'	11
5	F-FISH84	Meadows Ck, Meadows	SA	IV	26	35º16'	138º39'	10
6	F-FISH84	Finniss R., Ashbourne	SA	IV	26	35º21'	138º47'	9
7	F-FISH84	Angas R., Strathalbyn	SA	IV	26	35º15'	138º53'	10
8	F-FISH84	L. Alexandrina drain, Milang	SA	IV	26	35º24'	139º01'	13
9	F-FISH84	L. Alexandrina, Hindmarsh Is.	SA	IV	26	35º32'	138º53'	16
10	PU00-08	Fyans Ck diversion, Fyans Ck	Vic.	IV	15	37º06'	142º33'	10
11	PU00-06	Mount Cole Ck, Warrak	Vic.	IV	15	37º17'	143º08'	1*
12	F-FISH83	Wimmera R., Elmhurst	Vic.	IV	15	37º10'	143º15'	6
13	PU99-33	Middle Ck trib., Warrenmang	Vic.	IV	8	36º58'	143º14'	10
14	PU00-01	Jews Harp Ck, Sidonia	Vic.	IV	6	37º11'	144º36'	10
15	PU9-208	Yea R., Yea	Vic.	IV	5	37º13'	145º26'	10
16	PU94-43	Swanpool Ck, Benella	Vic.	IV	4	36º44'	146º01'	10
17	PU99-79	Meadow Ck, Moyhu	Vic.	IV	3	36º34'	146º24'	10
18	PU99-81	Gap Ck, Kergunyah	Vic.	IV	2	36º19'	147º01'	10
19	PU94-47	Murray R. lagoon, Albury	NSW	IV	9	36º06'	146º56'	10
20	PU99-82	Coppabella Ck, Coppabella	NSW	IV	1	35º44'	147º43'	10
21	F-FISH98	Lachlan R. trib., Dalton	NSW	IV	12	34º04'	149º01'	8
22	F-FISH98	Henry Ck, Salt Creek	SA	II	39	36º00'	139º53'	10
23	PU00-16	Mosquito Ck, Langkoop	Vic.	II	39	37º05'	140º57'	9
24	F-FISH21	Drain L, Robe	SA	II	39	37º10'	139º49'	8
25	F-FISH83	Lake Bonney, Millicent	SA	II	39	37º39'	140º19'	5
26	F-FISH83	Ewens Ponds, Port McDonnell	SA	II	39	38º02'	140º47'	9
27	F-FISH83	Piccaninnie Ponds, Pt McDonnell	SA	II	38	38º03'	140º57'	10
28	PU00-14	Glenelg R., Glenisla Crossing	Vic.	II	38	37º09'	142º15'	10
29	PU00-17	Merino Ck, Merino	Vic.	II	38	37º43'	141º33'	8
30	PU00-19	Stokes R., Digby	Vic.	II	38	37º48'	141º31'	2*
31	PU00-20	Surrey R., Heathmere	Vic.	II	37	38º00'	141º37'	10
32	PU00-21	Shaw R., Yambuk	Vic.	II	37	38º02'	142º04'	10
33	PU00-22	Merri R., Grassmere	Vic.	II	36	38º16'	142º32'	10
34	PU00-23	Mount Emu Ck, Panmure	Vic.	II	36	38º20'	142º46'	10
35	PU00-24	Curdies R., Curdie	Vic.	II	35	38º27'	142º57'	10
36	PU00-25	Kennedy Ck, Kennedy Creek	Vic.	II	35	38º32'	143º14'	10
37	PU02-92	Gellibrand R. floodplain	Vic.	II	35	38º43'	143º13'	10
38	F-FISHY2	Gnarkeet Ck, Lismore	Vic.	II	34	37º58'	143º28'	7
39	PU00-26	Pirron Yaloak Ck, Pirron Yaloak	Vic.	II	34	38º21'	143º25'	10
40	PU02-107	Thompson Ck	Vic.	II	35	38º16'	144º17'	7
41	PU00-29	Waurn Ponds Ck, Geelong	Vic.	II	33	38º11'	144º21'	8
42	PU00-03	Deep Ck, Lancefield	Vic.	II	30	37º16'	144º43'	10
43	PU99-89	Diamond Ck, Tonimbuk	Vic.	II	28	38º01'	145º44'	10
44	PU99-88	Powlett R.	Vic.	II	27	38º33'	145º41'	10
45	PU02-75	Bald Hills Ck	Vic.	II	27	38º45'	145º58'	6*
46	PU99-77	Berrys Ck, Berrys Creek	Vic.	II	27	38º24'	146º04'	10

Site	Field code	Locality	State	DD	RB	Latitude (S)	Longitude (E)	*n*
47	PU99-75	Pebble Ck, Foster	Vic.	II	27	38º37'	146º13'	8
48	PU02-73	Darby R., Wilsons Prom.	Vic.	II	27	38º58'	146º16'	3*
49	PU02-70	Freshwater Lake Ck, Wilsons Prom.	Vic.	II	27	38º55'	146º27'	2*
50	PU02-69	Miranda Ck, Wilsons Prom	Vic.	II	27	38º55'	146º27'	3*
51	PU02-98	Billy Ck	Vic.	II	27	38º33'	146º30'	5*
52	PU99-74	Macks Ck, Calrossie	Vic.	II	27	38º30'	146º40'	10
53	PU02-96	Tarra R.	Vic.	II	27	38º36'	146º42'	5*
54	PU99-72	Merrimans Ck, Hiamdale	Vic.	II	27	38º16'	146º44'	10
55	PU99-78	Morwell R., Yinnar	Vic.	II	26	38º19'	146º20'	10
56	PU99-87	Prospect Ck	Vic.	II	24	37º47'	147º32'	10
57	PU99-85	Snowy R. lagoon, Orbost	Vic.	II	22	37º42'	148º27'	10
58	PU99-84	Maramingo Ck, Genoa	Vic.	II	21	37º26'	149º38'	10
59	F-FISH82	Harcas R., West Montagu	Tas.	III	14	40º47'	144º55'	10
60	F-FISH82	Macquarie R., Ross	Tas.	III	18	42º01'	147º30'	10
61	F-FISH98	Boobyalla R., Gladstone	Tas.	III	19	41º02'	147º49'	4
62	F-FISH98	Gladstone Lagoon, Gladstone	Tas.	III	19	40º56'	148º01'	9
63	F-FISH98	Icena Ck trib, Gladstone	Tas.	III	2	40º58'	148º09'	8
64	F-FISH84	Flinders Island	Tas.	III	1	39º57'	148º10'	5
65	F-FISH82	Ansons R. trib.	Tas.	III	2	41º09'	148º11'	10

Table 5-2. Summary of pairwise comparisons of allele frequency between all SEC/Tas sites. The lower triangle presents the number of statistically-significant differences for $p < 0.05$. The upper triangle summarizes the statistical outcome of a global test across all polymorphic loci, based on Fisher's method (** $0.001 \leq p < 0.05$; *** $p < 0.001$; ns = not significant; ^ = found to be statistically 'heterogeneous').

Site	10	12	22	23	24	25	26	27	28	29	31	32	33	34	35	36	37	38	39	40	41	42	43	44	52	59	60	61	62	63
10	-	ns	ns	ns	ns	ns	***	ns	ns	ns	***	**	ns	***	***	***	***	***	***	***	***	*	*	*	***	ns	***	ns	ns	ns
12	0	-	**	*	*	ns	ns	ns	ns	ns	***	ns	ns	***	ns	***	***	***	***	***	ns	*	ns	*	**	ns	***	ns	*	ns
22	0	0^	-	ns	ns	ns	ns	ns	ns	ns	***	*	ns	***	**	***	***	***	***	***	***	***	***	ns	***	ns	***	ns	ns	ns
23	0	0^	0	-	ns	ns	ns	ns	ns	ns	***	ns	ns	***	**	***	***	***	***	***	***	***	***	*	***	ns	***	ns	ns	ns
24	0	0^	0	0	-	ns	ns	ns	ns	ns	***	*	ns	***	*	***	***	***	***	***	*	***	***	*	***	ns	***	ns	ns	ns
25	0	0	0	0	0	-	ns	ns	ns	ns	ns	ns	ns	***	ns	*	***	***	**	***	ns	***	ns	ns	***	ns	***	ns	ns	ns
26	0^	1	0	0	0	0	-	ns	*	ns	**	***	ns	***	***	***	***	***	***	***	***	***	***	***	***	*	***	ns	ns	ns
27	0	0	0	0	0	0	0	-	ns	ns	***	*	ns	***	ns	***	***	***	***	***	***	***	*	ns	***	ns	***	ns	ns	ns
28	0	0	0	0	0	0	0^	0	-	ns	***	ns	ns	***	*	***	***	***	***	***	*	ns	ns	ns	***	ns	***	ns	ns	ns
29	0	1	0	0	0	0	0	0	0	-	***	ns	ns	***	**	***	***	***	***	***	*	***	***	***	***	ns	***	ns	ns	ns
31	1	1	1	1	1	1	1	1	1	1	-	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	ns	***	***
32	1	0	1	1	0^	0	1	0^	1	0	0	-	ns	***	ns	***	***	***	***	***	**	ns	**	ns	***	ns	***	ns	**	***
33	0	0	0	0	0	0	0	0	0	0	1	0	-	***	ns	***	***	***	***	**	ns	***	ns	ns	**	ns	***	ns	ns	ns
34	4	2	5	5	5	4	5	5	4	5	4	3	4	-	***	***	***	**	***	***	***	***	***	***	***	***	***	***	***	***
35	1	0	1	1	1	0	1	1	1	1	2	1	0	4	-	***	***	***	**	ns	ns	**	**	***	***	ns	***	ns	**	*
36	1	1	2	1	1	1	1	1	1	1	2	2	1	3	1	-	ns	***	***	***	***	***	***	***	***	***	***	ns	***	***
37	1	1	1	1	1	1	1	1	1	1	2	2	2	4	2	1	-	***	***	***	***	***	***	***	***	***	***	ns	***	***
38	2	1	2	2	2	2	2	2	2	2	2	1	2	1	2	1	1	-	***	***	***	***	***	***	***	***	***	***	***	***
39	3	0^	3	3	3	1	2	3	3	3	3	3	2	4	1	2	4	2	-	*	**	***	***	***	***	***	***	ns	***	***
40	1	1	2	2	1	1	2	1	1	2	2	1	1	5	0	2	2	3	1	-	ns	***	***	***	***	***	***	***	***	***
41	1	1	2	2	1	0	2	1	1	2	2	1	0	3	0	1	2	2	1	0	-	ns	**	***	***	ns	***	ns	***	**
42	1	0^	1	1	1	1	1	1	1	1	2	1	1	4	1	2	2	3	4	1	1	-	ns	ns	***	ns	***	***	***	***
43	0	0	1	1	1	1	1	1	0	1	1	1	0	4	1	2	2	2	3	1	1	0	-	ns	ns	ns	***	ns	***	***
44	0	0^	1	1	0^	0	1	0	0	1	1	1	0	4	1	1	1	2	3	1	1	0	0	-	**	ns	***	ns	**	**
52	2	1	2	2	2	1	2	2	2	2	2	2	2	5	2	3	4	4	5	2	2	1	1	1	-	***	***	***	***	***
59	0	0	0	0	0	0	0^	0	0	0	1	1	0	4	1	1	1	2	3	1	1	1	0	0	1	-	***	ns	ns	ns
60	1	2	1	1	1	1	1	1	1	1	2	3	1	6	2	2	2	3	4	3	3	2	2	2	2	1	-	***	***	***
61	0	0	0	0	0	0	0	0	0	0	1	0	0	2	0	1	1	1	0	1	0	1	1	0	1	0	1	-	ns	ns
62	0	0^	0	0	0	0	0	0	0	0	1	2	0	5	1	1	1	2	3	1	2	1	1	1	2	0	1	0	-	ns
63	0	0	0	0	0	0	0	0	0	0	1	0^	0	5	1	1	1	2	3	1	1	1	1	0^	2	0	1	0	0	-

Table 5-3. Summary of pairwise comparisons of allele frequency between all MDB lineage sites. The lower triangle presents the number of statistically-significant differences for $p < 0.05$. The upper triangle summarizes the statistical outcome of a global test across all polymorphic loci, based on Fisher's method (*** $p < 0.05$).

Site	1	2	3	4	5	6	7	8	9	13	14	15	16	17	18	19	20	21
1	-	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***
2	4	-	ns	ns	***	***	***	***	***	***	***	***	***	***	***	***	***	***
3	4	0	-	ns	***	***	***	***	ns	***	***	***	***	***	***	***	***	***
4	3	0	0	-	***	***	***	ns	ns	***	***	***	ns	***	***	***	***	***
5	5	3	1	1	-	**	***	***	***	***	***	***	***	***	***	***	***	***
6	2	3	2	1	1	-	***	***	***	***	***	***	***	***	***	***	***	***
7	3	3	4	4	5	3	-	***	***	***	***	***	***	***	***	***	***	***
8	3	3	1	0	2	3	4	-	ns	***	***	***	ns	***	***	***	***	***
9	4	1	0	0	2	2	4	0	-	***	***	***	ns	***	***	***	***	***
13	2	3	4	3	3	5	4	2	2	-	***	***	***	***	***	***	***	***
14	3	3	4	2	3	4	5	2	2	2	-	***	***	***	***	***	***	***
15	4	6	6	6	6	7	6	4	5	3	4	-	***	***	***	***	***	***
16	3	2	1	0	1	2	4	0	0	2	3	5	-	***	***	***	***	***
17	5	1	2	2	2	3	3	2	4	3	2	5	2	-	***	***	***	***
18	3	2	2	2	3	4	4	2	2	2	2	4	1	3	-	ns	***	***
19	4	3	2	2	4	5	5	3	2	3	3	4	2	4	1	-	***	***
20	4	4	3	3	4	5	5	2	3	3	2	4	2	3	2	2	-	***
21	3	5	5	5	4	6	5	4	4	1	4	3	4	4	3	3	3	-

Table 5-4. Summary of pairwise comparisons of allele frequency between all *N.* 'flindersi' sites. The lower triangle presents the number of statistically-significant differences for $p < 0.05$. The upper triangle summarizes the statistical outcome of a global test across all polymorphic loci, based on Fisher's method (** $0.001 \leq p < 0.05$; *** $p < 0.001$; ns = not significant; ^ found to be statistically heterogeneous). All significance values were Bonferroni-adjusted to correct for multiple tests.

Site	47	54	55	56	57	58	64	65
47	-	***	ns	*	ns	***	***	***
54	1	-	***	***	***	***	***	***
55	0	1	-	ns	ns	***	***	***
56	0^	2	1	-	*	***	***	***
57	1	1	1	1	-	***	***	***
58	3	3	3	3	3	-	***	***
64	2	1	2	2	2	4	-	***
65	4	3	4	4	4	2	2	-

Table 5-5. Summary of quantitative analyses of populations structure in *Nannoperca australis s.l.* [1]Nodes refer to those identified in Figure 5-5 (UPGMA dendrogram). [2]Mean and range (bracketed) for the number of statistically-significant differences in allele frequency detected among pairwise comparisons of populations (raw data in Tables). [3]Confidence intervals (99% CI) shown in brackets for F_{IS} and F_{ST} (** $p < 0.01$).

Hierarchical level	Node[1]	Sig. diffs[2]	F_{IS} (99% CI)[3]	F_{ST} (99% CI)
N. '*australis*'	A		-0.027 (-0.073 to 0.014)	0.544** (0.414 to 0.678)
all MDB	B+C	2.97 (0-7)	-0.010 (-0.079 to 0.061)	0.456** (0.340 to 0.571)
MDB - exclude sites 15,21	C	2.58 (0-5)	-0.013 (-0.088 to 0.063)	0.389** (0.261 to 0.492)
MDB - cluster of 6 similar sites	D	0.53 (0-3)	0.036 (-0.044 to 0.160)	0.127** (0.060 to 0.182)
all SEC/Tas	E	1.19 (0-6)	-0.043 (-0.090 to 0.010)	0.383** (0.276 to 0.493)
SEC/Tas - exclude sites 34,38,39,60	F	0.72 (0-4)	-0.027 (-0.081 to 0.035)	0.316** (0.151 to 0.442)
SEC - cluster of 15 similar sites	G	0.02 (0-1)	-0.001 (-0.078 to 0.096)	0.138** (0.061 to 0.240)
N. 'flindersi'	H	2.14 (0-4)	-0.069 (-0.148 to 0.064)	0.740** (0.113 to 0.864)
exclude sites 58,64,65	I	0.90 (0-1)	-0.074 (-0.158 to 0.071)	0.343** (0.031 to 0.584)
cluster of 4 similar sites	J	0.67 (0-1)	0.053 (-0.128 to 0.087)	0.162** (0.030 to 0.224)

6. REDISCOVERY OF THE SOUTHERN PURPLE-SPOTTED GUDGEON

6.1. INTRODUCTION

The southern purple-spotted gudgeon *Mogurnda adspersa* (Castelnau) is an attractive small fish (< 150 mm), being one of the first species to excite early naturalists and aquarists in Australia, and later abroad (Gale 1914; Freund 1918; Hale 1928). Wild fish display strong counter-shading, having a pale brown dorsal surface and iridescent blue flanks with brick-red spots. Spotting plus red stripes on the gills are diagnostic among Australian gudgeons (Allen *et al*. 2002). Colour intensifies and darkens during spectacular courting displays in spring and summer before pairs choose a nesting site. Between 200-1300 adhesive eggs are attached to solid surfaces which the male guards and fans until semi-pelagic larvae hatch after *c*. 10 days (Gale 1914; Blewett 1929; Llewellyn 2006). Populations currently assigned to *M. adspersa* are distributed along most of the eastern seaboard of Australia, stretching into the Murray-Darling Basin (MDB) west of the Great Dividing Range. Habitat is variable but predominantly restricted to smaller environments with dense physical and biological cover (Blewett 1929; Merrick and Schmida 1984; Moffat and Voller 2002; Pusey *et al*. 2004). Localised adult movements have been reported (Boxall *et al*. 2002; Llewellyn 2006).

Mogurnda adspersa is one of many freshwater fishes to have undergone a dramatic decline in the highly-modified MDB. At present, it is known to occur only in a few small populations in upper tributaries of the Darling River in New South Wales and Queensland (northern Basin). Once common, although patchy, across much of the southern Basin, including the Murray and Murrumbidgee rivers, the species disappeared by the early 1970s (Scott *et al*. 1974; Llewellyn 2006; Lintermans 2007). In the mid 1990s, a few individuals were discovered in the Cardross Lakes, Victoria, occurring in localised habitat separated from the main river (Raadik 2001). Following a decade of major water level lowering and salinisation, the population could not be secured and is now almost certainly lost. Recent molecular data indicate a east-west genetic divergence across the Great Dividing Range, implying added evolutionary and conservation significance for remnant MDB fish (Faulks *et al*. 2008).

This chapter reports on the re-discovery of *M. adspersa* in the lower River Murray in South Australia, at a site near the terminus of the system, some 2500 river kilometres from the nearest known population. The nature and basic ecology of the population are documented from field sampling, as part of evolving conservation measures in the face of unprecedented environmental

change. The combined effects of drought and water abstraction led to the probable extirpation of the wild population soon after its discovery.

The genetic focus of this chapter is more restrictive, concentrating on the MDB which nevertheless represents a large portion of the species range in southern Australia. Genetic characterisation using a combined nuclear and matrilineal analysis will also guide priority setting and recovery efforts for the newly discovered Lower Murray population. Given an elapsed 30-year period of sightings for a species subject to a reasonable level of scientific and recreational survey effort (Glover 1987; Lloyd 1987; Wedderburn 2000), a more recent origin involving deliberate re-introduction (translocation) had to be considered as a possible explanation. Furthermore, a translocated population of Darling catchment *M. adspersa* was known to exist in an artificial habitat only tens of kilometres from the wild population at the Murray Bridge Army Range (Pierce 1997; Hammer and Walker 2004), clouding any management response. As a recent molecular assessment indicated strong sub-structuring between the different Darling River catchments (Faulks *et al*. 2008), it was hypothesised that distinct gene pools exist for the extreme south and north of the MDB, with any close similarity likely to reflect translocation of northern derived fish.

6.2. METHODS

Study region

The lower River Murray, at the terminus of the MDB (Figure 6-1), is a deep channel carved through marine limestone over the last *c*. 1My (Twidale *et al*. 1978). The current environment contains the main river and laterally-connected wetlands that are inundated periodically but permanently connected artificially to the river *via* elevated water levels associated with weirs and barrages (Walker and Thoms 1993). The flow regime includes pronounced temporal variability, although the more recent trend has been for prolonged low flows owing to heavy abstraction (Maheshwari *et al*. 1995; Walker *et al*. 1995) and a severe drought (Figure 6-2). During the study, environmental conditions in the River Murray near Murray Bridge were characterised by low transparency from turbid water (colloidal particles), conductivity between 300-600 μS cm^{-1}, high pH and carbonate hardness, and temperatures between 8-20°C, with values fluctuating more widely in shallow wetlands. Winds in the region drive significant daily fluctuations in water level and available fish habitat, as much as 0.2 m. Northerly winds push water downstream, lowering levels in the wetland and southerly winds, which prevail in the evening, elevate wetland levels. The effect appears linked to water movement between the Lower Lakes and River Murray. With a 'normal' pool level in the Murray (*c*. 0.7 mAHD), these fluctuations basically inundate or expose fringing vegetation, with higher rivers inundating swampy areas.

Field sampling

Mogurnda adspersa was rediscovered in South Australia by Todd Goodman in late 2002, in a wetland on the Murray between Murray Bridge and Mannum. After initial correspondence, an *ad hoc* research and monitoring program was established by the author with appropriate permission (PIRSA exemption 9902081). Annual observations on the wild population were made during 2003-2006 and more frequently in 2007-2008, as water levels dropped dramatically.

Various sampling methods were trialled for capture, including those routinely used in baseline sampling along the River Murray (fyke nets, bait traps, back-pack electrofishing: Smith *et al*. 2007). However, only dip netting proved to be consistently effective at capturing the species. Night-time spotlighting was sometimes useful for determining continued presence, but was limited in effectiveness owing to low visibility. Sampling was always undertaken mindful that the population and habitat were highly restricted and vulnerable to interference (e.g. Knight *et al*. 2007). Fish were handled minimally and carefully (e.g. on wet surfaces) with total length measured and health condition visibly determined before a return to the point of capture. Fish were sexed based on males having a more robust body, rounded head, and thin and pointed

urogenital papilla (Llewellyn 2006). Examination of other suitable *M. adspersa* habitat was made opportunistically in the surrounding area and regionally between 2003 and 2007.

Conservation actions in response to falling water levels included some *in situ* measures by way of installing small steel cages (recycling containers) filled with rock and placed strategically in deeper sections of core habitat (although these too ultimately dried). Options to maintain water levels through artificial structures and pumping were considered by management (DWLBC and PIRSA Fisheries) but were thought likely to be difficult to achieve and ineffective in long-term conservation, and were not attempted. An *ex situ* captive maintenance program was established with fish rescued and transferred to aquaria in early 2007 (see Hammer 2007b).

Molecular approach and samples

The presence of an existing population genetic framework for *M. adspersa* in the northern MDB allowed a targeted investigation of the native or introduced status of the newly-discovered South Australian population (Faulks *et al*. 2008). Collaborative links were formed to extend this recent mtDNA analysis to wild and translocated populations of *M. adspersa* from the southern MDB, and allozyme electrophoresis was undertaken to provide a comparative nuclear data set. Hence the desired outcomes included confirmation that the SA population was part of the MDB genetic population, that it differed from known MDB locations and the translocated Army Range fish as the most likely source of any potential introduction, and that the distinctness and variability of the population could be characterised to assist in recovery efforts such as captive breeding.

Genetic material included fin clips from three wild South Australian fish collected in 2003, and rescued fish which died due to existing disease (2007). The latter were snap frozen in liquid nitrogen and stored at -70°C at the Australian Biological Tissues Collection (ABTC), South Australian Museum. A sample of translocated fish at the Murray Bridge Army Range wetland was collected on 1/8/2003, returned live to the laboratory, euthanased in clove oil and stored at -70°C. Several existing MDB collections of *M. adspersa* were available in the ABTC with varying numbers of fish for each of six populations. A small tissue sample was obtained from a single fish from Cardross Lakes in Victoria, preserved in alcohol and held at the Victorian Museum. A specimen fixed in alcohol in the late 1960s was obtained from a researcher that observed *M. adspersa* at sites in close proximity to the rediscovery (Murray Bridge) (S. Doyle pers. comm.). The method of preservation dictated the number of fish available for allozyme analysis. The location and sample sizes of populations for molecular analyses are shown in Figure 6-1 and Table 6-1.

Allozyme electrophoresis

All available frozen samples were screened at a large number of loci, maximising the amount of information that could be obtained for comparative purposes and increasing the likelihood of detecting rare or unique alleles for characterising within-population variation (i.e. covering elements of both an *overview* and *population* study: see Chapter 4).

Homogenates comprised a small piece of caudal muscle sonicated in an equal volume of buffered lysing solution (0.02M Tris-HCl, pH 8.0, with 0.2% 2-mercaptoethanol and 0.02% NADP). After centrifugation for 10 min. at 10,000 g, supernatant fluids were stored at -20°C as 10-20 μL aliquants in glass capillary tubes. Allozyme electrophoresis was conducted on cellulose acetate gels (Cellogel^{TM}), following Richardson *et al*. (1986). Thirty five enzymes or non-enzymatic proteins produced zymograms of sufficient intensity and resolution for genetic interpretation: ACON, ACP, ADA, ADH, AK, ALD, CA, CK, ENOL, EST, FDP, FUM, GAPD, GLO, GOT, GP, GPI, GSR, IDH, LDH, MDH, ME, MPI, NDPK, PEPA, PEPB, PEPD, PGAM, 6PGD, PGK, PGM, PK, SORDH, TPI and UGPP. Details of enzyme and locus abbreviations, enzyme commission numbers, electrophoretic conditions, and stain recipes are in Richardson *et al*. (1986) and Bostock *et al*. (2006). Allozymes were designated alphabetically and multiple loci, where present, were designated numerically, both in order of increasing electrophoretic mobility (i.e. *Sordh*^{a}, *Sordh*^{b}, *Acon1*, *Acon2*).

The genetic affinities of individuals were explored using principal co-ordinates analysis (PCO), implemented on a pairwise matrix of Rogers' genetic distance (Rogers 1972) using PATN (Pattern Analysis Package, DOS version, Belbin 1994). The genotypic data were examined for statistical evidence of any deviation from Hardy Weinberg expectations or linkage disequilibrium within populations, and any heterogeneity of allele frequencies between populations using pairwise comparisons for statistically-significant differences plus a global statistical test across all polymorphic loci, based on Fisher's method (Fisher 1948). These tests involved estimating exact probabilities using GENEPOP version 3.4 (Raymond and Rousset 2003), with all probability values adjusted for multiple tests using the sequential Bonferroni correction factor (Rice 1989). F-statistics were used to provide an overall measure of within site variability and between population divergence, and F_{IS} and F_{ST} values plus their associated 99% confidence intervals were calculated using the program FSTAT version 2.9 (Goudet 2000). Observed heterozygosity levels (H_O, direct count method) were calculated as a measure of within population diversity.

Mitochondrial DNA

The mtDNA data presented herein reflect a collaboration with Leanne Faulks, Macquarie University, who sequenced tissues, analysed data and assisted in the preparation of the methods and results sections, as an extension of a recent published study (Faulks *et al*. 2008). DNA was extracted, three gene regions of the mitochondrial genome amplified (339 base pairs of the control region and 802 base pairs of ATPase 6/8), and gene products were sequenced using an automated method. Data were cleaned, aligned and submitted to GenBank (Accession numbers: DQ219317-39). Phylogenetic reconstructions were carried out using maximum likelihood and neighbor joining methods whereby the strength of tree nodes was determined by bootstrap analysis, and tree constructed using the TVM as selected by Modeltest. Genealogical relationships within *M. adspersa* were investigated by constructing a haplotype network with the statistical parsimony method (see methods in Faulks *et al*. 2008).

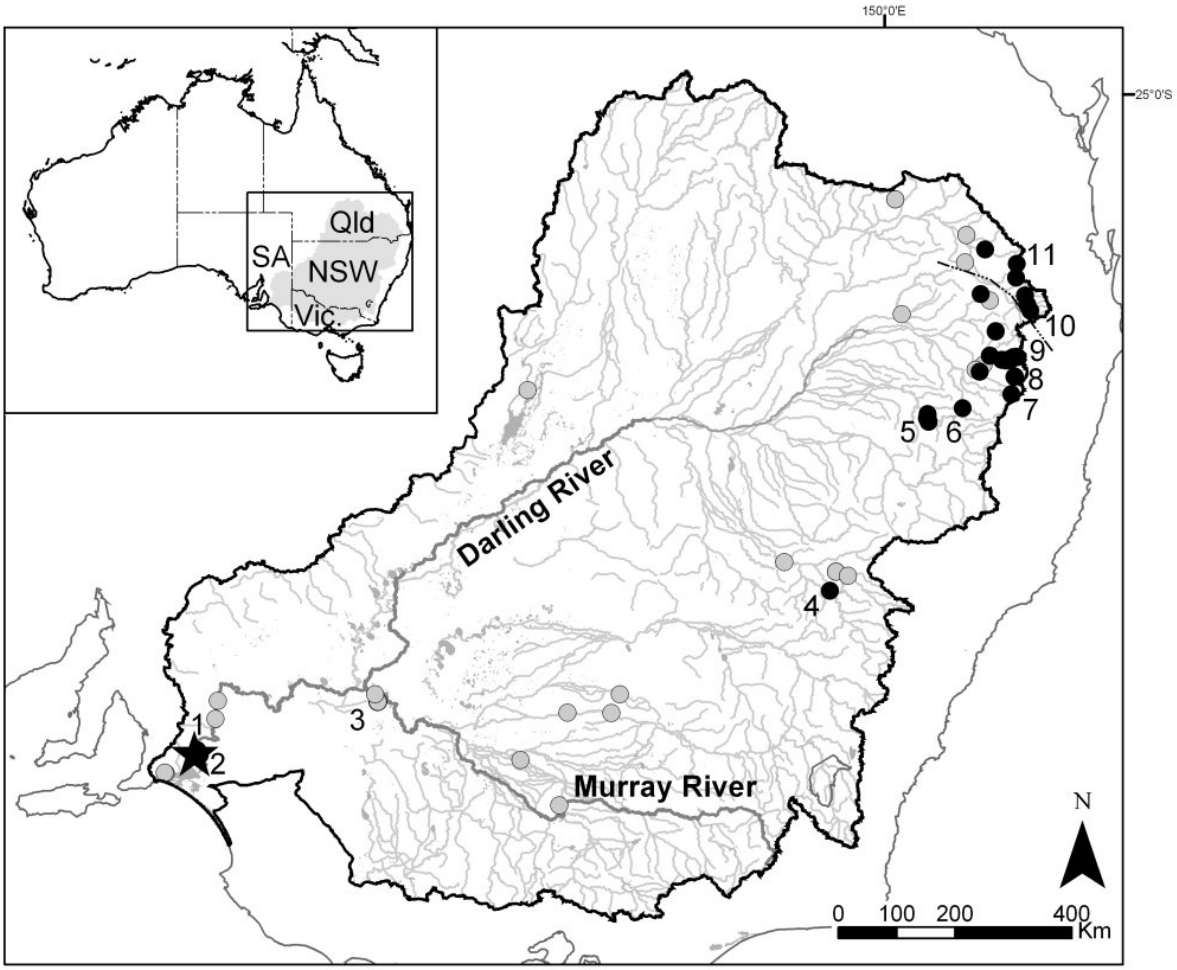

Figure 6-1. Distribution of *Mogurnda adspersa* in the Murray-Darling Drainage Division (Basin) including historic records (grey circles), extant populations (black circles) and with the star indicating the site of the population rediscovered on the lower River Murray, South Australia (adapted from Lintermans 2007). The dashed line reflects separate flow paths of proximate sites to the Condamine River or Border Rivers.

6.3. RESULTS

Population status

The distribution surrounding the initial point of capture was restricted to two inlets between the wetland and the main River Murray channel and a small nearby drain connected to the main channel (total river linear length of *c*. 600 m, estimated area of occupancy < 0.05 km^2). Searches at many other locations in the relevant reach of river, including historic known locations, did not expand the known range, and most apparently suitable habitat was eliminated by the near-complete loss of lateral connectivity (wetland drying) along the lower Murray below Blanchetown by summer 2007-2008. Over a five-year period, 160 *M. adspersa* were captured from the study wetland (this is likely to include repeat captures). Estimates as of January 2007 when habitat had just started to contract, allowing a more complete census, suggested that up to 100-200 fish may have been present at the main habitat with low numbers at the other two locations (certainly less than 500 adult fish with full habitat availability). By April 2008, no water remained in any previously occupied wetland habitat, and searches in the adjoining main river edge were unsuccessful, suggesting the extirpation of the population (functionally if not literally). Around 56 wild adults remain in captivity (aquaria), as the basis of a recovery program (see Hammer 2007b).

Biology

Fish were sampled from a variety of microhabitats. Core habitat prior to 2007 was banks reinforced with rock and comprising overhanging and emergent vegetation (grasses and *Triglochin*). Other dense stands of emergent macrophytes (e.g. *Schoenoplectus*) and submerged vegetation (*Vallisneria* and *Ceratophyllum*) also held fish. The two channel habitats recorded lower fish numbers and these had unidirectional flow through dense willows and high levels of underwater cover from woody debris and willow roots. As water levels fell throughout 2007, edge rock and emergent vegetation was isolated (critical level of 0.3 mAHD), with submerged aquatic vegetation and individual rocks becoming important cover. Aquatic vegetation was virtually eliminated *via* exposure/desiccation and bird feeding by late May 2007 (≤ 0.1 mAHD). Sampling continually investigated willow habitat lining the main channel of the River Murray near known habitat, but this remained unoccupied until late autumn 2007 (two individuals recorded), suggesting this is sub-optimal habitat.

Most adult and juvenile fish were captured in very shallow water (0.1-0.5 m depth). They appeared to have cryptic behaviour, occurring in dense cover and in areas otherwise difficult to sample. The position of capture points close to the main channel and providing low velocity water exchange, but never in stagnant areas of the wetland, suggests some preference for higher

dissolved oxygen or the food and habitat such conditions provide. Fish appeared to position themselves to actively hunt in shallow areas, especially at night, with a diet likely to comprise larger macroinvertebrates and small fish. An 80 mm male was captured with an unspecked hardyhead *Craterocephalus stercusmuscarum fulvus* in its mouth, and fish in captivity have good recognition and selectivity for small fish, glass shrimp (*Paratya*) and larger macroinvertebrates. *Mogurnda adspersa* was commonly recorded alongside various gudgeons (*Philypnodon* spp. and *Hypseleotris* spp.), *C. s. fulvus* and eastern gambusia *Gambusia holbrooki*. Fyke nets set near known edge habitat (day and night) seldom recorded individuals, suggesting minimal movement during more stable conditions. However, observations under conditions of exposed core habitat due to low water levels showed active lateral movement at a local scale in response to water level variation. Fish had a strong fidelity to preferred cover and moved actively back into local areas whenever water returned (fyke catches and night observations).

Demographic data were developed to examine life history and track population trends based on late summer/early autumn catches (n = 93 fish) (Figure 6-3). A population model indicates young of year fish (0+) as a cluster between 19-41 mm, with subsequent peaks showing a strong 1+ size grouping between 50-70 mm (out-rearing of captive spawned larvae in ponds matched this size grouping), through to possible successive generations up to 4+ or older fish appearing at intervals to a maximum recorded size of 100 mm. Recruitment was documented at the site in all years *via* the presence of small juveniles captured in late summer with a relatively tight band of juvenile (0+) fish followed by progressive peaks in length data. A particularly strong cohort evident in the 2007 catch data tracked to spawning in spring 2005. Examination of the hydrograph (Figure 6-2) distinguished subsequent summer and autumn 2005-2006 conditions of a higher minimum annual water level compared to preceding years (0.7 mAHD), and the extra inundation of habitat thus appears to have aided recruitment into the adult population. The sex ratio of the sampled wild population (and subsequently in captivity) was skewed 2:1 toward males. This may reflect sampling bias or a genuine pattern.

Fish health was examined by visual inspection. Initially, only low levels of external parasites were detected (i.e. occasional fish with the parasitic copepod *Lernaea*) and all fish were in a healthy state. As environmental stress developed in 2007, so did the incidence of diseased fish. In January 2007, one of 33 fish was diseased, with a large ulceration on the side of a 61mm male. A month later, as water levels became low (0.2 mAHD through most of the wetland) and exposure to warm summer conditions and increased inter-specific interaction occurred, the number of diseased fish increased dramatically to 16 of 42 fish. This included *Lernaea*, severe fungal infections, finrot and/or lesions.

Molecular data

Fifty putative loci were interpretable in the allozyme study, 35 of which (*Acp2*, *Acp3*, *Adh1*, *Adh2*, *Ak*, *Ald1*, *Ald3*, *Ca2*, *Ck*, *Enol*, *Fdp*, *Fum*, *Gapd*, *Got1*, *Gp*, *Gpi1*, *Gpi2*, *Ldh*, *Mdh1*, *Mdh2*, *Me1*, *Mpi*, *Ndpk1*, *PepA*, *PepB*, *PepD1*, *PepS*, *Pgam*, *Pgm2*, *6Pgd*, *Pgk*, *PGm-1*, *Pk1*, *Pk2* and *Ugpp*) were invariant amongst all 74 individuals screened (allozyme profiles are presented in Table 6-2). PCO grouped individuals to discrete clusters matching their geographic origins to the northern or southern MDB, with additional separation within the 'northern' population group aligning with a Condamine River *versus* Border Rivers split with only minimal overlap (Figure 6-4). Fish from the translocated Army Range population aligned with the northern MDB samples, with no indication of overlap in genotypic profiles with putative wild Lower Murray fish. Furthermore, examination of the raw allelic profiles identified alleles unique to the Lower Murray at five loci (*Acon2*, *Ada*, *Me2*, *Ndpk2*, *PepD2*), albeit in moderate to low frequency and subject to the caveat of small samples sizes for some northern MDB sites (Table 6-2).

Due to inadequate sample sizes for two populations (n = 1 for sites 5 and 9), quantitative statistical analyses were limited to a three way regional comparison of the Lower Murray (site 1), Condamine River tributary (site10), and a grouping of three Border Rivers sites (sites 6-8) combined due to their close geographic and genetic similarity (Figure 6-1 and Figure 6-4). There were no statistically-significant departures from the null expectation of panmixia under Hardy Weinberg expectations or linkage disequilibrium within any of the three regions. Subsequent statistical comparison revealed the three populations to have divergent allele frequencies (between four and five significant differences between pairwise comparisons: Table 6-3) and to have limited gene flow as indicated by large and significantly-positive F_{ST} values (Table 6-4). Heterozygosity estimates were low (population mean 0.023 ± 0.014), although the values for particular populations including the Lower Murray were moderate, with values > 0.04 (Table 6-2).

Twenty-six individuals from the southern MDB were incorporated into the broader mtDNA analysis of Faulks *et al*. (2008), increasing the final sample size to 92 fish. In total, nine haplotypes were identified in the MDB (Table 6-5), most of which were population specific, including two haplotypes specific to the southern MDB (H and I). This extended analysis supports the previous observation of moderate phylogeographic structure in the MDB, which notably includes division between the northern and southern populations (Table 6-5 and Figure 6-6). The translocated population in South Australia (Army Range) comprises a mix of northern MDB haplotypes (A, C and E) and thus has little similarity to wild populations in the southern MDB. The historic 1960s specimen did not amplify with the primers employed.

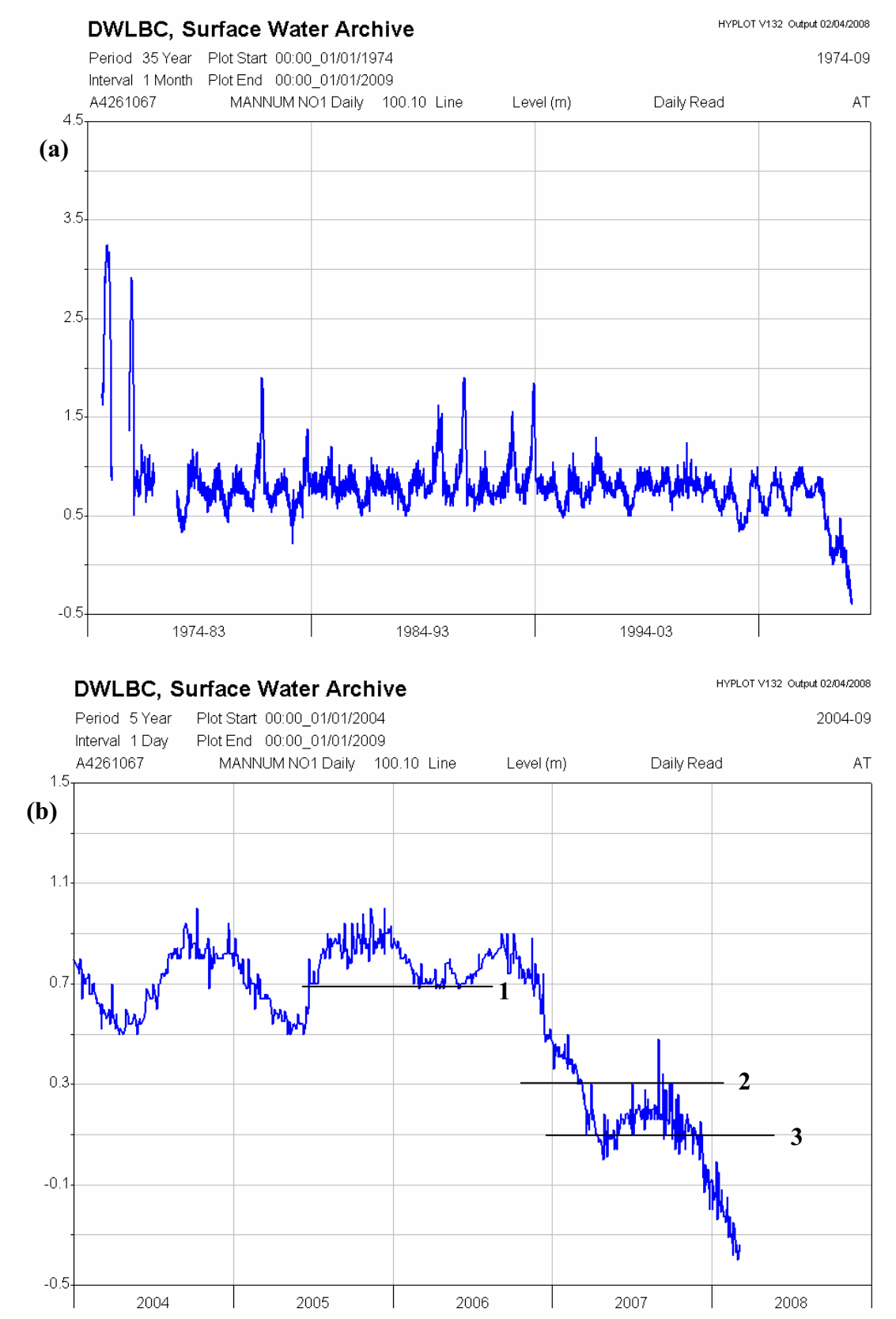

Figure 6-2. Representative hydrographs (water level in metres AHD) for the lower River Murray indicating (1) longer term 1974-2008 minimum levels and variability and (2) patterns for recent years 2004-2008 including dramatic water level decline (DWLBC 2008). Important water levels linking to field observations of *M. adspersa* biology are indicated: (1) elevated levels corresponding to a strong cohort, (2) loss of core habitat and (3) loss of wetland habitat.

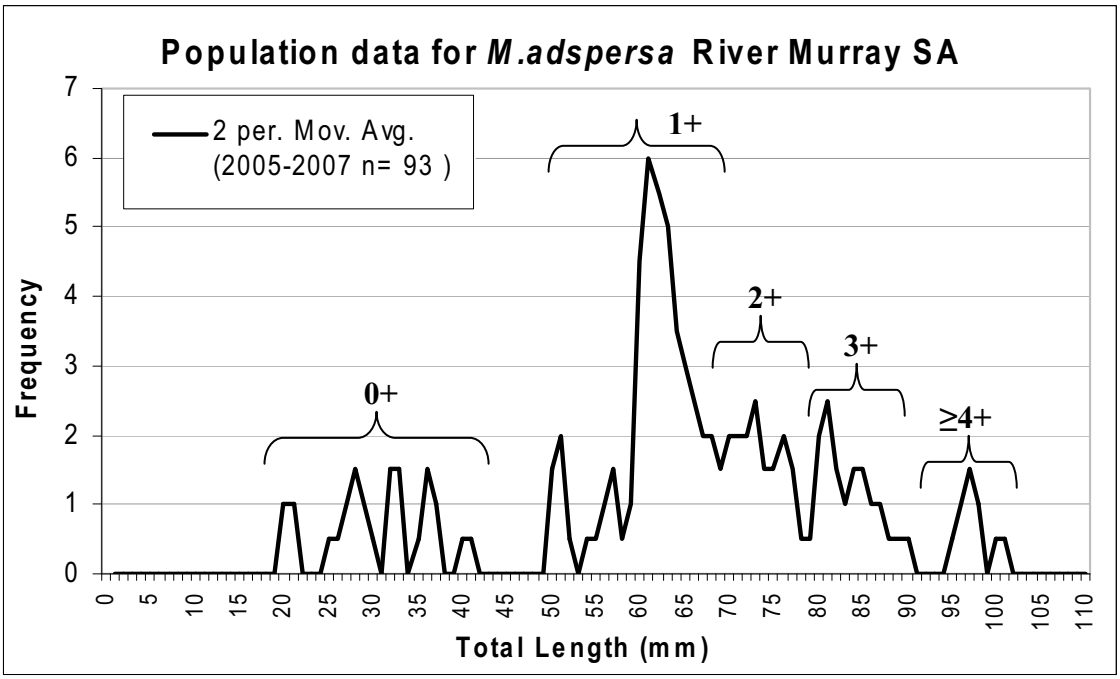

Figure 6-3. Demographic data collected for *M. adspersa* summer 2005-2007. Length data indicates a likely population model of: young of year (0+) fish ranging from 20-40 mm, 1+ fish 50-70 mm then less obvious older cohorts at intervals up to a maximum size of 100 mm.

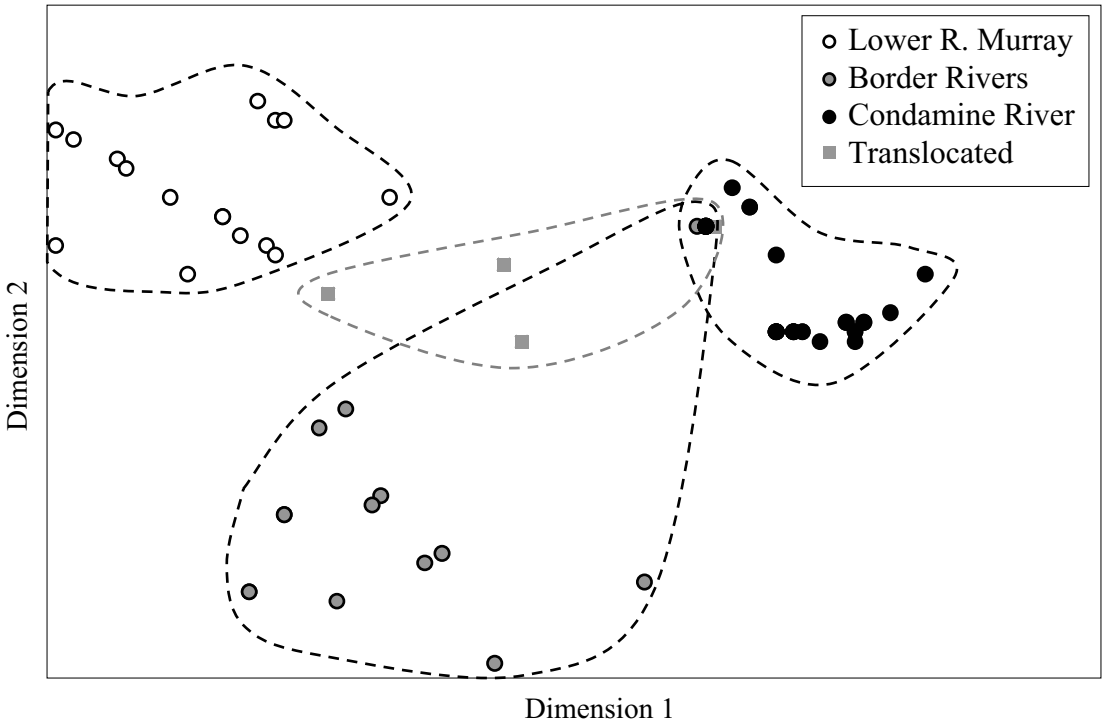

Figure 6-4. Principal Coordinates Analysis of the 74 specimens from the allozyme study. The relative PCO scores have been plotted for the first and second dimensions, which explain 38% and 23%, respectively, of the total variance. Geographic regions and the translocated population are coded with different symbols and highlighted with envelopes.

Figure 6-5. Maximum Likelihood tree showing geographic relationships among *M. adspersa* haplotypes from the Murray-Darling Basin, based on mtDNA control region and ATPase 6/8 sequence data. The out-group is a popualtion from the southeastern coast of Queensland. Bootstrap values greater than 50% are presented above the branches. Bold text indicates new haplotypes identified in addition to those presented in Faulks *et al*. (2008). Italicized text indicates the translocated population.

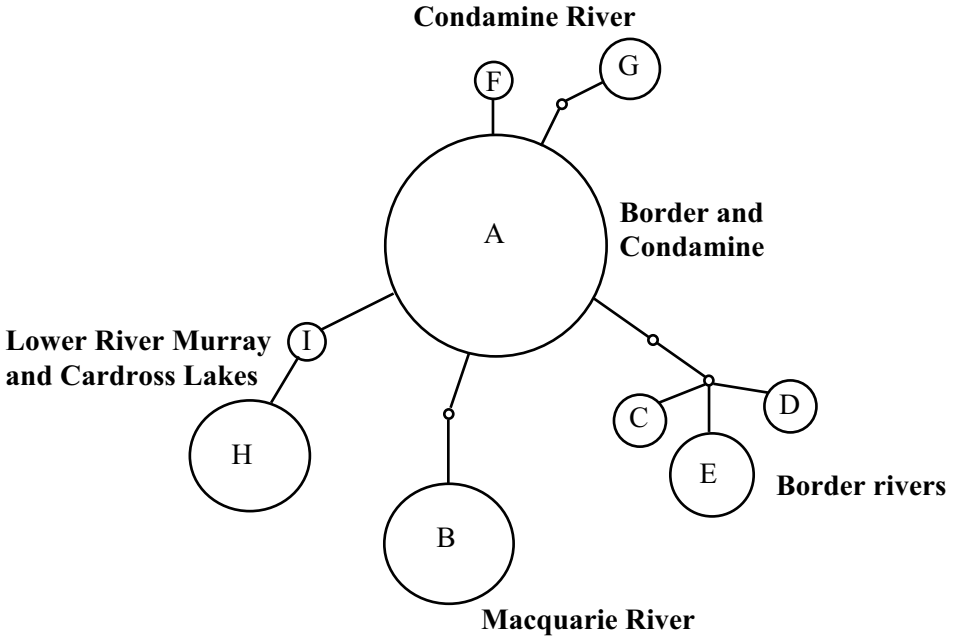

Figure 6-6. Network inferring the relationships among *Mogurnda adspersa* populations in the Murray-Darling Basin based on mtDNA control region and ATPase 6/8 haplotypes. Letters correspond to haplotypes in Table 6-5 and the size of circles reflects the overall frequency of each haplotype. Each discrete line indicates a single mutational difference, and small circles along lines represent missing haplotypes (not sampled or extinct).

6.4. DISCUSSION

Despite intensive surveys in this and other studies, southern Murray-Darling Basin *M. adspersa* are known from only one small wetland on the lower River Murray. The last verified record of *M. adspersa* in South Australia was in 1973 from near Blanchetown (SA Museum specimen), heralding a rapid decline of a once-common species (Glover 1987; Pierce 1997). The current finding of a remnant population is a significant discovery and a second opportunity for conservation in the Lower Murray. Combined ecological and genetic data were gathered at a critical point to inform future recovery. Both allozyme and mtDNA datasets provided no indication that the translocated population known to occur in the same region (Army Range) was the source of the wild fish, and instead revealed that southern MDB fish were genetically distinct when compared to those sampled from Darling River tributaries.

Taxonomic considerations

This chapter investigated population structure for all known extant locations within the MDB. While subtle genetic heterogeneity is evident, populations nevertheless appear to represent the same taxon. However, the taxonomic relationship of MDB *M. adspersa* to those found in other parts of its extensive range (most coastal rivers in Queensland) remains to be determined. There are strong indications that *M. adspersa* is a species complex: extensive levels of mtDNA divergence have been found between MDB fish and coastal Queensland (Faulks *et al*. 2008) and between different populations in Queensland coastal drainages (Hurwood and Hughes 1998; Faulks *et al*. 2008), while unpublished allozyme studies have revealed a suite of six diagnosable taxa among the populations surveyed thus far (M. Adams and colleagues, South Australian Museum, unpublished). Clearly, a comprehensive molecular genetic overview of *M. adspersa*, inclusive of populations from across the range, different river basins, and distinct parts of catchments (especially upland compared to lowland: Hurwood and Hughes 1998), is required to determine how many species are actually present.

Genetic sub-structure

The threatened nature of *M. adspersa* limited the availability of some samples in the allozyme analyses, nevertheless the combined genetic approach provided a complementary picture of genetic structure for extant populations and the presumed recently extinct Cardross Lakes population. Significant differences in allelic profiles for three regional comparisons concurred with divergent mtDNA haplotypes, and together these two independent genetic datasets confirm the presence of subtle phylogeographic structure comprising five MDB sub-populations. These include a southern MDB sub-population comprising the Lower Murray and Cardross Lakes.

Whether this gene pool extended across the whole southern Murray-Darling Basin, or was further sub-divided, could not be assessed herein due to a lack of contemporary samples suitable for allozyme and mtDNA analyses, from areas such as the Murrumbidgee River. More specialised techniques using primers designed for short but informative mtDNA portions may help in future to reconstruct the historic phylogeny based on historic museum specimens. The existence of regional populations displaying limited gene flow is not surprising for this species, given habitat specialisation and no evidence for large scale adult dispersal. Larvae are semi-pelagic for only a short period and there is informed speculation that spawning is restricted to low- or no-flow conditions (Llewellyn 2006), limiting dispersal of this life stage.

The overall observed levels of variation within populations (i.e. allozyme heterozygosity and haplotype diversity) were low but did vary between populations, suggesting varying population histories such as recent or historic fragmentation, differing patterns of colonisation or founder effects and contrasting effective population sizes. Consideration of these patterns rather than simply the overall divergence of regions and populations could also be an important part of genetic management practices such as restocking and translocation (Faulks *et al*. 2008). The Lower Murray site did display a higher level of allozyme diversity and numerous rare alleles, two genetic attributes likely to suffer as a consequence of the genetic bottlenecking accompanying the recent decline (if not extinction) in the wild, and which merit some consideration within the captive breeding and reintroduction program. This genetic variability may also be indicative of the adaptive potential which has allowed the persistence of this population until now.

Biogeographic patterns

While MDB populations are currently fragmented by significant habitat change and degradation, there is some suggestion of natural isolation between regional sub-populations in recent evolutionary history, based on distinctive mtDNA haplotypes and unique nuclear genetic components. Habitat specialisation and large distances of unfavourable habitat after the last glacial maximum *c*. 15-10 Kya may have contributed to divergence (e.g. drift and adaptation). Longer-term dispersal and colonisation routes or their converse, biogeographic barriers, require broader analysis across the Australian range of *M. adspersa*. Certain elements have already been hypothesised, including the role in waterfalls in limiting gene flow (Hurwood and Hughes 1998) and low divides allowing historic colonisation of the MDB across the Great Dividing Range (Unmack 1999; Faulks *et al*. 2008). A Lower Murray sub-population matches other species examined (Chapters 3, 4, 5 and 8), and suggests that some biogeographic phenomenon (e.g. possible periods of aridity or disconnection of the Murray: Chapter 5) lead to the divergence of local gene pools.

Ecology

A salient question is how the small lower Murray population of *M. adspersa* escaped extirpation when the species has disappeared across a much broader area. The local water-level fluctuation, providing flushing flows and oxygenation at regular intervals, is largely lacking from the heavily regulated, stable river environments upstream of Lock 1 at Blanchetown (Walker and Thoms 1993), and a rare combination of swamp habitat with dense physical and biological cove apparently presents ideal habitat for this cryptic species. Water-level data indicate that critical lows observed in 2007 have not occurred since the species was more common, in the 1970s (Figure 6-2), and this wetland habitat has been continuously available in the intervening period. Thus, features of local habitat and hydrology, with an element of chance, might have combined to present a refuge.

The field observations noting habitat specialisation and specific behaviour imply that the possible persistence of *M. adspersa* at other MDB locations cannot confidently be excluded; sampling requires specialised techniques and the species is sometimes hard to detect even in its known habitat. Consequently, previous baseline surveys or opportunistic collections in the region may have missed or overlooked the species, and targeted efforts should be continued. The prolonged and continuing low water levels and related habitat degradation do, however, reduce the prospects for other finds in this region.

The ecology of the southernmost population in the species appears similar to that observed elsewhere, in that there is a strong requirement for cover, both physical and biological, occurring in small or off-channel habitat (Moffat and Voller 2002; Pusey *et al*. 2004; Llewellyn 2005). Survival in cool winter temperatures (i.e. < 15°C) appears unique to the MDB (Briggs 1998), and a key reason for occurrence at the southernmost latitude may be the temperature buffer offered by the large River Murray volume (although shallow wetland areas would be cooler). Feeding, survival and reproduction in such high turbidity (low water transparency) are unique to lower Murray population and may represent a local adaptation.

The demographic data provide some interesting insights into local flow-ecology relationships. A tight band of juvenile fish followed by progressive peaks in length data suggests that while spawning could occur through summer (e.g. based on suitable warm conditions: Gale 1914; Blewett 1929; Legget and Merrick 1987), the bulk of successful recruitment occurs in a defined period (Llewellyn 2006). This likely corresponds to the onset of suitable warm temperatures for spawning in spring and water levels are generally higher (e.g. suitable sheltered areas and food for larvae). The strong presumed cohort of fish tied to protracted high water levels in late 2005

reflected a stronger Murray flow over a three-month period. Such responses, combined with moderate fecundity and repeat spawning (Gale 1914; Llewellyn 2006), indicate the potential for populations to rebuild, provided that favourable conditions return.

Conservation

The subtle genetic distinctiveness in nuclear and matrilineal data sets implies the presence of several discrete Management Units (MUs) within the MDB (Moritz *et al*. 1995). Of these, the Lower Murray MU is geographically and environmentally the most distinctive, and thus requires special conservation attention and management response.

The rediscovery of a remnant population of a presumed extinct species demonstrates that ecological assets can persist in highly-degraded systems. However, the rediscovery has coincided with unprecedented environmental change and rapid population decline, highlighting one of the key risks in working with endangered species. Despite the best efforts of recovery programs, when reduced to small single populations, chance can play a large role in conservation outcomes (Soulé 1987). In this case, factors such as skewed sex ratios, elimination of specific habitat and disease were observed, and an influx of predators, human collection for the aquarium trade and inappropriate research are among other factors that could easily contribute to local extinction. The current research program stemmed largely from the voluntary effort and dedication of individuals with very limited resources, rather than a decisive and intensive management response.

Captive maintenance has become a significant component of conserving Lower Murray *M. adspersa*. Undertaking transfer of fish into captivity is clearly most effective on a proactive basis to avoid periods when fish are already stressed. Fortunately, *M. adspersa* is a small species that adapts well to captivity, so preserving some part of the gene pool *ex situ* will be possible in the short term. Nevertheless, captive breeding programs have inherent risks such as gradual or chance population losses, and loss of genetic diversity and natural behaviour, and should not be seen as a long-term replacement for natural habitat, but a temporary measure to avoid catastrophe (Philippart 1995).

6.5. TABLES

Table 6-1. *M. adspersa* locality and sample size information for the allozyme and mtDNA studies.

Site	Field code	Locality	State	RB	Latitude (S)	Longitude (E)	*n* Allozymes	*n* mtDNA
1	F-FISHY4	Lower R. Murray	SA	26	35º03'	139º19'	19	16^
2	F-FISH98	Army Range*	SA	14	35º08'	139º21'	10	9^
3	NMV-A22791	Cardross Lakes	Vic.	14	34º18'	142º07'	-	1^
4	LF1	Wuluuman Ck	NSW	21	32º36'	149º04'	-	17
5	PU5	Halls Ck	NSW	18	29º52'	150º35'	1	14
6	F-FISH19	Inverell	NSW	16	29º47'	151º07'	4	4
7	PU97-38	Deepwater R.	NSW	16	29º18'	151º55'	5	3
8	PU4	Severn R.	NSW	16	29º34'	151º52'	3	2
9	PU3	Tenterfield Ck	NSW	16	28º59'	151º57'	1	17
10	PU97-41	Farm Ck	Qld	22	28º17'	152º10'	31	2
11	LF2	Toowoomba	Qld	22	27º33'	151º57'	-	7

*Known translocated population ^ Samples extending the analysis of Faulks *et al*. (2008)

Table 6-2. Allozyme profiles for MDB *M. adspersa* populations, sample sizes in parenthesis. Another 35 invariant loci are not included (see list in text).

	Southern MDB	Border Rivers					Condamine River	Translocated
Locus	1 (19)	5 (1)	6 (4)	7 (5)	8 (3)	9 (1)	10 (31)	2 (10)
Acon1	b	b	b	B	b	b	b^{96},a	b
Acon2	d^{94},b^{3},a	d	d	d	d	d	d	d
Ada	b^{91},c	b	b	b	b	b	b	b
Ca1	b	b	b^{87},a	b	b	b	b	b
Glo	c	c	c^{75},a	c	c	c	c	c
Got2	b	b	b	b	b	b	b^{84},a	b
Gsr	b	b	b	b	b	b	b^{97},c	b
Idh1	b	a^{50},b	a	a^{67},b	-	b	b	b^{95},a
Idh2	b^{97},a	a^{50},b	b	a^{90},b	a^{83},b	b	b	b
Me2	d^{50},b^{31},a	b	b	b	b	b	b	b
Ndpk2	b^{90},a	b	b	b	b	b	b	b
PepD2	c^{75},d	c	c	c	c	c	c	c
Sordh	a^{87},b	a	a	a^{50},b	a	b	b	b^{70},a
Tpi1	c	c	c	c^{90},d	c	c	c	c
Tpi2	c^{97},d	d	c^{50},d	d^{83},c	-	c	c	c
H_O	0.041	0.040	0.035	0.040	0.009	0.000	0.010	0.010
S.E.	0.018	0.028	0.023	0.019	0.009	0.000	0.010	0.008

Table 6-3. Summary of pairwise comparisons of allele frequency between the three major Murray-Darling Basin regions sampled in allozyme analyses. Sites with $n = 1$ were excluded. The lower triangle presents the number of statistically-significant differences for $p < 0.05$. The upper triangle summarizes the statistical outcome of a global test across all polymorphic loci, based on Fisher's method. (*** $p < 0.001$).

Region	Sites	Lower Murray	Border Rivers	Condamine
Lower Murray	1	-	***	***
Border Rivers	6/7/8	5	-	***
Condamine	10	4	4	-

Table 6-4. Summary of quantitative analyses of population structure among the three Murray-Darling Basin regions. [1]Mean and range (bracketed) for the number of statistically-significant differences in allele frequency detected among pairwise comparisons (raw data shown in Table 6-2). [3]Confidence intervals (99% CI) shown in brackets for F_{IS} and F_{ST} (** $p < 0.01$).

Comparison	Sites	Sig. diffs[1]	F_{IS} (99% CI)[3]	F_{ST} (99% CI)
Lower Murray *v.* Border Rivers *v.* Condamine River Basin	1, 6/7/8, 10	4.3 (4-5)	0.015 (-0.150 to 0.243)	0.595** (0.256 to 0.737)

Table 6-5. Frequencies of composite mtDNA haplotypes of *M. adspersa* in each sampling location, sample sizes in parenthesis. Haplotypes represent 1141bp of mtDNA sequence that includes 339bp of the control region and 802bp of ATPase 6 and 8 (GeneBank Accession numbers DQ219317-39)

	---- Southern MDB ----		**Macquarie R**	**---------- Border Rivers ----------**					**------ Condamine R ------**		**Translocated**
	1	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**2**
Haplotype	**(16)**	**(1)**	**(17)**	**(14)**	**(4)**	**(3)**	**(2)**	**(17)**	**(2)**	**(7)**	**(9)**
A				0.6				1.0	0.5		0.7
B			1.0								
C							1.0				0.2
D						1.0					
E				0.4	1.0						0.1
F									0.5		
G										1.0	
H	0.9	1.0									
I	0.1										

7. COMPARING CO-OCCURRING *PHILYPNODON*

7.1. INTRODUCTION

The genus *Philypnodon* forms part of a speciose eleotrid radiation (gudgeons or sleepers) in Australasia (Merrick 2006). Two species are described, both endemic to southeastern Australia: the flathead gudgeon *Philypnodon grandiceps* (Krefft) and the long-recognised (*sensu* Hoese and Larson 1980) but only recently described dwarf flathead gudgeon *P. macrostomus* Hoese & Reader (as discussed later, the name applies formally to only a small part of the range, but has been used for convenience herein to relate to all populations). *Philypnodon grandiceps* is among the most common of fishes in mainland southeastern Australia, being ubiquitous to coastal drainages from southeast Queensland through to the Light River in South Australia and the inland southern Murray-Darling Basin (MDB), with isolated occurrences also in the northern MDB and a few systems of northern and southern Tasmania (Larson and Hoese 1996; Hoese and Reader 2006). *Philypnodon macrostomus* has a similar but patchy distribution, although it is conspicuously absent from coastal systems west of Wilsons Promontory to the Murray Mouth and from Tasmania (Unmack 2001; Hammer 2002). Outlying populations of *P. macrostomus* in coastal catchments in the western range include the Hindmarsh River (Hammer 2006a) and the Onkaparinga and Torrens river systems, the latter possibly introduced (Hammer and Walker 2004). Similarly, recent additional records have been made for both *P. grandiceps* and *P. macrostomus* from the Condamine River, an upper Darling River tributary (M. Hutchison, pers. comm.).

Philypnodon species primarily occur in lowland freshwater environments including rivers, streams, wetlands and lakes, with common records also from saline freshwater habitats and estuarine areas (freshwater stragglers: Geddes 1987; Schiller *et al*. 1997; Hammer 2004). In more northerly drainages they are higher upstream in catchments (Pusey *et al*. 2004). Biological information is somewhat confused owing to the relatively recent recognition of *P. macrostomus* as a second, co-occurring congener. Both taxa spawn on hard sub-strata prepared and guarded by the male, with semi-pelagic larva hatching after 4-7 days during a protracted spring-summer spawning period (Llewellyn 1971; Legget and Merrick 1987; Gehrke 1992). *Philypnodon grandiceps* is the larger of the two species (maximum length 150 mm *v*. 65 mm) and is regarded as an ecological generalist with broad habitat and physiological tolerances, features attributed to the species flourishing in altered environments (Humphries and Lake 2000; Gehrke *et al*. 2002; Hammer 2004; McNeil and Closs 2007). *Philypnodon macrostomus* appears to have narrower habitat requirements, being more crypto-benthic in dense physical or biological cover, at least

within the MDB (Lloyd 1987; Hammer 2004). Large migrations of smaller *P. grandiceps* have been recorded at the freshwater-tidal interface (see Pusey *et al*. 2004).

Smaller individuals of the two species are similar in appearance, although colouration, head shape and morphology (e.g. mouth position and extent) are some of the characters useful for field identification. There has been no suggestion of hybridisation between the two species, although this is extensive in related groups across the same region (Bertozzi *et al*. 2000). Both *Philypnodon* species are considered widespread and secure (Larson and Hoese 1996; Allen *et al*. 2002), although there is some conservation recognition for *P. macrostomus* in the western range, where it is less common (Hammer 2004; Hammer and Walker 2004; Hammer *et al*. 2007a), and both are reported to have declined in the MDB (Schiller and Harris 2001).

Philypnodon species might be expected to display relatively modest genetic sub-structuring compared to the previously examined groups, based on their broad spawning period to broadcast semi-pelagic larvae, and occurrence in lowlands and estuaries, attributes likely to enhance exposure to drainage coalescence (and hence gene flow) during low sea-level or other phenomena such as freshwater flood plumes (Unmack 2001). Moreover, the subtle differences in distribution and habitat preference between *P. grandiceps* and *P. macrostomus* also argue for a null hypothesis that the two species will display differing levels and patterns of genetic divergence across their respective ranges, with the latter species showing more genetic sub-structure. However, a confounding factor may prove to be human-mediated gene flow, as *P. grandiceps* in particular is collected and transported as live bait, especially in western Victoria (e.g. see angling guides for that area).

7.2. METHODS

Sampling and analyses

Sampling was designed to cover the range of both nominal *Philypnodon* species, with maximal overlap to allow comparison of observed genetic patterns between the two species. For *P. grandiceps* this included coastal catchments north of the Fitzroy River (Queensland) through to the Light River (South Australia), in the southern MDB, and northern Tasmania. A sample was obtained from southern Tasmania (lower Derwent River), but only after all analyses were completed (Figure 7-1 and Table 7-1). Reports of the species from Kangaroo Island are erroneous (cf. Larson and Hoese 1996; Allen *et al*. 2002). *Philypnodon macrostomus* was sampled in: coastal catchments from north of Baffle Creek (Queensland) through to Wilsons Promontory (Victoria), with an additional recently-discovered population to the west, Lang Lang River, supplied by T. Raadik; South Australian Gulf Drainage Division (SAG), and the MDB, but mostly limited to the Lower Murray with attempts to locate and or source fish from the upper Murrumbidgee, Macquarie and Condamine rivers proving unsuccessful (Figure 7-2 and Table 7-2). A presumed *P. grandiceps* × *macrostomus* hybrid, detected visually during other sampling by the author at the Angas River mouth (South Australia), was also screened.

The analysis of *Philypnodon* incorporated the principles of an *overview study* to examine species boundaries and broad population structure, and thereby incorporated a large number of loci, small sample sizes per locality, and numerous localities spread across the geographic range (as recommended by Richardson *et al*. 1986). Where possible, sample sizes for genetically distinctive sites identified in initial gel stages were increased to $n = 5$.

Allozyme electrophoresis

Homogenates comprised a small piece of caudal muscle sonicated in an equal volume of buffered lysing solution (0.02M Tris-HCl, pH 8.0, with 0.2% 2-mercaptoethanol and 0.02% NADP). After centrifugation for 10 min at 10,000 g, supernatant fluids were stored at -20°C as 10-20 μL aliquants in glass capillary tubes. Allozyme electrophoresis was conducted on cellulose acetate gels (Cellogel^{TM}), following Richardson *et al*. (1986). Thirty-five enzymes or non-enzymatic proteins produced zymograms of sufficient intensity and resolution to permit allozymic interpretation: ACON, ACP, ADA, ADH, AK, ALD, AP, CA, CK, ENOL, EST, FDP, FUM, GAPD, GDA, GLO, GOT, GP, GPI, GSR, IDH, LDH, MDH, ME, MPI, PEPA, PEPB, PEPD, PGAM, 6PGD, PGK, PGM, PK, TPI, and UGPP. Details of enzyme and locus abbreviations, enzyme commission numbers, electrophoretic conditions, and stain recipes are in Richardson *et al*. (1986) and Bostock *et al*. (2006). Alphabetic and numerical designations were assigned to

allozymes and multiple loci respectively, both in order of increasing electrophoretic mobility (i.e. *Acon*[a], *Acon*[b], *Adh1*, *Adh2*).

Data analysis

The initial unit for analysis was individual specimens, with genetic affinities explored using stepwise principal co-ordinates analysis (PCO), implemented on a pairwise matrix of Rogers' genetic distance (Rogers 1972) using PATN (Pattern Analysis Package, DOS version, Belbin 1994). Homogenous genetic groupings from a site were treated as a distinct Operational Taxonomic Unit (OTU). Two between-OTU estimates of genetic similarity were calculated, namely (1) percentage fixed differences (%FD, Richardson *et al*. 1986), allowing a 10% tolerance, and (2) Nei's unbiased Distance (Nei D, Nei 1978). The genetic affinities of OTUs or populations were displayed visually as UPGMA (unweighted pair-group method of arithmetic averages) dendrogram and neighbor joining (NJ) networks constructed from Nei D values using the NEIGHBOR routine in PHYLIP 3.5c (Felsenstein 1993) and drawn using TREEVIEW 1.6.0 (Page 1996). Allele frequencies, heterozygosity levels (H_O, direct count method) and genetic distances were calculated using BASIC programs written by M. Adams.

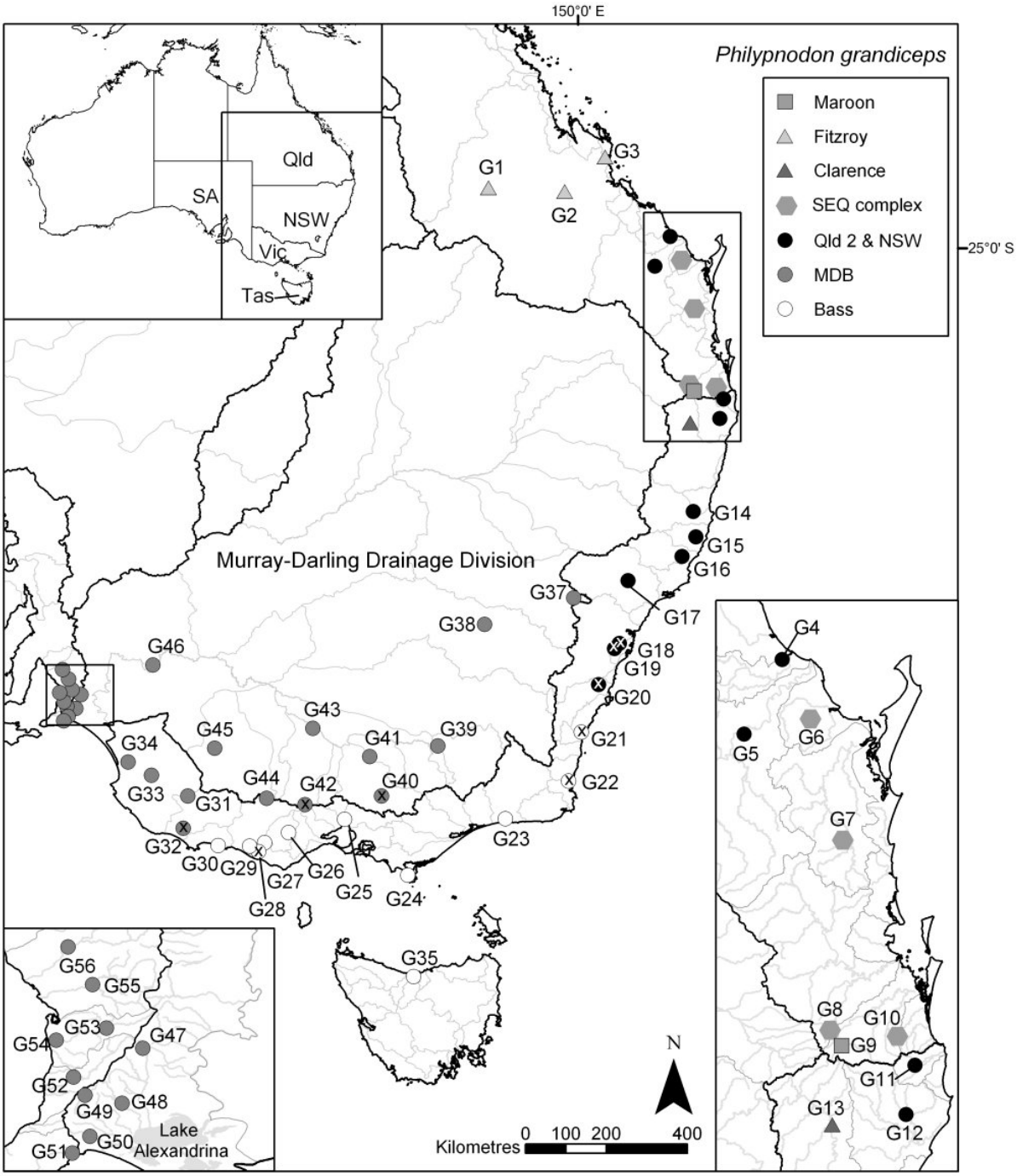

Figure 7-1. Geographic relationships of *Philypnodon grandiceps* samples subject to allozyme electrophoresis. Also shown are major drainage divisions and river basins (AWRC 1976). Sites codes as per Table 7-1. Four taxa are denoted with different symbols, and sub-groups with different shading, codes as per the text (the southern taxon is represented with circles). Sites marked with (x) indicate populations with genetic interchange between sub-groups.

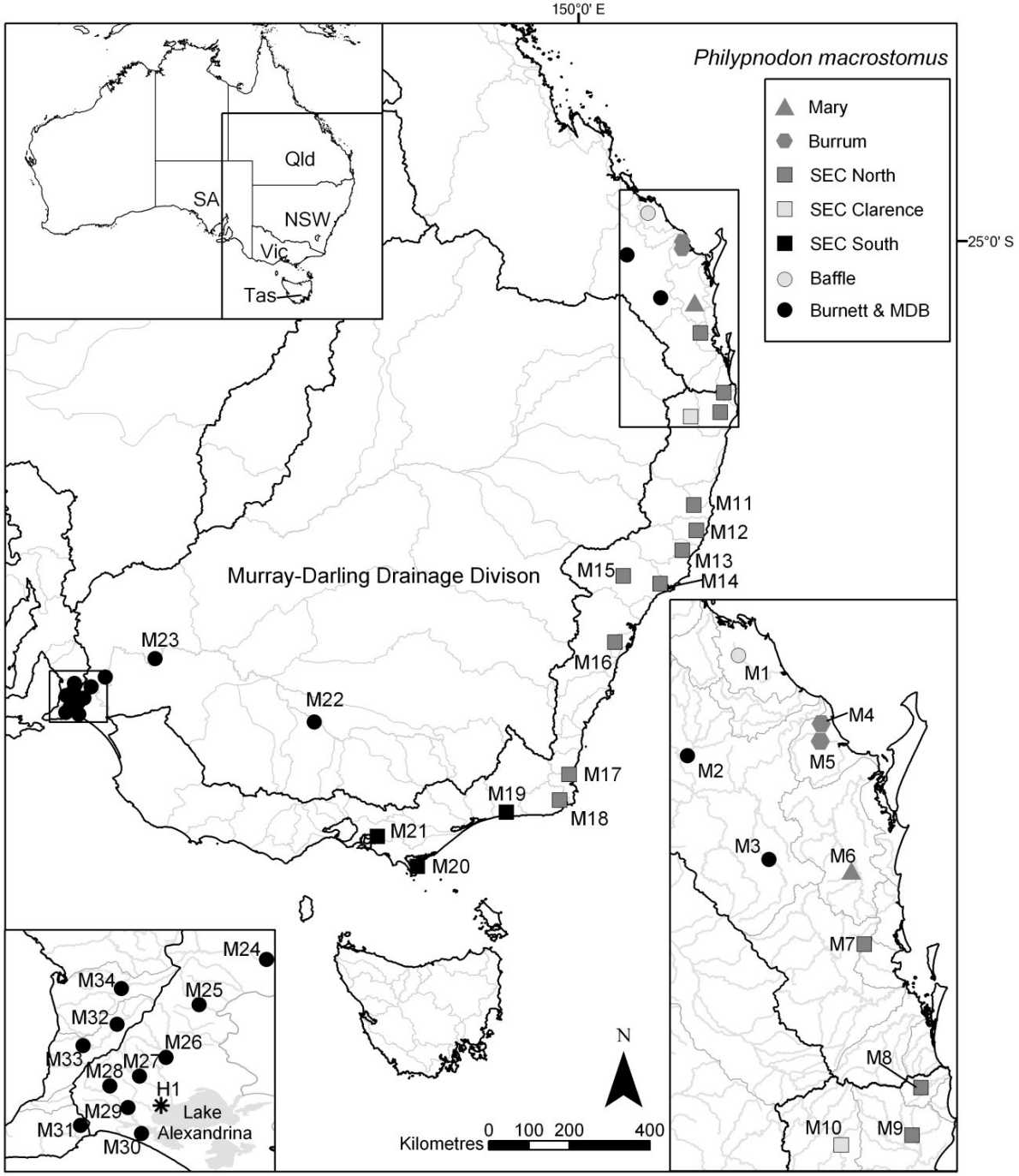

Figure 7-2. Geographic relationships of *Philypnodon macrostomus* samples subject to allozyme electrophoresis. Also shown are major drainage divisions and river basins (AWRC 1976). Sites codes as per Table 7-2. Four taxa are denoted with different symbols and sub-groups with different shading, codes as per the text (the SEQ/MDB taxon is represented with circles). Site H1 marked with (✳) indicates a hybrid individual between *P. macrostomus* and *P. grandiceps*.

7.3. RESULTS

Between-species comparisons

Some 269 fish were successfully scored at 49 putative loci including 146 *P. grandiceps* (55 sites), 122 *P. macrostomus* (34 sites) and a putative hybrid (site H1) (see Appendix 4 for allozyme profiles). The larger number of sites for *P. grandiceps* reflects its wider distribution, but comparative coverage within the range of *P. macrostomus* was reasonable, with 15 locations where the two were collected sympatrically and another six instances where they were collected from the same river basin (cf. Figure 7-1 and Figure 7-2).

An initial PCO (not shown) grouped individuals to one of two well-separated and discrete clusters (i.e. matching the nominal species), with a single intermediate individual corresponding to the suspected hybrid individual (its allozyme profile was consistent with it being an F_1 hybrid, see Appendix 4). The primary *P. grandiceps versus P. macrostomus* divergence is large (51 %FD, Nei D = 0.87), as illustrated by a UPGMA dendrogram among all non-hybrid populations (Figure 7-3).

Both the UPGMA dendrogram and the NJ tree (not shown) reveal that the two species harbour similar levels of within-taxon genetic diversity. More significantly, they also share two unexpected biographic patterns, both at odds with any null hypothesis that each represents a single evolutionary species. First, the most genetically divergent sites are those from the northeastern portion of their respective geographic ranges (i.e. sites G1-13 in *P. grandiceps* and sites M1-6 in *P. macrostomus*: Figure 7-1 to Figure 7-3). Second, in both cases these northeastern sites comprise a mosaic of some very distinctive populations and others that display clear genetic affinities with southern populations (e.g. northern sites G4-5 in *P. grandiceps* and sites M1-3 in *P. macrostomus*: Figure 7-1 to Figure 7-3).

Major genetic groupings in P. grandiceps

An initial PCO on all 146 individuals (not shown) failed to unequivocally identify any well-defined genetic groupings in the first two dimensions. Such a result could, in principle, reflect (1) the presence of a large number of diagnosable but similarly-distinctive genetic lineages (which become resolved only in deeper dimensions), (2) complex patterns of genetic admixture among several otherwise genetically-distinctive lineages, or (3) an absence of significant genetic sub-structure (Horner and Adams 2007). The presence of multiple fixed differences among most northern sites (Appendix 4) plus non-trivial genetic distances and obvious population sub-structure involving many sites (Figure 7-3) ensures that only (1) and (2) apply here.

It is clear that the genetic affinities among populations of *P. grandiceps* are too complex for stepwise PCO to identify lineages from first principles (i.e. starting with all individuals). Instead, two different regional stepwise PCOs were carried out, one restricted to all east coast populations north of and including the Clarence River (sites G1-13), and the other focussing on the 'southern' region (sites G14-G55, all coastal sites south of the Clarence River plus inland MDB), but also including the four northern sites with obvious genetic affinities to southern populations (site G5-6 and G11-12: Figure 7-3).

A PCO of the 52 fish representing the 13 northern sites identified four major genetic groupings and several distinctive sub-groups, with no intermediate or hybrid forms (Figure 7-4; Appendix 4). These taxa and sub-groups (denoted with letters) were: (1) Maroon Dam (Logan River: site G9), (2) Clarence/Fitzroy (a = site G13, Clarence River; b = sites G1-3, Fitzroy River), (3) a composite of divergent sub-groups from Southeastern Queensland, herein 'SEQ complex' (a = site G8, upper Brisbane River (Moogerah Lake); b = site G10, Coomera River; c = site G6, Isis River; d = site G7, Mary River), and (4) 'southern' sites G5-6 and G11-12 from proximate river basins in Queensland and northern NSW respectively (Figure 7-1). All were diagnosable by at least two fixed differences (i.e. at least 4%FD, 73% of pairwise comparisons ≥ 10%FD) and often harboured autapomorphic or private alleles rather than just alternate genotype frequencies for widespread alleles (Table 7-3 and Table 7-4). Maroon Dam in particular was distinctive, showing at least five and up to 15 fixed differences (10-31%FD) from all other groupings, some involving alleles not detected in other populations (fixed for $Acon^{e}$ and Acp^{a}; near fixed or shared with one other population for $Enol^{b}$, $PepD2^{e}$, $Pgam^{b}$, $Pgm2^{g}$). Such differentiation, which holds even at the local scale between river basins, is also apparent in the UPGMA dendrogram of Figure 7-3. No clear geographic pattern is evident, as group 4 is interspersed within the other three groups, and sites at the extreme ends (i.e. Clarence and Fitzroy) belong to the same taxon (Figure 7-1 and Figure 7-3).

The second PCO focused on the 'southern' region (or taxon), including sites from the remainder of the range (all coastal sites south of the Clarence River plus inland MDB) plus those identified in major grouping 4 above. These latter four sites are a natural cluster (Figure 7-4), and their inclusion also allows the 'northern' and 'southern' analyses to be cross-referenced. Four diagnosable sub-groups emerge in this analysis, although there are nine sites displaying the genetic characteristics of between-group admixture (Figure 7-5 and Table 7-3). The distribution of the four sub-groups (excluding the nine genetically-intermediate sites) follows a neat geographic distribution: (4a) the two Queensland sites G5-6 (herein 'Qld2' sub-group), (4b) coastal NSW from site G11-20 excluding the Clarence, site G13 (herein 'SEC north' sub-group),

(4c) the MDB, SAG, and Millicent Coast and Glenelg river basins (herein 'SEC south' sub-group), and (4d) coastal Victoria from site G21 through to the Shaw River (site G30), plus Tasmania (site G35) (Bass Strait drainages, herein 'Bass' sub-group). Areas of admixture include proximate coastal sites at the boundaries between 4b and 4d (sites G18-22), between 4c and 4d (site G32 and 38), and across inland divides between 4c to 4b (site G18) and between 4d to 4c (sites G40 and 42) (Figure 7-1 and Table 7-1).

In summary, *P. grandiceps* has complex genetic structure involving four major genetic groups which can be split into eight diagnosable populations in the northern part of its range, plus a widespread 'southern' taxon which encompasses three sub-groups plus a number of genetically-intermediate sites in regions where the geographic distribution of these sub-groups abut (i.e. 11 diagnosable groups in total: Figure 7-3 and Table 7-4).

Major genetic groupings in P. macrostomus

An initial PCO of all *P. macrostomus* individuals identified four major groups (Figure 7-6), all diagnosable by two or more fixed differences and with no intermediate or hybrid forms (Table 7-5 and Table 7-6). A clear outlier is the Mary River (site M6), although the two-dimensional PCO belies its distinctness in the third dimension (not shown), displaying 6-9 fixed differences (13-19%FD) from all other sites and autapomorphic alleles at four loci (*Gpi2*^{c}, *Idh1*^{b}, *PepB2*^{b}, *Pgm2*^{f}: Table 7-5). A second major genetic group from the Burrum River Basin (herein Burrum taxon) was represented by sub-groups from (a) the distinctive Gregory River (site M5) and (b) Elliot River (site M4). These are portrayed as the next most-basal cluster (after the Mary) in the UPGMA dendrogram of Figure 7-3. The two additional major groups included: (3) a southeastern Queensland and MDB taxon (herein SEQ/MDB) represented by the sub-groups (a) Baffle Creek (site M1) and (b) widespread populations on both sides of the Great Dividing Range, namely sites M2-M3 (Burnett River Basin) and M22-34 (MDB/SAG), and (4) a broad coastal grouping south of the Clarence through to Lang Lang River (sites M6-21: herein SEC).

Variation within the widespread SEC taxon was explored with a second PCO, and this distinguished (a) the Clarence River (herein 'SEC Clarence'), and identified a more subtle split (i.e. no fixed differences but a reasonably high Nei D: Table 7-6) between (b) southern sites (sites M19-21, herein 'SEC south') and (c) northern sites, herein 'SEC north' (Figure 7-7). Hence four major groups with seven sub-groups were recognised within *P. macrostomus* (i.e. eight diagnosable, or near-diagnosable (sites M19-M21), groupings: Figure 7-3).

Genetic sub-structure within major groups

Although the overview study provides clear evidence of numerous major genetic divisions and further sub-groupings (lineages) within both *P. grandiceps* and *P. macrostomus*, the small sample sizes employed places major limits on how much information can be obtained on within-group genetic diversity. Certainly both nominal species display significant sub-structure in the northern part of their coastal range, with contrasting broad and relatively homogenous groups in the south, especially in the MDB. Indeed, many groups comprise a single site, sampled for only three to five individuals, and thus between-site measure of genetic diversity are not assessable. Only three sub-groups in each of *P. grandiceps* and *P. macrostomus* are represented by more than two sites, and in most cases only 1-3 individuals per site have been characterised.

It nevertheless remains instructive to assess levels of within-site variability, since these are largely unaffected by small sample sizes (Nei 1978). Comparison between MDB lineages of each species reveals that they harbour quite different levels of within site variability (H_O = 0.063 ± 0.021 for *P. grandiceps* and 0.017 ± 0.012 for *P. macrostomus*). Overall, H_O values in the 11 'pure' sub-groups identified within *P. grandiceps* were low to moderate (range 0.017 to 0.067), whereas generally higher values were present in the proposed sites of admixture (range 0.057-0.104) when compared to their putative pure parental forms (Table 7-3), thus supporting the hypothesis that these sites are zones of admixture. The MDB was one of few regions with low H_O values in *P. macrostomus*; these values were otherwise moderate to high, especially for central coastal sub-groups (Clarence and northern SEC) and the Elliot River site (0.070-0.080: Table 7-5).

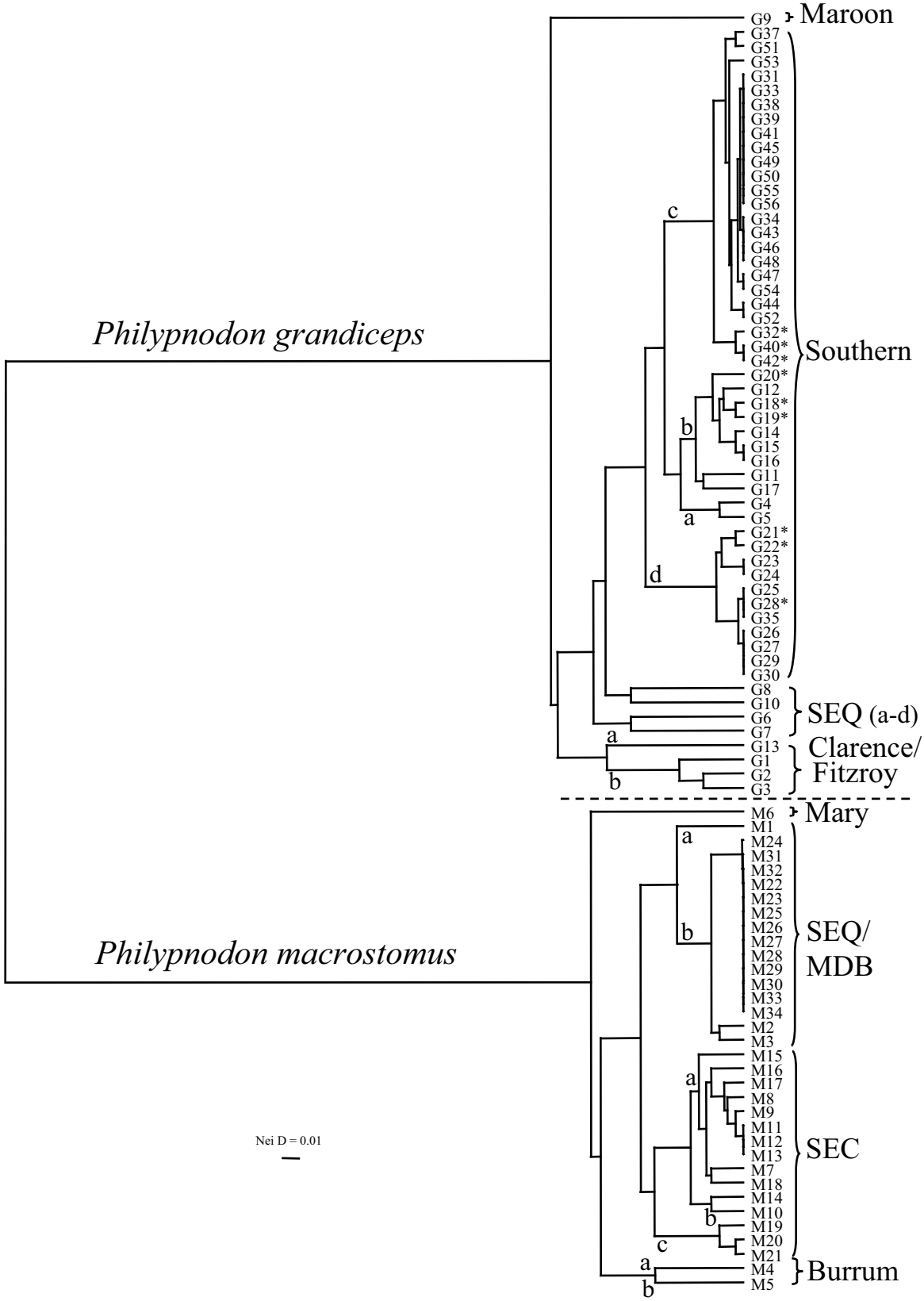

Figure 7-3. UPGMA dendrogram depicting genetic affinities between and within *Philypnodon grandiceps* and *P. macrostomus* based on pairwise Nei Distance values. Major genetic groupings (taxa) are labelled (e.g. Maroon) and letters (a-d) indicate sub-groups or lineages within taxa (e.g. Burrum is represented by two lineages (a) Gregory and (b) Elliot). * indicates populations with genetic exchange across sub-groups.

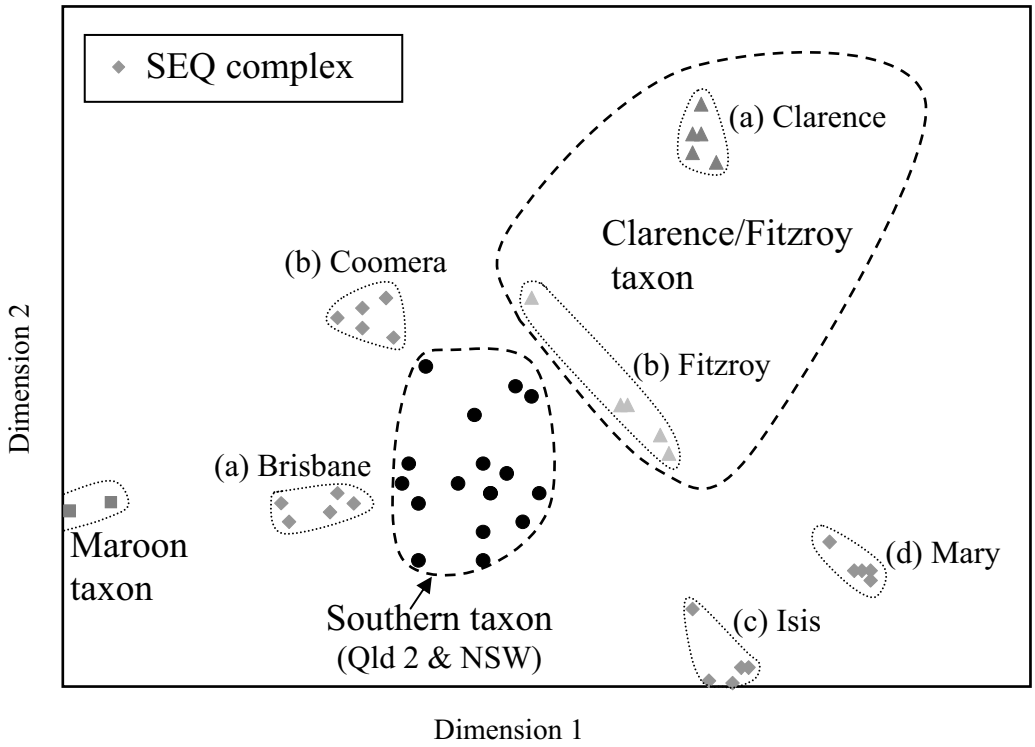

Figure 7-4. Principal Coordinates Analysis of 52 individuals representing 13 northern populations of *P. grandiceps*. The relative PCO scores have been plotted for the first and second dimensions, which explain 22% and 12%, respectively, of the total variance. Shown are major genetic groupings (taxa) and sub-groups, names match Figure 7-1 and Figure 7-3.

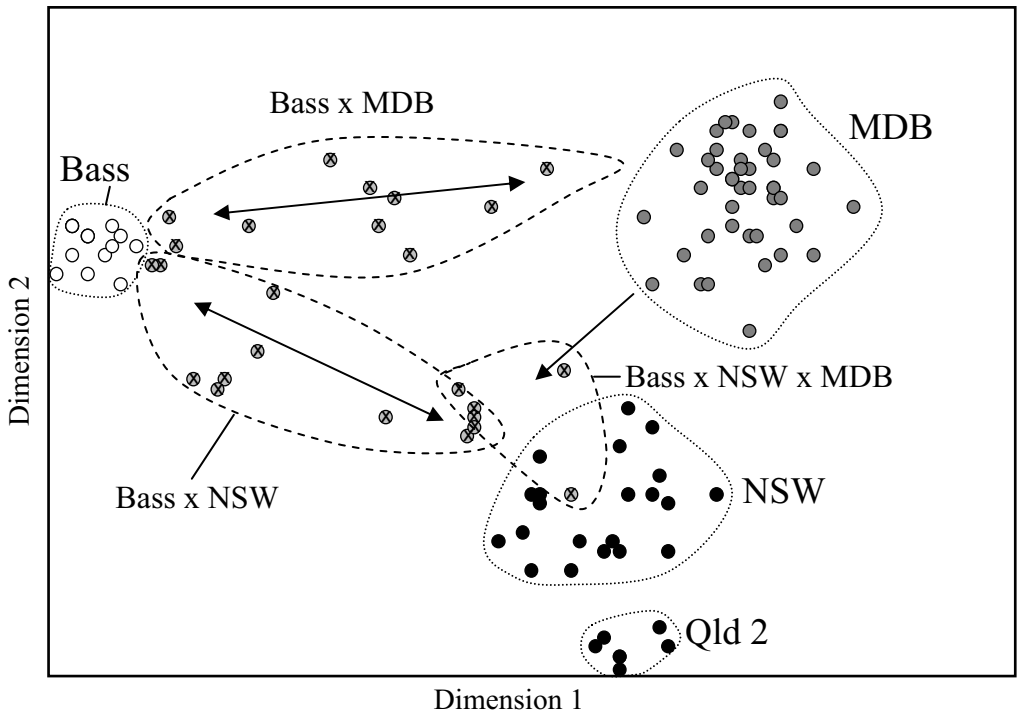

Figure 7-5. Principal Coordinates Analysis of 111 individuals representing 43 southern populations of *P. grandiceps* and four key northern sites. The relative PCO scores have been plotted for the first and second dimensions, which explain 25% and 21% of the total variance, respectively. Names for major genetic groupings match Figure 7-1 and Figure 7-3 (sites codes are found in the latter). Sites of admixture between major groupings are highlighted (x).

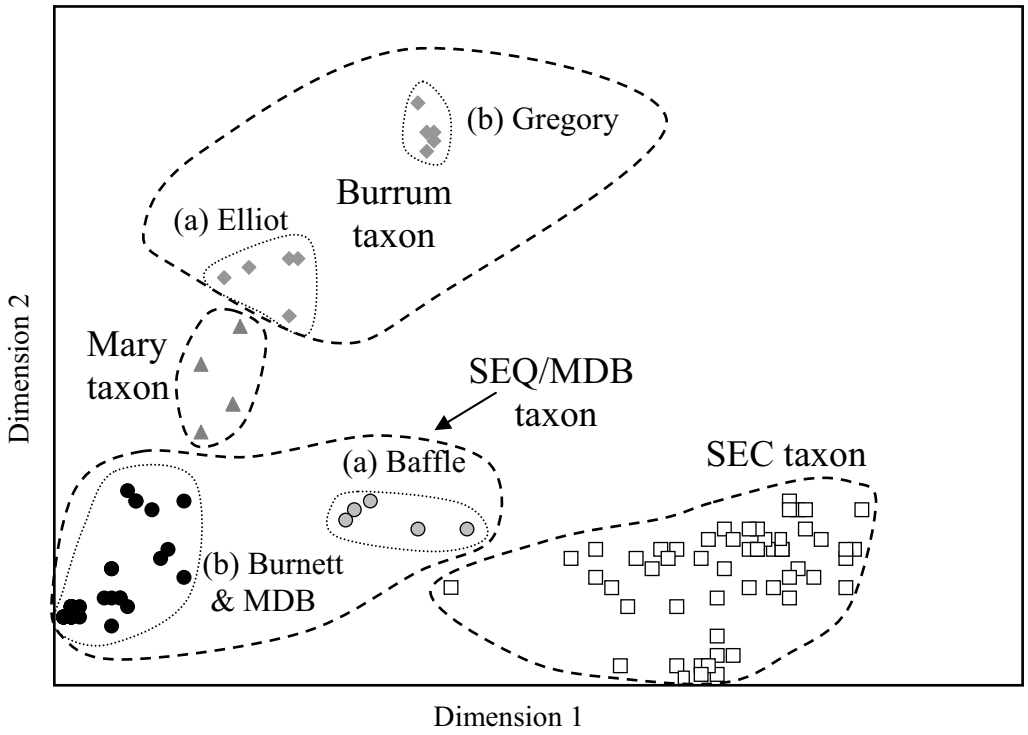

Figure 7-6. Principal Coordinates Analysis of 122 individuals representing 34 populations of *P. macrostomus*. The relative PCO scores have been plotted for the first and second dimensions, which explain 27% and 12% of the total variance, respectively. Shown are major genetic groupings (taxa) and sub-groups, names match Figure 7-2 and Figure 7-3. Note the Mary major grouping is distinctive in a third dimension of the ordination.

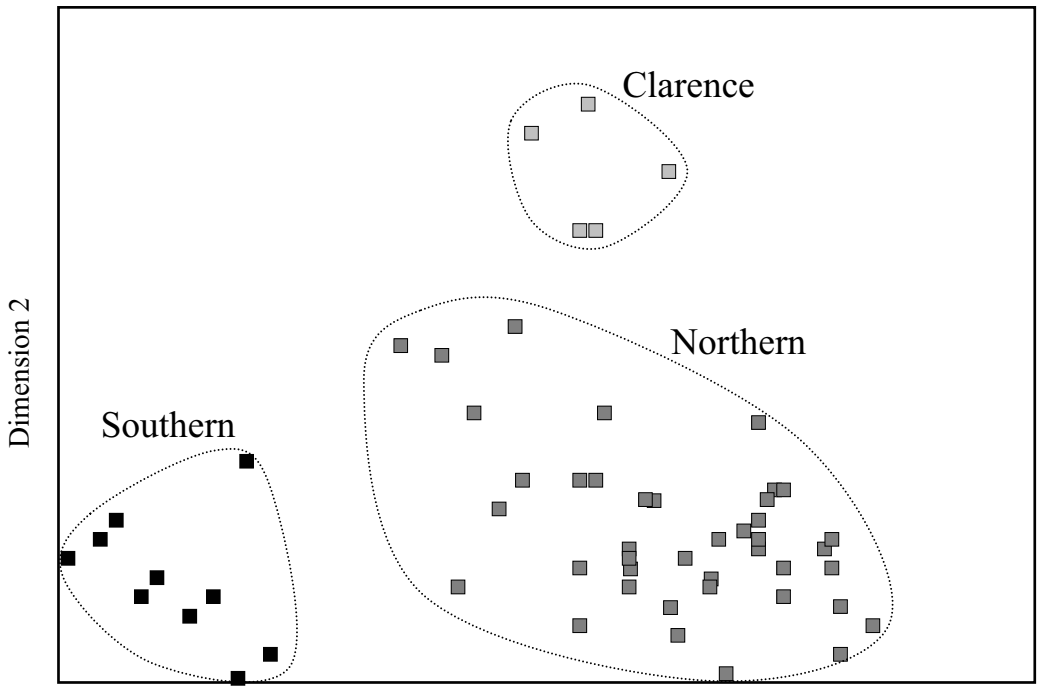

Figure 7-7. Principal Coordinates Analysis of 57 individuals representing 15 populations of *P. macrostomus* within the SEC lineage. The relative PCO scores have been plotted for the first and second dimensions, which explain 21% and 10% of the total variance, respectively. Names for lineages match Figure 7-2.

7.4. DISCUSSION

Results confirm that the genus *Philypnodon* comprises two distinctive taxa corresponding to the two currently recognised species. However, both *P. grandiceps* and *P. macrostomus* display major genetic divisions, suggesting that each is a species complex with discrete evolutionary dissimilar units. As a consequence, the management and ecology of these 'common' species are likely to require major reassessment.

Taxonomic considerations

The genetic sub-structure noted within each of *P. grandiceps* and *P. macrostomus* clearly surpasses the expectations for single species. The northern coastal portion of their range harbours considerable heterogeneity in proximate sites of abutting drainages and river basins, with rather chaotic patterns in regard to the distribution and scale of distinctive units. Unlike other groups examined thus far, divergent genotypes do not conform to broad and neatly-divided geographic clusters, hence sample size and related confidence in discrimination is low for most major groupings and indeed sub-groups (especially the SEQ complex of *P. grandiceps*). Thus, resolution of the species boundaries requires more in-depth spatial coverage in coastal Queensland and northern New South Wales, with a basic prescription of low sample sizes (n = 5-10) from multiple populations, especially for gaps in coverage, longitudinally within drainages (i.e. upper *v*. lower), and at drainage divides.

Two basic outcomes might be expected from increased spatial sampling, namely (1) additional sites appear intermediate to existing populations screened, with the resultant clinal patterns inferring the presence of a single genetically-heterogenous species, and/or (2) the original genetic distinctions are reaffirmed, with the extra insight perhaps even revealing additional species-level diversity. Assessing relationships in the north should in turn provide clarity for the position of other major groupings across the remainder of southeastern Australia. Congruent molecular (i.e. mtDNA), morphological and/or ecological datasets are the key to the ultimate taxonomic resolution of such species complexes.

Further treatment of species boundaries is warranted only after further investigation, but there are key outcomes worth flagging. The four major genetic groups within both *Philypnodon* species represent provisional species-level divisions, given that all are diagnosable at multiple nuclear genetic markers (albeit based on small sample sizes). Maroon Dam on the upper Logan River is particularly distinctive within *P. grandiceps s.l.*, with other major groupings corresponding to (2) the combined Fitzroy and Clarence Rivers, (3) a complex of divergent populations (possibly species) in south east Queensland, and (4) remaining populations from coastal New South Wales,

Victoria, Tasmania and South Australia and the MDB (this grouping coincides with the holotype of *P. grandiceps*). There has been some suggestion of other morphotypes within the taxonomic history of *P. grandiceps* (apart from recognition of the dwarf flathead gudgeon), but all fall within the range of the widespread southern form (e.g. *P. nudiceps* (Castelnau) and *P. melbournensis* (Sauvage) both from the Yarra River/Melbourne region) (Eschmeyer 2008).

The Mary River form displays all the characteristics of a novel species within the dwarf flathead gudgeon (i.e. large genetic distance, high proportions of fixed differences including unique alleles). Other divisions include (2) the Burrum River Basin, (3) Baffle, Burnett and MDB (including SAG coastal streams), and (4) coastal populations south of the Mary through to Lang Lang River (SEC). The description of *P. macrostomus* is only meant to apply for populations in the Coffs Harbour region of NSW (types from the Clarence River Basin and possibly also Bellinger River Basin, accurate details not provided) as a future point of reference and comparison in the face of considerable morphological variability (Hoese and Reader 2006). How widely the name *P. macrostomus* applies is unresolved but, based on the allozyme data, it would appear to apply at least across the range of the SEC taxon. As part of their description of *P. macrostomus*, Hoese and Reader (2006) refer to a morphologically-distinct population from the Mary River, further supporting the conclusion, based on the allozyme data, that this river basin houses a novel species of dwarf flathead gudgeon. The morphological summary also highlights an unusual form taken at the Macquarie River Basin (Cudgegong River), part of the inland MDB. Clearly the Macquarie and indeed another Darling tributary, the upper Condamine, remain key targets in addition to northern coastal populations for future molecular investigations.

Genetic sub-structure

Contrasting the patterns of genetic sub-structure between *P. grandiceps* and dwarf flathead gudgeon complexes, there are basic similarities in that relatively deep divisions and high within species diversity occur in each, the most distinctive sites occur in the northern coastal range, and southern populations are more homogenous and are genetically allied to northern populations. A difference concerns the nature of the alignments. *Philypnodon grandiceps* extends as four groups from the southern end of Queensland, whereas *P. macrostomus* has an inland group (MDB) tied closely to the northern most Queensland populations (especially Burnett River Basin) and a coastal group tied to southern Queensland populations (i.e. extending south of the Mary). Nevertheless, the presence of the widespread southern form of *P. grandiceps* in the Burnett suggests possible dispersal routes to the south and east. The other major contrast is that *P. macrostomus* also has a simpler geographic pattern of genetic structure in the northern range, while the alignment of major groups and sub-groups in *P. grandiceps* is chaotic.

The most curious pattern within *P. grandiceps* concerned the similarity of the geographically separate Fitzroy and Clarence systems, either end of a grouping of divergent southeast Queensland populations (Maroon and SEQ complex) and also interspersed by four sites from the widespread southern taxon. Whether this relationship is real, or a chance pattern based on convergence and/or the shared retention of alleles present in an ancestral gene pool, cannot be determined from these allozyme data. Clearly, there is unlikely to be any recent gene flow between these regions, however allozymes can occasionally reveal the genetic signature of relationships that date back over quite long periods of evolutionary time (Hillis *et al*. 1996). The presence of the widespread southern taxon in southeast Queensland, on the other hand, could indicate the presence of a coastal form, in contrast to more divergent populations occurring in upstream areas. This sort of pattern is seen in another eleotrid, *Mogurnda adspersa*, further north on the eastern seaboard (Atherton Tablelands: Hurwood and Hughes 1998), and such a distribution, and potential overlap of forms (e.g. sympatry as in *Retropinna*: Chapter 3), represent key hypotheses for further sampling and analysis.

Lineages within the widespread southern taxon of *P. grandiceps* do have geographically distinct boundaries, including a split between Queensland and NSW, at southern NSW/Victoria and the Glenelg River Basin, albeit with some fuzzy boundaries due to admixture among adjacent populations across sub-groups. Finally, the site from northern Tasmania was indistinguishable from sites across the Tasman in Victoria, suggesting either dispersal *via* ongoing or recent natural movement (e.g. last inter-glacial maximum) or translocation (e.g. transportation between adjacent ports). The affinity of the Hobart population (Derwent River) collected during the study, but after analyses were complete, is of similar interest.

Biogeographic patterns

The varied patterns of distribution, genetic structure and affinity of diagnosable groups within the two nominal *Philypnodon* species suggest that contrasting intrinsic characteristics (and perhaps chance) have interplayed differently with current conditions, historic division, and changes in Australian aquatic environments. Again, limitations in fully determining genetic patterns from complex local structure in the northern range inhibit confident discussion of major extrinsic factors that may have shaped patterns. Instead, these patterns provide testable hypotheses for further investigation and complementary analyses across species and faunal groups.

The SEC compared to northern population divide in *P. macrostomus* shares a point of division involving the Mary River Basin, common to several other species studied thus far using molecular tools, including species of *Retropinna* which occur symatrically in the system (CEQ *v.*

SEQ: Chapter 3), major lineages in the rainbowfish *Rhadinocentrus ornatus* (Page *et al*. 2004), lineages in a southern taxon of the Pacific blue-eye *Pseudomugil signifier* (Wong *et al*. 2004), and sub-species of the hardyhead *Craterocephalus stercusmuscarum* (McGlashan and Hughes 2001). Further, a mtDNA overview of carp gudgeons (Thacker *et al*. 2007) points to splits in the eastern distribution of taxa, namely (1) *Hypseleotris klunzingeri* has three major groups aligning to (a) SEQ/SEC (Clarence to Brisbane rivers), (b) CEQ (Mary River north to Baffle Creek) and (c) Fitzroy, (2) *Hypseleotris* sp. 5 (Midgley's carp gudgeon) has two lineages which split between the Mary and Brisbane Rivers (i.e. I and J), and (3) *H. galii* (firetail gudgeon) has two major lineages that occur from the Mary south (i.e. F and G *v*. E) (see Thacker *et al*. 2007).

Beyond being a point of division, the Mary also houses its own unique taxa, including a likely novel species of dwarf flathead gudgeon, a divergent lineage of *P. grandiceps*, the Mary River cod *Maccullochella peelii mariensis* (Rowland 1993) and a monotypic genus of freshwater turtle (*Elusor macrurus*: Cann 1998), and is the main natural habitat for the ancient lungfish *Neoceratodus fortseri*. Similarly, two distinctive populations within *P. grandiceps* match local uniqueness in other fishes, namely the Fitzroy River which has a separate sub-species of golden perch *Macquaria ambigua oriens* (Musyl and Keenan 1992) and lineage of *H. klunzingeri* (Thacker *et al*. 2007), and the Clarence River which has an endemic freshwater cod *Maccullochella ikei* (Rowland 1993) and turtle *Emydura macquarii bingjing* (Cann 1998). Clearly, the general area between the Fitzroy River Basin and Clarence River is a biodiversity hotspot for freshwater biota.

Biogeographic patterns outside of the northern region associated with the widespread forms of both *Philypnodon* include: (1) a potential split aligning to the McPherson Range in major groupings of the southern *P. grandiceps* taxon (similar to *Retropinna*: Chapter 3), (2) Wilson Promontory as the rough distribution limit for *P. macrostomus* SEC taxon (*P. grandiceps* shows limited alignment, and instead breaks further up the coast at the eastern edge of Gippsland), (3) Glenelg River represents the discontinuation of the MDB and Bass lineages of *P. grandiceps* thus matching *Nannoperca obscura* (Chapter 4), and (4) the coastal distribution of *P. grandiceps* shows no indication of a MDB *versus* Millicent Coast River Basin split as per *Retropinna* (Chapter 3), but in contrast to *Nannoperca* species (Chapters 3 and 4).

In general, the Great Dividing Range does appear as an inland barrier (i.e. distinct major groupings of both nominal *Philypnodon* species), but with areas of colonisation and/or admixture suggesting historically recent dispersal routes across the geographic high. The most obvious of these is the similarity of Burnett River Basin populations to the widespread MDB group in

P. macrostomus, with the limited heterogeneity in the MDB indicative of origins and founder effect from the coast. Indeed the area of southeastern Queensland abutting the MDB is a postulated area of drainage exchange between a number of other native fishes (Musyl and Keenan 1992; Rowland 1993; McGlashan and Hughes 2001; Unmack 2001; Thacker *et al*. 2007; Faulks *et al*. 2008; Jerry 2008). Areas of admixture in *P. grandiceps* occur in the east and south near the major cities of Sydney and Melbourne respectively. It is unclear without historic reference and wider spatial and temporal sampling whether this is the result of natural movement across the divide, or as a result of human-mediated translocation (e.g. as bait fish, water transfers).

Ecology

Unlike other species examined, a level of tolerance to marine conditions is apparent within *Philypnodon*, and this forms the basis of the original hypothesis of moderate or less-pronounced sub-structure in this genus as opposed to other groups examined. However, the general ecological assumptions of high dispersal ability are only partially supported by the molecular data, with two seemingly different scales in operation. Firstly, there is a region of high sub-structure that is complicated by the presence of multiple, species-level taxa within each nominal species of *Philypnodon*, and their unacknowledged presence clouds any attempted interpretation of existing ecological data. Secondly, some areas do show similarity across drainage divides involving widespread genetic groupings, and thus support the idea of higher coastal-mediated gene flow under current and/or low sea levels. Perhaps the best support for potential marine dispersal occurs near divides in the sub-groups of southern *P. grandiceps*, where admixture between proximate coastal river basins has occurred. Nevertheless, structure also exists where it might not be expected (e.g. breaks in *P. grandiceps* along the southern coastline). Such discrepancies highlight overall complex patterns and the difficulty in drawing ecological generalisations across such a heterogenous group.

More work is required to refine ecological information both between and within the two species groups of *Philypnodon*. Although the two were assumed to be closely related by virtue of the dwarf flathead gudgeon being a cryptic and only recently-recognised species, where ecological data may be interchangeable, the divergence between *P. grandiceps s.l.* and *P. macrostomus* complex strongly suggests otherwise. Comparative ecological studies in Queensland in particular need to be mindful of the presence of divergent lineages between and within *Philypnodon* species and of the potential of upland *versus* coastal ecological forms in *P. grandiceps s.l.* Moreover, despite being genetically distinctive, *P. grandiceps s.l.* and dwarf flathead gudgeon are clearly capable of producing viable F_1 hybrids in the wild, as witnessed in the Lower Murray. Although

only a minor level of hybridisation was detected, field ecologists should be mindful of its potential occurrence, particularly in altered or degraded habitats (e.g. Fisher *et al*. 2006).

Conservation

Despite *Philypnodon* being treated as a widespread, vagile, generalist genus, the current taxonomic framework does not reflect its true biological diversity. The situation in its northern range argues strongly for more intensive examination to identify distinctive conservation units. For example, within the provisional four species identified in each of *P. grandiceps* and *P. macrostomus*, there are numerous sub-groups that operate as provisional ESUs, pending support from their matrilineal genealogies and more detailed spatial sampling. As with *Retropinna*, abundance in some habitats is no guarantee that some isolated, distinctive, and threatened taxa do not exist. The significant sub-structure observed implies that translocation between drainages, especially across drainages divisions and river basins, should be discouraged to prevent unnatural mixing, and may require tightening of policies and practice with regard to the use of *P. grandiceps* as live bait, and interbasin water transfers.

7.5. TABLES

Table 7-1. Locality and sample size information for *Philypnodon grandiceps*. Site numbers match those in Figure 7-1. DD = Drainage Division; RB = River Basin (AWRC 1976). Nine sites marked with (^{x}) indicate populations with genetic exchange across sub-groups.

Site	Field code	Locality	State	DD	RB	Latitude (S)	Longitude (E)	*n*
G1	PU01-53	Fairbairn Dam, Emerald	Qld	I	30	23°39'	148°04'	1
G2	PU01-55	Dawson R., Moura	Qld	I	30	23°43'	149°46'	2
G3	PU02-49	Maryvale Ck, Maryvale Stn	Qld	I	30	22°57'	150°40'	2
G4	PU02-39	Yandaran Ck, Avondale	Qld	I	35	24°44'	152°07'	5
G5	PU99-55	Burnett R., Mingo Crossing	Qld	I	36	25°23'	151°46'	2
G6	PU97-48	Isis R., Childers	Qld	I	37	25°15'	152°22'	5
G7	PU02-33	Amamoor Ck, Amamoor	Qld	I	38	26°20'	152°39'	5
G8	PU02-27	Moogerah Lake, Moogerah	Qld	I	43	28°02'	152°32'	5
G9	PU02-28	Maroon Dam, Maroon	Qld	I	44	28°10'	152°38'	5
G10	PU02-24	Coomera R., Flying Fox	Qld	I	45	28°05'	153°08'	5
G11	PU02-22	Oxley R., Eungella	NSW	II	1	28°21'	153°18'	5
G12	PU02-17	Leycester Ck, Leycester	NSW	II	3	28°47'	153°13'	5
G13	PU 99-43	Clarence R., Tabulam	NSW	II	4	28°53'	152°33'	5
G14	PU02-13	Hickeys Ck, Millbank	NSW	II	6	30°51'	152°37'	3
G15	PU99-38	Hastings R., Wauchope	NSW	II	7	31°25'	152°41'	2
G16	PU02-09	Cedar Party Ck, Wingham	NSW	II	8	31°52'	152°22'	2
G17	PU02-08	Goorangoola Ck, Mt Pleasant	NSW	II	10	32°24'	151°10'	4
G18^{x}	F-FISH53	Sydney	NSW	II	12	33°39'	150°59'	3
G19^{x}	F-FISH53	Georges R., Liverpool	NSW	II	13	33°55'	150°52'	3
G20^{x}	PU02-58	Kangaroo R., Kangaroo Valley	NSW	II	15	34°43'	150°31'	3
G21^{x}	PU02-60	Mogo Ck, Mogo	NSW	II	16	35°47'	150°08'	3
G22^{x}	PU99-83	Millingandi Ck, Milligandi	NSW	II	20	36°52'	149°51'	3
G23	PU02-65	Snowy R. Lagoon, Orbost	Vic.	II	22	37°42'	148°27'	2
G24	PU02-73	Darby R., Wilsons Prom.	Vic.	II	27	38°58'	146°16'	4
G25	F-FISHADD4	Steele Ck, Melbourne	Vic.	II	29	37°43'	144°52'	2
G26	PU00-28	Woady Yaloak R., Cressy	Vic.	II	34	38°01'	143°37'	2
G27	F-FISHY2	L. Bullen Merri, Camperdown	Vic.	II	34	38°14'	143°05'	1
G28^{x}	PU00-24	Curdies R., Curdie	Vic.	II	35	38°26'	142°57'	2
G29	PU02-112	Mt Emu Ck, Panmure	Vic.	II	36	38°19'	142°45'	1
G30	PU02-113	Shaw R, Yamnbuck	Vic.	II	37	38°18'	142°03'	2
G31	PU00-15	Glenelg R., Burke Bridge	Vic.	II	38	37°12'	141°23'	2
G32^{x}	F-FISH99	Glenelg R., Dartmoor	Vic.	II	38	37°55'	141°17'	2
G33	F-FISH99	Cockatoo Lake, Naracoorte	SA	II	39	36°44'	140°34'	2
G34	F-FISH90	Cortina Lakes, Cortina	SA	II	39	36°27'	140°03'	1
G35	F-FISH98	Mersey R., Latrobe	Tas.	III	16	41°14'	146°24'	2
G36	F-FISHY4	Derwent R., New Norfolk	Tas.	III	4	42°46'	147°04'	0
G37	TR02-497	Cudgegong R., Rylstone	NSW	IV	21	32°47'	149°58'	3
G38	PU99-36	Lake Forbes, Forbes	NSW	IV	12	33°23'	147°59'	2
G39	PU02-55	Murray R., Albury	NSW	IV	9	36°05'	146°56'	3
G40^{x}	TR02-433	Goulburn R., Alexandra	Vic.	IV	5	37°12'	145°41'	4
G41	F-FISH52	Reedy Swamp, Shepparton	Vic.	IV	5	36°20'	145°26'	2
G42^{x}	TR02-209	Bullarook Ck, Creswick	Vic.	IV	7	37°24'	143°99'	2
G43	F-FISH52	Black Swamp, Cohuna	Vic.	IV	9	35°42'	144°09'	2
G44	PU00-06	Mount Cole Ck, Warrak	Vic.	IV	15	37°15'	143°08'	2
G45	PU01-60	Wimmera R., Jeparit	Vic.	IV	15	36°08'	141°58'	1
G46	F-FISH99	R. Murray, Berri	SA	IV	26	34°17'	140°36'	1

Site	Field code	Locality	State	DD	RB	Latitude (S)	Longitude (E)	*n*
G47	TR02-159	Bremer R., Harrogate	SA	IV	26	34°57'	139°00'	1
G48	F-FISHY2	Angas R., Strathalbyn	SA	IV	26	35°15'	138°53'	1
G49	TR02-170	Meadows Ck, Meadows	SA	IV	26	35°12'	138°41'	2
G50	F-FISHADD2	Currency Creek, Mt Compass	SA	IV	26	35°26'	138°43'	2
G51	F-FISHY2	Hindmarsh R., Victor Harbor	SA	V	1	35°31'	138°37'	4
G52	F-FISH94	Onkaparinga R., Clarendon	SA	V	3	35°06'	138°37'	2
G53	F-FISHY2	Torrens R., Cudlee Creek	SA	V	4	34°50'	138°48'	2
G54	F-FISHADD1	Torrens R., Adelaide	SA	V	4	34°54'	138°32'	3
G55	F-FISHADD4	Gawler R., Gawler	SA	V	5	34°36'	138°44'	2
G56	F-FISHY2	Light R., Hamley Bridge	SA	V	5	34°23'	138°35'	2

Table 7-2. Locality and sample size information for *Philypnodon macrostomus*. Site numbers match those in Figure 7-2. DD = Drainage Division; RB = River Basin (AWRC 1976). Site H1 represents a F1 hybrid individual between *P. macrostomus* and *P. grandiceps*.

Site	Field code	DD	RB	Locality	State	Latitude (S)	Longitude (E)	*n*
M1	PU02-50	I	34	Baffle Ck, Miriam Vale	Qld	24°21'	151°36'	5
M2	PU02-51	I	36	Burnett R., Ceratodus	Qld	25°16'	151°08'	5
M3	PU99-52	I	36	Barambah Ck, Murgon	Qld	26°14'	151°53'	5
M4	PU02-38	I	37	Elliott R., Elliott	Qld	24°59'	152°22'	5
M5	PU02-37	I	37	Gregory R., Goodwood	Qld	25°09'	152°22'	5
M6	PU02-33	I	38	Amamoor Ck, Amamoor	Qld	26°20'	152°39'	5
M7	PU97-71	I	43	Delaney Ck, D'Aguilar	Qld	27°01'	152°46'	5
M8	PU02-22	II	1	Oxley R., Eungella	NSW	28°21'	153°18'	3
M9	PU02-17	II	3	Leycester Ck, Leycester	NSW	28°47'	153°13'	5
M10	PU99-43	II	4	Clarence R., Tabulam	NSW	28°53'	152°33'	5
M11	PU02-13	II	6	Hickeys Ck, Millbank	NSW	30°51'	152°37'	5
M12	PU99-38	II	7	Hastings R., Wauchope	NSW	31°25'	152°41'	5
M13	PU02-09	II	8	Cedar Party Ck, Wingham	NSW	31°52'	152°22'	1
M14	F-FISH95	II	9	Limeburners Ck, Limeburners Ck	NSW	32°37'	151°53'	2
M15	PU02-07	II	10	Bowmans Ck, Ravensworth	NSW	32°26'	151°03'	1
M16	F-FISH53	II	12	Goerges R., Liverpool	NSW	33°55'	150°52'	5
M17	PU 99-83	II	20	Millingandi Ck, Millingandi	NSW	36°52'	149°51'	5
M18	PU 99-84	II	21	Maramingo Ck, Genoa	Vic.	37°26'	149°38'	4
M19	PU02-65	II	22	Snowy R. Lagoon, Orbost	Vic.	37°42'	148°27'	5
M20	PU02-69	II	27	Miranda Ck, Wilsons Prom.	Vic.	38°55'	146°27'	2
M21	F-FISHY2	II	28	Lang Lang R., Lang Lang	Vic.	38°15'	145°34'	3
M22	F-FISH52	IV	9	Black Swamp, Cohuna	Vic.	35°42'	144°09'	3
M23	F-FISH99	IV	26	R. Murray, Berri	SA	34°17'	140°36'	4
M24	F-FISHY4	IV	26	Marne R., Black Hill	SA	34°42'	139°29'	2
M25	F-FISHY2	IV	26	Reedy Ck, Palmer	SA	34°55'	139°10'	3
M26	F-FISHY4	IV	26	Bremer R., Langhorne Creek	SA	35°10'	139°01'	1
M27	F-FISH52	IV	26	Angas R., Strathalbyn	SA	35°15'	138°53'	3
M28	F-FISHY4	IV	26	Finniss R., Ashbourne	SA	35°18'	138°45'	2
M29	F-FISH84	IV	26	Finniss R., Lake Alexandrina	SA	35°24'	138°50'	2
M30	F-FISH94	IV	26	Hindmarsh Is., Lake Alexandrina	SA	35°31'	138°54'	1
M31	F-FISHY4	V	1	Hindmarsh R., Victor Harbour	SA	35°29'	138°36'	5
M32	F-FISHY4	V	3	Onkaparinga R., Verdun	SA	35°00'	138°47'	2
M33	F-FISH94	V	3	Onkaparinga R., Clarendon	SA	35°06'	138°37'	3
M34	F-FISHY2	V	4	Torrens R., Cudlee Creek	SA	34°50'	138°48'	5
H1	F-FISHY4	IV	26	Angas R. mouth, Milang	SA	35°23'	139°00'	1

Table 7-3. Allele frequencies at all loci for the 11 diagnosable groupings (and three hybrid assemblages) identified for *P. grandiceps*. All were invariant at 16 loci for the following alleles: *Adh2*^{c}, *Ak*^{a}, *Ald2*^{b}, *Ap*^{b}, *Ck*^{a}, *Est*^{a}, *Fdp*^{c}, *Fum*^{b}, *Gapd*^{a}, *Got1*^{d}, *Got2*^{b}, *Gsr*^{b}, *Idh2*^{b}, *Ldh*^{a}, *Tpi1*^{b}, and *Ugpp*^{a}. Sample sizes are shown in parenthesis. Site codes for hybrid assemblage are shown in Table 7-1 and Figure 7-1.

Taxon	**---------- Southern ----------**						**--Clarence/Fitzroy--**		**---------- SEQ complex ----------**				**Maroon**
Sub-group	**Bass x MDB**	**Bass x NSW**	**Bass/MDB x NSW**	**Bass G23-30**	**MDB G31-56**	**NSW G11-17**	**Qld 2 G4-5**	**Clarence G13**	**Fitzroy G1-3**	**Isis G6**	**Mary G7**	**Brisbane G8**	**Coomera G10**	**Maroon G9**
Locus	**(10)**	**(12)**	**(3)**	**(16)**	**(42)**	**(21)**	**(7)**	**(5)**	**(5)**	**(5)**	**(5)**	**(5)**	**(5)**	**(5)**
Acon	g^{70}, h^{25},j	g	g	g	h^{77},g	g^{62},f^{31},d^{5},b	d	d	f^{90},d	g^{70},f	d	i^{70},f	f	e
Acp	b	b	b	b	b	b	b	b	b	b	b	b	b	a
Ada	d	d^{92},f	d	d	d	d^{95},c	d^{86},c	c^{50}, d^{30},a	d	d^{90},c	b	d	c^{80},d	d
Adh1	c	b^{45}, c^{42},a	b^{50},c	c^{91}, a^{6},b	c^{79},b	b^{83},c	b	b	b^{80},c	b	b	b^{90},c	b	c^{90},b
Ald1	a	a	a	a	a	a	a	a	a	a	a^{80},b	a	a	a
Ca	c	c	c^{50},e	c	c	e^{55},c	e	e^{90},d	e^{90},c	c	c	e	e	e
Enol1	a^{85},c	a	a	a^{50},c	a	a^{90},b	a	a	a	a	a	a	a	b
Enol2	a	a	a	a	a	a	a	a^{90},c	a	a	a	a	a	a
Gda	a^{55},b	a^{50},b	a	b	a	a	a	a^{80},b	a	a	a	a	b	a
Glo	b	b	b	b	b	b	b	a	a	b	b	b	b	b
Gp	a	a	a	a	a	a	a	a	b	a	a	a	a	a
Gpi1	g	g	g	g	g	g	g	g	g	g	g	g	d^{90},g	g
Gpi2	h^{75}, i^{15},e	h^{79},i	h^{67},e	h	e^{49}, i^{45},h	h^{54},i^{19}, g^{17},f	h	h	h^{70},j	h	i	h^{90},i	h^{80},i	h
Idh1	e	e^{87},c	e^{67},a	e^{62},c	e	e	e	e	e	e	e	e^{60},a	e	e
Mdh1	d	d	d	d	d	d^{86},c	d	d	d	d	d	d	d	d
Mdh2	c	c	c	c	c	c^{90},e	c	e	c	c	c	c	c	c
Mdh3	b	b	b	b	b	b	b	b	b	b	a	b	b	b
Me	c	c	c	c	c	c	c	c	c	c	c^{90},a	c	c	c
Mpi	f^{85},e	f	f^{67},e	f	e^{62},f	f^{98},g	f^{86},e	f	e^{80},f	e^{80},f	e^{50},f	f	f	f
PepA1	c	c^{79}, b^{17},f	c^{50}, d^{33},f	c^{91},b	c^{99},b	c^{62}, f^{33},d	a^{86},b	c	c^{80},a	b	b	e	c	c
PepA2	f^{65}, d^{30},e	f^{71},e	e^{83},d	f	d^{76},e^{21},f	e^{81}, f^{17},c	e	c	e^{80},c	c	c	e	e^{80}, d^{10},c	e

Taxon	**---------- Southern ----------**							**--Clarence/Fitzroy--**		**---------- SEQ complex ----------**				**Maroon**
Sub-group	**Bass x MDB**	**Bass x NSW**	**Bass/MDB x NSW**	**Bass G23-30**	**MDB G31-56**	**NSW G11-17**	**Qld 2 G4-5**	**Clarence G13**	**Fitzroy G1-3**	**Isis G6**	**Mary G7**	**Brisbane G8**	**Coomera G10**	**Maroon G9**
Locus	**(10)**	**(12)**	**(3)**	**(16)**	**(42)**	**(21)**	**(7)**	**(5)**	**(5)**	**(5)**	**(5)**	**(5)**	**(5)**	**(5)**
PepB	c	c^{79},e	c	c	c	c^{88}, e^{10},a	c^{86},e	f	f	f	f	e^{80},c	c	c^{90},e
PepD1	c	c	c	c	c	c^{95},d^{5}	c	d	c	c	c	c	c	c
PepD2	g	g^{75},d	g^{83},d	g	g	g^{55}, d^{43},f	d^{71},g	d	d^{80},b	g	d	e^{90},g	g^{60},f	e
Pgam	a	a	a	a	a	a	a	a	a	a	a	b^{90},a	a	b
6Pgd	d^{95},b	d	d	d	d^{83},b	d^{93},b^{5},a	d	d	d	d	d	d	d	d
Pgk	d	d	d	d	d	d	f^{71},d	d	f^{80},d	f	d	d	d	d
Pgm1	d	d	d	d	d	d^{95},f^{3},b	d	d	d	d	d	d^{80},b	b^{60},d	b
Pgm2	e^{75},f	e	e^{83},f	e^{97},g	f^{61},e	e^{60},f^{26}, g^{12},d	e	e	e^{40}, h^{40},d	e^{60},g	e	e	e	g^{90},e
Pk1	b^{55},a^{40},c	b^{62},a	a^{50},b	b	a	a	a	a	a	a	a	a	a	a
Pk2	c^{95},b	b^{63},c	b^{83},c	c^{94},b	b^{50},c	b^{93},c	b^{93},a	b	b	b	b^{70},c	b	b	b
Tpi2	d	d	d	d	d	d	d^{93},c	d	d	d	d^{90},c	d	d	d
H_O	0.079	0.057	0.104	0.017	0.063	0.067	0.036	0.029	0.046	0.025	0.042	0.054	0.054	0.013
S.E.	0.026	0.019	0.033	0.008	0.021	0.016	0.016	0.016	0.017	0.015	0.020	0.021	0.023	0.007

Table 7-4. Pairwise genetic comparisons among the 11 diagnosable genetic groupings identified in *Philypnodon grandiceps*. The lower triangle represents %FD, and the upper triangle is Nei's unbiased D. Bass includes site 35 from Tasmania, and sites where admixture of Bass, MDB and NSW combinations was detected have been excluded from analysis (see Table 7-1 and Figure 7-1).

Taxon	**---------- Southern ----------**				**Clarence/Fitzroy**		**---------- SEQ Complex --------**				**Maroon**
Sub-group	**Bass G23-30**	**MDB G31-56**	**NSW G11-17**	**Qld 2 G4-5**	**Clarence G13**	**Fitzroy G1-3**	**Isis G6**	**Mary G7**	**Brisbane G8**	**Coomera G10**	**Maroon G9**
G23-30	-	0.13	0.13	0.21	0.30	0.30	0.21	0.29	0.24	0.19	0.28
G31-56	8	-	0.07	0.15	0.25	0.19	0.14	0.19	0.18	0.17	0.23
G11-17	4	0	-	0.05	0.15	0.12	0.11	0.16	0.08	0.09	0.16
G4-5	16	8	4	-	0.15	0.12	0.13	0.17	0.10	0.12	0.21
G13	22	18	12	12	-	0.15	0.21	0.19	0.21	0.20	0.32
G1-3	20	14	6	8	8	-	0.13	0.22	0.18	0.19	0.27
G6	16	10	8	8	16	10	-	0.14	0.19	0.21	0.30
G7	22	14	12	12	16	16	12	-	0.23	0.25	0.38
G8	18	10	6	8	18	12	14	20	-	0.14	0.15
G10	14	8	4	8	16	12	14	20	10	-	0.21
G9	22	18	12	18	27	20	24	31	10	18	-

Table 7-5. Allele frequencies at all loci for eight diagnosable sub-groups identified in *P. macrostomus* (* note geographically sperate populations from the MDB and Burnett River Basin are shown separately but represent a single sub-group). All populations were invariant at 15 loci for the following alleles *Acp*^{b}, *Adh1*^{b}, *Ak*^{a}, *Ald1*^{a}, *Ald2*^{a}, *Ck*^{a}, *Enol1*^{a}, *Est*^{b}, *Gapd*^{a}, *Glo*^{b}, *Idh2*^{a}, *Ldh*^{a}, *Pk1*^{a}, *Pk2*^{b}, and *Ugpp*^{b}. Sample sizes are shown in parentheses.

Taxon	**--------- SEQ/MDB ----------**			**---------- SEC ----------**			**----- Burrum -----**		**Mary**
Sub-group	**MDB***	**Burnett***	**Baffle**	**Clarence**	**North**	**South**	**Elliot**	**Gregory**	**Mary**
	M22-34	**M2-3**	**M1**	**M10**	**M7-16**	**M17-21**	**M4**	**M5**	**M6**
	(37)	**(10)**	**(5)**	**(5)**	**(41)**	**(10)**	**(5)**	**(5)**	**(5)**
Locus									
Acon1	d	d	e^{70},d^{20},a	e^{60},d	d^{62},e^{32},b	d^{94},c	d	d	d
Ada	d^{69},g	d^{40},g^{40}, c	c^{63}, g^{25},d	d^{60},f	d^{44},b^{34}, c^{20},a	c^{50},d^{38}, b^{6},f	c^{60},d	c^{50}, d^{30},e	d^{90},e
Adh2	b	b	b	b	b^{94},a	b	b	b	b
Ap	b^{99},a	b	b	b	b	b	b	b	b
Ca	d	d	d^{90},a	d	d^{99},b	d	d	d	d
Enol2	a	a	a	a	a	a	a^{50},b	b	a^{60},b
Fdp	d^{99},c	d	d	b^{60},d	d^{99},a	d	d	d	d
Fum	b	b	b	b	b^{91},a	b^{87},a	b	b	b
Got1	a	a	a	a	a^{91},b^{7},c	b^{87},a	a	a	a
Got2	c	d^{80},c	c	c	c^{99},d	c	a	c	c
Gp	b	b	b	a	a	a	b	a	b
Gpi1	e	e	f	g	e^{86},f^{12},a^{1},c	e	b	b	e
Gpi2	d	d	d	d	d^{93},g^{6},b	d	d^{90},a	b^{60},d	c
Gsr	d^{90},c	c^{85},d	c	c	c^{97},a^{2},d	c	c	c	d
Idh1	f	d^{50,} f^{50}	f	f	f^{97},g	f	d^{60},f	d	b
Mdh1	b	b	b	b	b^{95},e^{3},a	b	b	b	b
Mdh2	a	a	a	a	a^{98},b^{1},d	a	a	a	a
Mdh3	b^{99},a	b	b	b	b	b	b	b	b
Me	d	d	d	d	b^{67},d	d^{62},b	c	c	c
Mpi	b	b	b	b	b^{80},c^{19},a	b	d^{60},b	b^{80},d	b
PepA1	b	b	b	b^{90},c	b^{87},c^{8},a	b^{94},c	b	b	b
PepA2	c	c	c	c	c^{93},b^{6},a	c	c	c	c
PepB1	b	b	b	b	b	b	b^{90},d	d	b
PepB2	a	a	a	d^{80},c	c^{57},d^{34},a	d^{94},a	a	a	b
PepD1	b	b	b	b	b^{99},a	b	b	b	b
PepD2	f	f	c	f^{60},c	c^{71},f	f	f	a	f^{70},a
Pgam	a	a	a	a	a^{95},c	a^{50},c	a	a	a
6Pgd	a	a	a	a	a^{99},c	a	a	a	b
Pgk	c	c	c^{90},a	c	c^{70},b^{19},e	b^{88},e	c	c	c
Pgm1	d^{96},c^{3},e	d	d	d^{90},f	d^{97},a^{2},c	a^{56},d	d	d	d
Pgm2	c	c	c	b^{50},c^{38},d	d^{60},b^{33},c^{5},a	d	c^{60},d	c	f
Tpi1	a	a	a	a	a^{99},c	a	a	a	a
Tpi2	b	b	b	b	b	b	b	a^{50},b	b
H_O	0.017	0.029	0.031	0.077	0.076	0.044	0.071	0.025	0.017
S.E.	0.012	0.020	0.017	0.028	0.018	0.015	0.028	0.015	0.013

Table 7-6. Pairwise genetic comparisons among eight diagnosable sub-groupings identified in *Philypnodon macrostomus* (* note MDB and Burnett represent a single sub-group but are shown separately given their geographic isolation). The lower triangle represents %FD and the upper triangle is Nei's unbiased D. Sites are coded according to their genetic lineage, as displayed in Figure 7-2 and Figure 7-3.

Taxon	**--------- SEQ/MDB ---------**			**---------- SEC ----------**			**------ Burrum -----**		**Mary**
Sub-group	**MDB* M22-34**	**Burnett* M2-3**	**Baffle M1**	**Clarence M10**	**North M7-16**	**South M17-21**	**Elliot M4**	**Gregory M5**	**Mary M6**
M22-34	-	0.03	0.08	0.11	0.10	0.14	0.12	0.20	0.14
M2-3	0	-	0.08	0.12	0.11	0.15	0.08	0.18	0.18
M1	6	4	-	0.09	0.09	0.18	0.13	0.19	0.24
M10	8	6	6	-	0.05	0.11	0.17	0.20	0.23
M7-16	6	6	6	2	-	0.07	0.15	0.19	0.20
M17-21	10	8	12	4	0	-	0.20	0.26	0.25
M4	8	6	8	10	10	12	-	0.11	0.19
M5	16	12	14	14	16	18	8	-	0.24
M6	12	12	18	18	16	18	16	18	-

8. THE ENIGMATIC FRESHWATER BLACKFISHES

8.1. INTRODUCTION

The freshwater blackfishes (genus *Gadopsis*) are native to cooler streams and rivers of southeastern Australia including the Murray-Darling Basin (MDB), coastal systems between the River Murray and east Gippsland, and northern Tasmania. They grow to a much larger size than the other study species (i.e. 300-600 mm *v*. < 100 mm) and accordingly have wider recreational and cultural value for angling and food (Lake 1967; Jackson *et al*. 1996). The mystery of being secretive and nocturnal excites the curiosity of biologists and naturalists (e.g. Sim *et al*. 2000), and they have proved to be an enigmatic group with regard to phylogeny and taxonomy. Curious anatomical features lead to early speculation of close relationships to marine groups, but more recently the genus has been allied to the percichthyids, either as a related family (Gadopsidae) or, as per current consensus, an outlying member of the Percichthyidae (see Jerry *et al*. 2001).

Two species are currently recognised, the river blackfish *G. marmoratus* Richardson and the two-spined blackfish *G. bispinosus* Sanger. *Gadopsis bispinosus* was described from the range of *G. marmoratus* in the mid 1980s, and is restricted to the highlands of the southeastern MDB (Sanger 1984; Lintermans 2007). The taxonomic history of *G. marmoratus* is confounded by limited and variable morphological characteristics for consistent discrimination, illustrated by the late identification of *G. bispinosus*, description and subsequent synonymy of a Tasmanian compared to mainland species (cf. Parrish 1966; Sanger 1986), and by long-proposed but still undescribed northern and southern forms on respective sides of the Great Dividing Range (Sanger 1984; Ryan *et al*. 2004). Genetic distinctiveness of northern and southern '*marmoratus*' is supported by the results of several partial studies (Sanger 1986; Ovenden *et al*. 1988; Jerry *et al*. 2001; Miller *et al*. 2004; Ryan *et al*. 2004), but a full overview of spatial genetic structure remains to be undertaken.

Gadopsis species are widely regarded as habitat specialists, requiring areas with high levels of physical cover including woody debris, rock or undercut banks (e.g. Koehn 1987; Koehn *et al*. 1994; Bond and Lake 2003). They have low fecundity, and spawning has been documented in confined spaces such as hollow logs and boulder crevices (Jackson 1978a; O'Connor and Zampatti 2006). Larvae are large and demersal, living in dense cover (noted for *G. marmoratus*: Jackson 1978a). A primary habitat division for *G. bispinosus* from *G. marmoratus* involves occurrence in clear, fast-flowing streams compared to slower flowing mid-reaches, with areas of overlap and sympatry at the transition (Sanger 1984; Curmi 1996; Lintermans 2007). Individual blackfishes are reported to have restricted movement patterns including small home ranges and

site fidelity (Koehn 1986; Khan *et al*. 2004), with radio-tracking documenting some localised (i.e. hundreds of metres) latitudinal and longitudinal movement (Koster and Crook 2008).

Clearance of surrounding lands and riparian zones (leading to siltation and habitat loss), instream modifications (e.g. snag removal, weir construction), hydrological changes, fish introductions and overfishing have negatively affected blackfishes (Jackson 1978b; Koehn and O'Connor 1990; Lintermans 1998; Hammer 2004; Bond and Lake 2005). *Gadopsis marmoratus* has declined across its range, especially in the MDB including the lower Murray, where catchment-scale extirpations have occurred (Lloyd and Walker 1986; Morris *et al*. 2001; Hammer *et al*. 2007a). For *G. bispinosus*, an upland distribution across different systems leaves it vulnerable to the vagaries of fragmentation and prone to genetic partitioning (e.g. Ovenden *et al*. 1988). Similarly, strong sub-structure should be expected in *G. marmoratus* due to habitat specificity, small home range, limited document dispersal in adults, and demersal larvae, although large body size (i.e. better swimming ability) and occurrence in larger rivers implies a potential for wider dispersal. Human-mediated gene flow in *Gadopsis* could confound natural patterns, given that there are reports of translocation for angling purposes (Jackson *et al*. 1996).

8.2. METHODS

Sampling and analyses

Samples were obtained from intensive surveys in the range of both *Gadopsis* species, primarily by back-pack electrofishing. *Gadopsis bispinosus* was sampled from four major river basins, and *G. marmoratus* from the MDB between the Condamine River in Queensland through to the Mount Lofty Ranges in South Australia; coastal systems from Henry Creek, SA to Back River, Victoria; and Tasmania from the Arthur River across to the Wye River plus the Derwent River in the south (Figure 8-1 and Table 8-1). Such extensive collection (61 localities from five Australian states and a territory) was aided by samples provided by T. Raadik (13 sites in Victoria), S. Ryan (5 sites from southwestern Victoria) and J. Lyon (Ovens River *G. bispinosus*) and by knowledge of localities in Victoria (T. Raadik), Australian Capital Territory (M. Lintermans) and Tasmania (J. Jackson). Fish were euthanased, then sampled (whole or *via* a small lateral tissue section), with tissues snap frozen in liquid nitrogen and subsequently stored at -70^{o}C. Fish providing tissue samples or individuals from the same localities were retained as voucher specimens, and have been deposited at the Australian, South Australian and Victorian museums.

For two sites in the Mount Lofty Ranges, in order to increase sample size for restricted and threatened populations, fin clips were taken of live fish that were returned to the point of capture. As with *Philypnodon*, the allozyme analyses followed the principles of an *overview study* to examine species boundaries and broad population structure, and thereby incorporated a large number of loci, small sample sizes per locality (n = 1-5 fish), and numerous localities across the geographic range (Richardson *et al*. 1986).

Allozyme electrophoresis

Homogenates comprised a small piece of caudal muscle sonicated in an equal volume of buffered lysing solution (0.02M Tris-HCl, pH 8.0, with 0.2% 2-mercaptoethanol and 0.02% NADP). After centrifugation for 10 min at 10,000 g, supernatant fluids were stored at -20°C as 10-20 µL aliquants in glass capillary tubes. Allozyme electrophoresis was conducted on cellulose acetate gels (Cellogel^{TM}), following Richardson *et al*. (1986). Thirty-six enzymes or non-enzymatic proteins produced zymograms of sufficient intensity and resolution to permit allozymic interpretation: ACON, ACP, ACYC, ADA, ADH, AK, ALD, AP, CK, ENOL, EST, FDP, FUM, GAPD, GLO, GOT, GP, GPD, GPI, GSR, IDH, LDH, MDH, ME, MPI, NDPK, PEPA, PEPB, PEPD, PGAM, 6PGD, PGK, PGM, PK, TPI and UGPP. Details of enzyme and locus abbreviations, enzyme commission numbers, electrophoretic conditions, and stain recipes are in Richardson *et al*. (1986) and Bostock *et al*. (2006). Alphabetic and numerical designations were

assigned to allozymes and multiple loci respectively, both in order of increasing electrophoretic mobility (i.e. *Acon*[a], *Acon*[b], *Adh1*, *Adh2*).

Data analysis

The initial unit for analysis was individual specimens with genetic affinities explored using stepwise principal co-ordinates analysis (PCO), as implemented on a pairwise matrix of Rogers' genetic distance (Rogers 1972) using PATN (Pattern Analysis Package, DOS version, Belbin 1994). Homogenous genetic groupings from a site were treated as a distinct Operational Taxonomic Unit (OTU). Two between-OTU estimates of genetic similarity were calculated, namely (1) percentage fixed differences (%FD, Richardson *et al*. 1986), allowing a 10% tolerance, and (2) Nei's unbiased Distance (Nei D, Nei 1978). The genetic affinities of OTUs or populations were displayed visually as UPGMA (unweighted pair-group method of arithmetic averages) dendrogram and neighbor joining (NJ) networks constructed from Nei D values using the NEIGHBOR routine in PHYLIP 3.5c (Felsenstein 1993) and drawn using TREEVIEW 1.6.0 (Page 1996). Allele frequencies, heterozygosity levels (H_O, direct count method) and genetic distances were calculated using BASIC programs written by M. Adams.

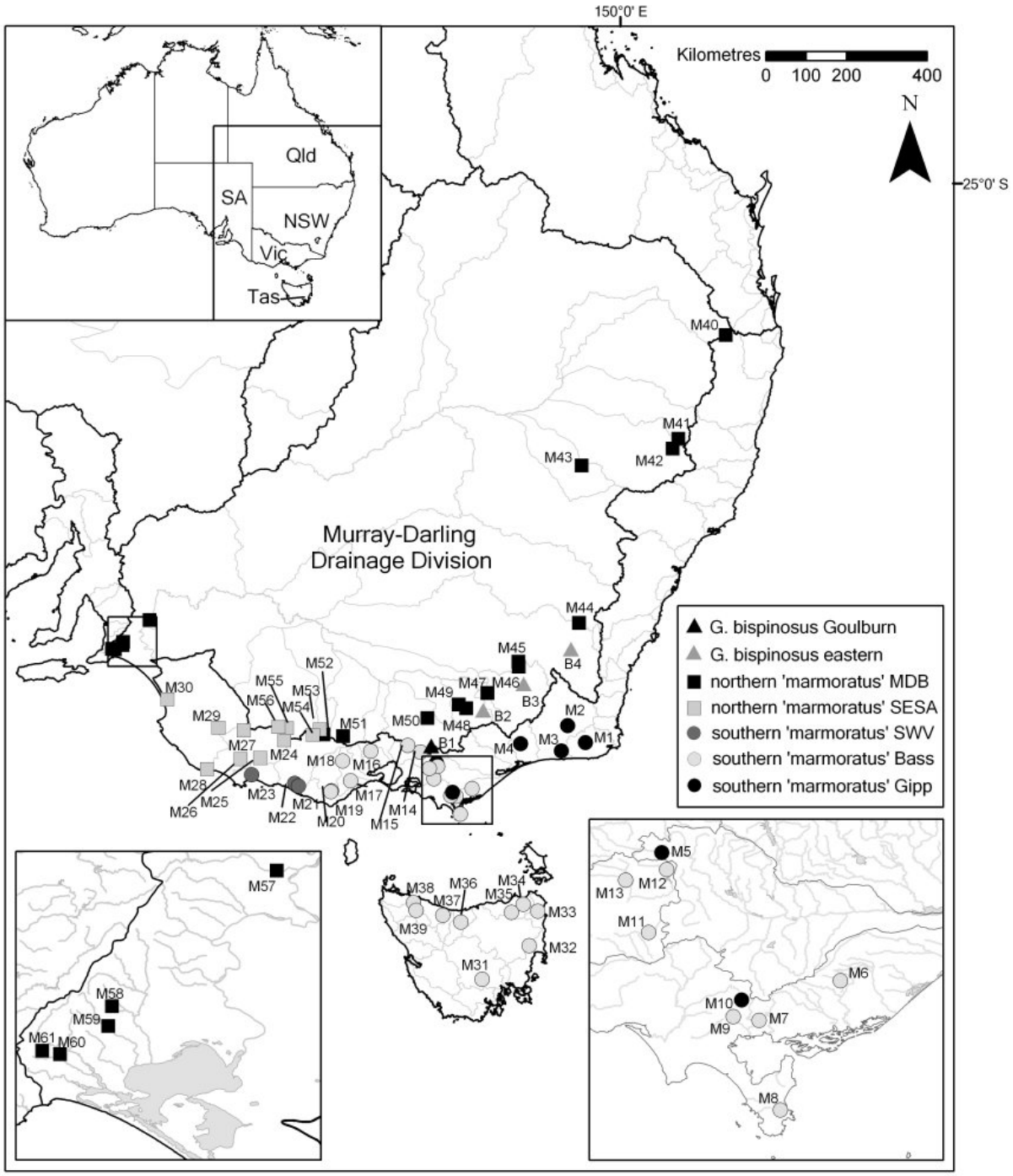

Figure 8-1. Geographic relationships of *Gadopsis* samples and their corresponding major genetic groups and lineages (abbreviations as per the text, sites codes as per Table 8-2). Also shown are major drainage divisions and river basins (AWRC 1976).

8.3. RESULTS

Major genetic groupings

Fifty putative loci were interpretable, 14 of which (*Ak*, *Ald*, *Ap1*, *Enol1*, *Fdp*, *Gapd1*, *Gapd2*, *Glo*, *Me*, *Ndpk1*, *Ndpk2*, *PepD2*, *Pk1*, and *Tpi1*) were invariant amongst 137 individuals screened (allozyme profiles are given in Appendix 5). An initial PCO (not shown) grouped individuals to one of two well separated and discrete clusters (i.e. no intermediate or hybrid forms were detected) in the first PCO dimension, although considerable additional heterogeneity was also evident for both clusters in the second and subsequent dimensions. These clusters aligned to the notional species *G. bispinosus* and *G. marmoratus* and were diagnosable at 13 loci (26%FD: Table 8-2). A PCO of the 14 *G. bispinosus* (not shown) revealed significant separation between sites, the most distinctive involving a Goulburn River sub-group diagnosable at three loci (6%FD: *Acp*, *Adh1*, *6Pgd*: Table 8-2).

An initial PCO of the 117 individuals assigned to *G. marmoratus* identified two primary clusters, aligning with abutting northern and southern major groupings (Figure 8-2). Examination of allele profiles between these taxa revealed six fixed differences (12%FD: *Acp*, *Acyc*, *Ada*, *Adh1*, 6*Pgd* and *Ugpp*), plus major differences in allele frequency and/or unique alleles at four other loci (*Acon3*, *Ap2*, *Mdh2* and *Pgm2*) (Table 8-2 and Table 8-3). Discrete lineages were also obvious within both northern and southern taxa (Figure 8-2), and these were supported by follow-up PCOs (not shown). Two sub-groups were evident within the northern taxon: (1) the MDB (excluding the Wimmera River Basin), and (2) the Millicent Coast, Glenelg and Wimmera river basins (herein SESA). Within the southern taxon, three sub-groups were obvious: (1) Portland Coast and Hopkins river basins (herein SWV), (2) a broad spread of locales in coastal Victoria, east of the Hopkins to Wilsons Promontory, and Tasmanian sites (herein Bass), and (3) Gippsland populations, one of which (Turtons Creek, site M10) was geographically adjacent to a Bass site (Deep Creek, site M9).

The distribution of all lineages is shown in Figure 8-1 and summaries of allele profiles and genetic divergence estimates between these sub-groups (%FDs and Nei D) are presented in Table 8-2 and Table 8-3. As none of the stepwise PCOs revealed any site to be genetically heterogeneous, individual sites were treated as OTUs for further comparisons. The genetic affinities among sites are displayed visually in the UPGMA dendrogram of Figure 8-3. Both this dendrogram and the corresponding NJ tree (analysis not shown) clearly portray the major genetic groups and lineages identified through PCO.

Genetic structure within groups

While the small sample sizes prevent a full, site based comparison, there is initial strong evidence for geographic patterns of sub-structure (Figure 8-3). All *G. bispinosus* sites show relatively-high levels of divergence, notably the Goulburn (as above), but all appear distinctive to varying degrees with at least one apparent (i.e. based on very small sample sizes) fixed difference (Appendix 5).

Within the MDB lineage of northern '*marmoratus*', Tookayerta Creek (sites M60-61) in the MLR was dissimilar to other proximate sites and distinctive at the drainage division level (i.e. one apparent fixed difference, major differences in allele frequency at four loci and many rare private alleles: Table 8-2). Surprisingly, populations from Queensland and northern NSW (sites M40-43) down to Victoria (sites M46-52) formed a homogenous 'widespread' population group. Several sites in southern NSW (Lachlan and upper Murray, sites M44-M45) and MLR catchments of the Lower Murray (sites M57-M59) displayed intermediate levels of genetic differentiation compared to the Tookayerta and widespread population groups. Little sub-structure was displayed in the other sub-group, SESA, with subtle distinction for two of three sites falling in Wimmera River Basin (i.e. sites M53 and M54). Site 55, Fyans Creek diversion, although within the Wimmera River Basin, was more similar to sites in the adjacent Glenelg River Basin.

The three sub-groups in southern '*marmoratus*' display varying degrees of genetic heterogeneity. SWV is comprised of two quite distinctive groups, one in the Hopkins River (including Mt Emu Creek, site M22) and the other in Darlot Creek (site M23), with three apparent fixed differences (Appendix 5). Gippsland shows a simple east *versus* west dichotomy. Bass has two contrasting scales of divergence: (1) the most distinctive sites are at the periphery, namely the Gellibrand River Basin in the west (sites M19-M20), the Ansons River in eastern Tasmania (the most divergent Bass population: site M33), and most sites at the eastern distribution (sites M6-M7, M9 and M12), and (2) sixteen central sites (Port Phillip Bay and Wilsons Promontory and the remainder of Tasmanian sites) together comprise homogenous population groupings.

Sufficient numbers of variable loci were detected in each taxon (16 in *G. bispinosus*, 20 in northern '*marmoratus*', and 12 in southern '*marmoratus*': Table 8-2) to permit a comparative assessment of observed heterozygosity, both within *Gadopsis* and other study groups. Given the very small sample sizes for most sites, H_O values were calculated for each lineage. All H_O values were low to moderate (range 0.015-0.042: Table 8-2), and there were no clear differences between the three taxa or lineages.

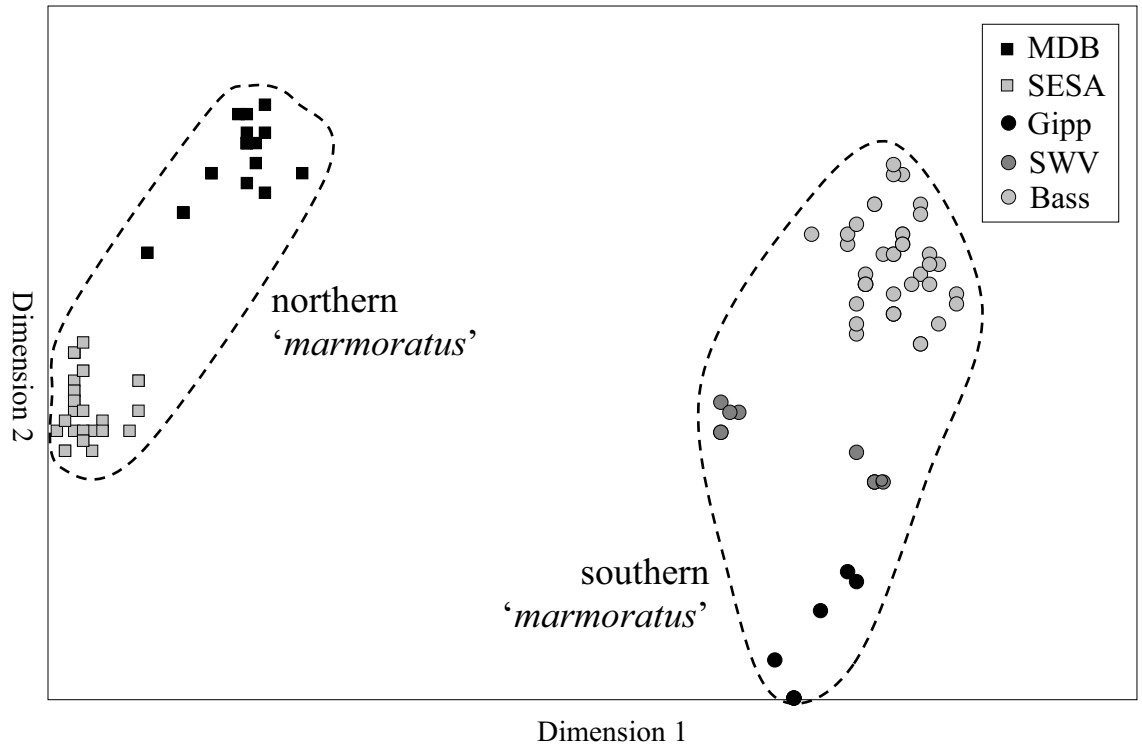

Figure 8-2. Principal Coordinates Analysis of 117 individuals representing 57 populations of *G. marmoratus*. The relative PCO scores have been plotted for the first and second dimensions, which explain 51% and 10% of the total variance, respectively. Names for lineages match Figure 7-1 and Figure 7-3 (Gipp = Gippsland).

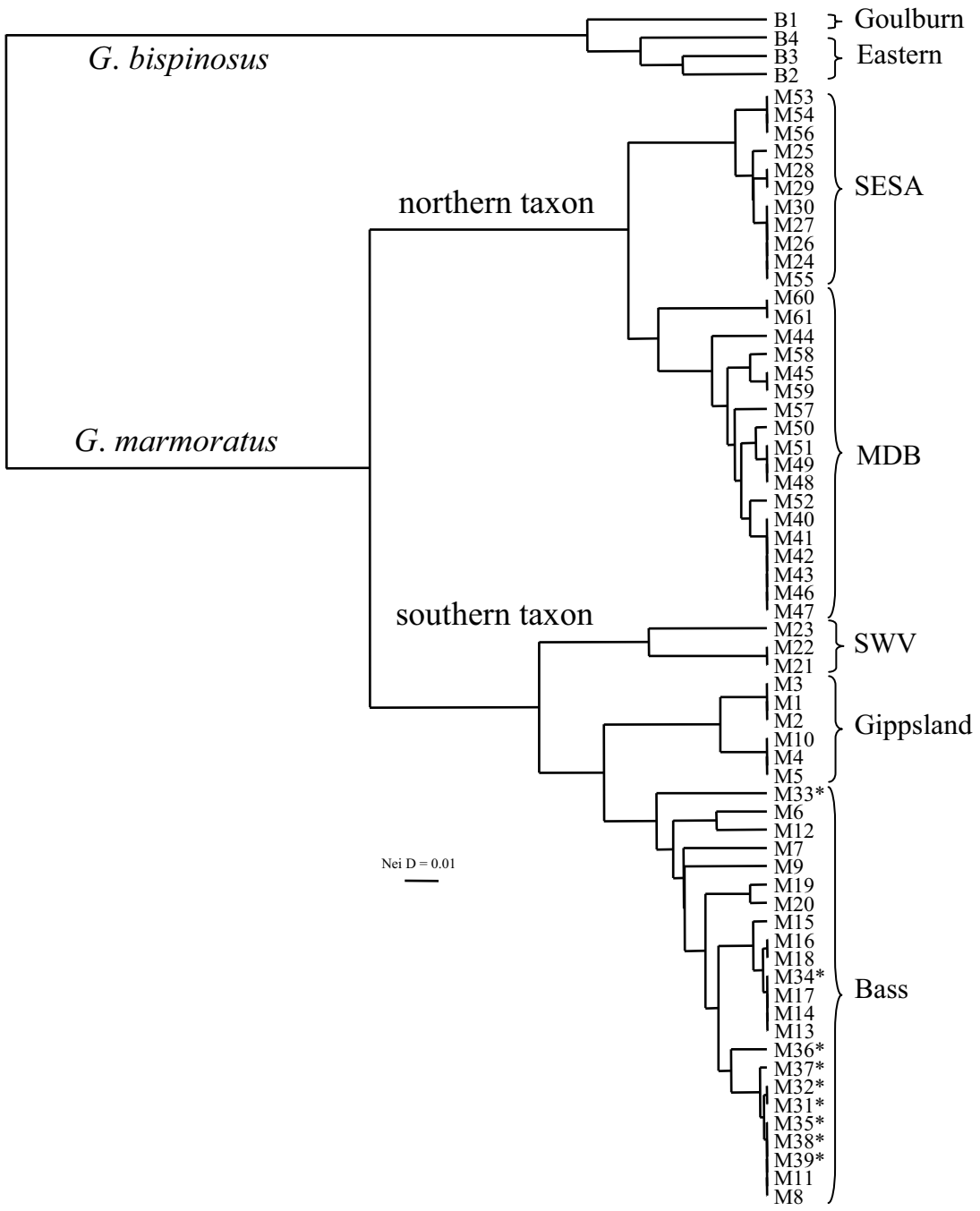

Figure 8-3. UPGMA dendrogram depicting genetic affinities between and within *Gadopsis* species based on pairwise Nei Distance values. Highlighted are major genetic groups (taxa) and/or lineages of *G. bispinosus* and *G. marmoratus s.l.* (*) denotes Tasmanian populations.

8.4. DISCUSSION

This chapter provides a genetic overview across the range of freshwater blackfishes, and demonstrates unequivocally the presence of distinct northern and southern species of *G. marmoratus*. Moreover, distinct genetic discontinuities with geographically-abutting lineages within *G. bispinosus* and *G. marmoratus s.l.* indicate the likely presence of multiple ESUs.

Taxonomic considerations

The allozyme overview study reinforces previously proposed species boundaries, confirming their broad application when examining the full distribution of the group. Accordingly, *G. bispinosus* is a valid and divergent species, there is clearly no Victorian *versus* Tasmanian split in *G. marmoratus*, and northern and southern taxa occur within *G. marmoratus* either side of the Great Dividing Range, then east and west of the Glenelg River (Sanger 1984). The degree of divergence within *G. marmoratus* matches that of a previous allozyme study (Ryan *et al*. 2004), although at a greater level of confidence, since the present study employs roughly double the number of loci (28 *v*. 50 herein) and samples the complete range (i.e. inclusion of 47 additional sites from central Victoria, Gippsland, Tasmania and the MDB). There is also congruent mtDNA support for species-level divisions based on representative samples for both taxa (Ovenden *et al*. 1988; Jerry 1997; Miller *et al*. 2004). Biological support is shown by the southern taxon growing to nearly twice the size of the northern taxon, but more comprehensive comparisons remain to be undertaken (Sanger 1984; Sanger 1986; Jackson *et al*. 1996). The only feature lacking for confident diagnosis appears to be reliable morphological characters (apart from size), but the neat geographic separation offers an interim means of field identification. The type locality for *G. marmoratus* is ambiguous ('rivers in the southern parts of Australia'), but could be from the River Murray, South Australia, and hence attributable to the northern species; the relevant type for the southern species is *G. gracilis* McCoy from the Yarra River (Eschmeyer 2008).

Genetic sub-structure

All three *Gadopsis* species display significant genetic sub-structure, both involving discrete lineages and more subtle, population groupings. A comprehensive assessment of the latter is constrained by the small sample sizes of the overview study, and a full characterisation remains for future study. Confirmation of the evolutionary distinctiveness of the Ansons River, Darlot River, Hopkins River and Tookayerta Creek populations is a high priority, while additional sample sizes and intervening sites in the range of *G. bispinosus* (e.g. Kiewa River, upper Mitta Mitta River) would also permit further examination of apparent high levels of sub-structure.

Congruent support for the distinct Goulburn River Basin population in *G. bispinosus* is provided by deep divergence at multiple mtDNA gene segments (Ovenden *et al*. 1993; Beitzel 2002)

The various *Gadopsis marmoratus s.l.* lineages align with neat geographic divides such as north and south of the Great Dividing Range, and east and west of the Glenelg River, Hopkins River and Wilsons Promontory. A minor exception involved the break between Bass and Gippsland lineages of southern *G. marmoratus*, where Turtons Creek represented an outlying Gippsland genetic type within the Bass geographic range. Turtons Creek abuts the headwaters of the La Trobe River (Gippsland lineage) and appears to share a relationship, either through natural or human-mediated dispersal, across the drainage divide. This pattern was also seen in *Nannoperca obscura* (Mt Emu Creek and Glenelg River similarity: Chapter 4), and in *N. australis* there was a similar interspersion of eastern populations within the western species of *N. australis* in the same area near Wilsons Promontory (Chapter 5). Another example of shared genetic similarity across different drainage boundaries included Fyans Creek (SESA lineage), a habitat with an artificial hydrological connection (diversion) between the Glenelg and Wimmera river basins. The presence of a Glenelg genetic type in the Wimmera may indicate recent accidental human-mediated gene flow across the drainage divide. The genetic similarity of southern Tasmania and the Wye River in eastern Tasmania to northern populations, but not the Ansons River, also suggests translocation, in this case for angling purposes (Jackson *et al*. 1996).

Estimates of observed heterozygosity applied to lineages indicated low to moderate levels of within population variability in *G. bispinosus*, and generally low levels within *G. marmoratus s.l.* The latter finding is broadly consistent with an extended population analysis (i.e. larger sample sizes) within southwestern Victoria (147 individuals from 14 populations across 28 allozyme loci: Ryan *et al*. 2004), although greater levels of polymorphism were revealed herein. Ryan *et al*. (2004) found just a single heterozygote in their analysis, whereas the present study identified 134 heterozygotes in 24 fewer individuals (but using twice the number of loci). Provided low heterozygosity estimates are validated by larger samples sizes (i.e. population studies), they may indicate low maintained effective population sizes (Nevo *et al*. 1984; Bazin *et al*. 2006), and thus match predictions derived from intrinsic characteristics such as low adult population density (i.e. home and territorial behaviour), and long generation time coupled with low fecundity (*K* selected species).

Biogeographic patterns

The observed genetic divisions in *Gadopsis* species offer further insight into the recent historic landscape of southern Australia. Firstly, *G. bispinosus* appears to have been isolated in the

Goulburn system for some time. Using mtDNA genetic distance estimates, the separation of a Goulburn haplotype was effected some 5-2 Mya (Ovenden *et al*. 1988; Beitzel 2002), matching uplift in the Pinnaroo Block, a period noted for drainage alteration and creation of lotic discontinuities between MDB drainages *via* Lake Bungunnia (Stephenson 1986). Nuclear gene flow also appears to have been limited for a significant period, if not as long as the mtDNA data suggest, implying effective long-term fragmentation in upland habitat. *Nannoperca australis* from the Goulburn system is also distinctive to some degree (most distinctive population in a lineage), but there is no similar distinction for *Retropinna* and *Philypnodon grandiceps*, and indeed *G. marmoratus*, which occur across the same region at lower altitudes.

The presence of an allozymically homogenous group of *G. marmoratus* across a wide area of the MDB suggests the opposite pattern to *G. bispinosus*, namely wide-dispersal through recent colonisation or episodic gene flow. Current environmental conditions in the northern half of the MDB in particular (i.e. mainly arid with warm, highly turbid waters), would not promote dispersal of *G. marmoratus*. Instead very different conditions must have prevailed in the past (e.g. a noted wetter period 6-5 Kya: White 2006) leaving populations recently fragmented to small sections of habitat along the cooler western edge of the Great Dividing Range. In contrast, the divergent population at Tookayerta Creek, a small catchment in lower Murray, appears to have been well isolated from gene flow, possibly reflecting long-term fragmentation above dense swamps in the lower catchment (similar to *N. australis*). Isolation of *Gadopsis* within the MDB appears to have been maintained by the physical barrier of the Great Dividing Range, with minor leakage into the Wimmera and potentially some historic dispersal between MDB and Millicent Coast River Basin (but not recent gene flow as per distinctiveness in *Nannoperca obscura* and *N. australis*).

The noted genetic break between MDB and SESA is congruent with moderate divergence in representative samples subjected to mtDNA sequencing (Ovenden *et al*. 1988; Miller *et al*. 2004). The species-level geographic divide east and west of the Glenelg system is similar to an ESU level split in *N. obscura* and might reflect a long-term barrier to gene flow between coastal systems in the area (e.g. Newer Volcanics, see Chapters 3-4). The homogenisation of central Victorian and northern Tasmanian populations indicates high connectivity and dispersal in the recent past, likely during or immediately after the last glacial maximum (similar to *Retropinna* and *N. australis*). Finally, alternate eastern and western drainage patterns around Wilsons Promontory through to eastern Tasmania appear to have limited gene flow, resulting in a major Gippsland lineage and distinctive populations in the Ansons River. These are patterns apparent also in *Retropinna* and *N. australis*.

Ecology

Taxonomic complexity within *Gadopsis* again confounds the interpretation of ecological data, but the 20 years since formal recognition of *G. bispinosus* and informal recognition of northern and southern forms of *G. marmoratus* has already allowed some assignment of biological data to taxon (Jackson *et al*. 1996; Lintermans 2007; Koster and Crook 2008). The noted habitat specificity *G. bispinosus* restricting it to upland fragments has support from matching moderate to high levels of genetic sub-structure. Based on homogenous groups in the northern and southern species of *Gadopsis marmoratus s.l*., the trait of larger body size, relative to preceding study species (Chapters 4-7), does appear to have facilitated wider dispersal and gene flow. This may not necessarily stem from greater vagility *per se*, but could reflect a species ecologically suited to the sorts of environments that promote wider dispersal (e.g. larger rivers) *via* population expansion and intermixing over generations. Nevertheless, the other and contrasting life-history traits of demersal larvae, habitat specificity and low vagility appear to have restricted gene flow in discontinuous historic environments.

Conservation

The likely inability of most blackfishes to move between disjunct habitats or those with unsuitable intervening connections has implications for their long-term survivorship as habitats are transformed by human agencies, leaving populations vulnerable to decline and extirpation. The lower and upper Murray, once thought to be connected by a continuous population in the main River Murray channel post-European settlement (e.g. Scott *et al*. 1974), are now fragmented due to an altered, static and turbid riverine environment. Likewise, in the SESA lineage the most northerly population at Henry Creek is fragmented and its habitat increasingly contracted by an extensive drainage scheme, and the genetic sample included in this study came from a diseased fish, being the last individual observed at the site despite intensive surveys (Hammer 2002, 2005, 2007c). Climate change could exacerbate hydrological change from significant abstraction, especially for small remanent populations (Bennett 2002; McInnes *et al*. 2003).

Seven major genetic groupings across the three *Gadopsis* species form putative ESUs awaiting a collaborative DNA study underway utilising the same samples as employed here (Unmack *et al*. in prep.). In the interim, representative samples from other partial studies support several lineages as true ESUs, namely Goulburn and eastern ESUs in *G. bispinosus* and MDB and SESA ESUs within northern *G. marmoratus* (Ovenden *et al*. 1988; Beitzel 2002; Miller *et al*. 2004). Population isolates and distinctive sites and regions represent notable MUs for conservation.

8.5. TABLES

Table 8-1. Locality and sample size information for *Gadopsis* species. Site numbers match those in Figure 8-1. DD = Drainage Division; RB = River Basin (AWRC 1976). Sample sizes in parenthesis represent fin clip samples for highly restricted populations.

Site	Field code	Locality	State	DD	RB	Latitude (S)	Longitude (E)	*n*
B1	PU02-05	Taggerty R., Marysville	Vic.	4	5	37º30'	145º46'	3
B2	F-FISHY4	Ovens R., Bright	Vic.	4	3	36º42'	146º55'	4
B3	F-FISH93	Cudgewa Ck, Cudgewa	Vic.	4	1	36º07'	147º49'	3
B4	F-FISH98	Cotter R., Vanities Crossing	ACT	4	10	35º20'	148º53'	4
M01	TR02-24	Back Creek, Noorinbee North	Vic.	2	21	37º25'	149º12'	2
M02	PU02-63	Delegate R., Delegate	NSW	2	22	37º02'	148º48'	2
M03	PU02-64	Brodribb R.	Vic.	2	22	37º36'	148º40'	1
M04	PU02-66	Haunted Stream	Vic.	2	23	37º27'	147º45'	2
M05	PU02-81	LaTrobe R., Noojar	Vic.	2	26	37º52'	145º53'	2
M06	PU02-97	Greig Ck, Yarrum	Vic.	2	27	38º26'	146º41'	2
M07	PU02-101	Tin Mine Ck	Vic.	2	27	38º37'	146º19'	1
M08	PU02-72	Blackfish Ck, Wilsons Prom.	Vic.	2	27	39º01'	146º25'	2
M09	PU02-95	Deep Ck, Forster	Vic.	2	27	38º36'	146º12'	2
M10	PU02-78	Turtons Ck	Vic.	2	27	38º32'	146º14'	2
M11	PU02-105	Minnieburn Ck	Vic.	2	28	38º14'	145º50'	2
M12	PU02-82	Tarago R.	Vic.	2	28	37º57'	145º54'	2
M13	PU03-05	Diamond Ck, Tonimbuk	Vic.	2	28	38º00'	145º43'	2
M14	TR02-268	Donnellys Ck, Healesville	Vic.	2	29	37º38'	145º32'	2
M15	TR02-16	Running Ck, Kinglake	Vic.	2	29	37º29'	145º14'	2
M16	TR02-210	Lerderderg R.	Vic.	2	31	37º37'	144º25'	2
M17	PU02-85&108	Barwon R., Winchelsea	Vic.	2	33	38º16'	143º58'	2
M18	TR02-373	Kuruc-A-Ruc Ck, Dereel	Vic.	2	34	37º50'	143º47'	2
M19	PU02-109	Loves Ck, Gellibrand	Vic.	2	35	38º30'	143º33'	2
M20	F-FISH93	Gellibrand R., Gellibrand	Vic.	2	35	38º31'	143º32'	2
M21	PU03-08&09	Brucknells Ck, Naringal East	Vic.	2	36	38º23'	142º48'	3
M22	PU02-112&03-07	Mount Emu Ck, Panmure	Vic.	2	36	38º20'	142º43'	2
M23	F-FISH93	Darlot Ck, Haywood	Vic.	2	37	38º08'	141º46'	3
M24	PU02-117/118	Wannon R., Grampians NP	Vic.	2	38	37º22'	142º29'	4
M25	F-FISH93	Muddy Ck, Hamilton	Vic.	2	38	37º46'	141º57'	2
M26	PU00-19	Stokes R., Digby	Vic.	2	38	37º48'	141º31'	2
M27	F-FISH93	Glenelg R., Harrow	Vic.	2	38	37º09'	141º35'	1
M28	F-FISH99	Ewens Ponds, Mt Gambier	SA	2	39	38º01'	140º46'	2
M29	TR02-194	Mosquito Ck, Langkoop	Vic.	2	39	37º06'	141º02'	2
M30	F-FISH98	Henry Ck, Kingston	SA	2	39	36º27'	139º53'	1
M31	F-FISHY4	Styx R., Bushy Park	Tas.	3	4	42º42'	146º54'	2
M32	F-FISH98	Wye R., Swansea	Tas.	3	2	41º57'	147º57'	2
M33	F-FISH98	Ansons R., Ansons Bay	Tas.	3	2	41º10'	148º08'	1
M34	F-FISH98	Boobyalla R., Winnaleah	Tas.	3	19	41º01'	147º49'	1
M35	F-FISH98	Great Forester R., Scottsdale	Tas.	3	19	41º12'	147º33'	1
M36	F-FISH98	Minnow R., Beulah	Tas.	3	16	41º25'	146º25'	2
M37	F-FISH98	Leven R., Gunns Plains	Tas.	3	14	41º16'	146º01'	2
M38	F-FISH98	Black R., Mawbanna	Tas.	3	14	40º59'	145º22'	2
M39	F-FISH98	Relapse Ck	Tas.	3	12	41º09'	145º26'	2
M40	PU99-46	Browns Ck, Killarney	Qld	4	22	28º21'	152º20'	2
M41	TR01-305	Molong Ck, Uralla	NSW	4	18	30º40'	151º16'	2
M42	PU99-38	McDonald R., Bendemeer	NSW	4	19	30º52'	151º09'	2

Site	Field code	Locality	State	DD	RB	Latitude (S)	Longitude (E)	*n*
M43	PU 99-37	Shawns Ck, Coonabarabran	NSW	4	20	31º15'	149º07'	2
M44	F-FISH98	Catherines Ck, Dalton	NSW	4	12	34º46'	149º04'	2
M45	TR02-455	Stony Ck, Carabost	NSW	4	10	35º37'	147º43'	2
M46	PU99-82	Coppabella Ck, Coppabella	NSW	4	1	35º44'	147º43'	1
M47	TR02-295	Kiewa R., Kergunyah	Vic.	4	2	36º19'	147º01'	1
M48	TR02-312	Scrubby R., Carboor East	Vic.	4	3	36º39'	146º33'	3
M49	PU99-80	King R., Moyhu	Vic.	4	3	36º34'	146º23'	2
M50	PU00-05	Seven Ck, Strathbogie	Vic.	4	5	36º52'	145º41'	2
M51	TR02-230	Birch Ck, Clunes	Vic.	4	7	37º17'	143º48'	2
M52	TR02-236	Avoca R., Mt Lonarch	Vic.	4	8	37º15'	143º22'	2
M53	TR02-245	Nowhere Ck, Elmhurst	Vic.	4	15	37º08'	143º17'	2
M54	PU00-06	Mount Cole Ck, Warrak	Vic.	4	15	37º15'	143º08'	1
M55	PU00-08	Fyans Ck diversion	Vic.	4	15	37º06'	142º33'	1
M56	F-FISH99	McKenzie R., Zumsteins	Vic.	4	15	37º04'	142º22'	4
M57	F-FISHY4	Marne R., Black Hill	SA	4	26	34º42'	139º29'	1(3)
M58	F-FISHY4	Rodwell Ck, Mt Barker	SA	4	26	35º11'	138º54'	1(3)
M59	F-FISH93	Angas R., Strathalbyn	SA	4	26	35º15'	138º53'	2
M60	F-FISHADD6	Tookayerta Ck, Mt Compass	SA	4	26	35º21'	138º43'	1
M61	TR02-172	Nangkita Ck, Mt Compass	SA	4	26	35º20'	138º39'	4

Table 8-2. Allele frequencies at all loci for the seven lineages (and the most distinctive MDB catchment: Tookayerta Creek, Lower Murray) identified for *Gadopsis* species. All individuals were invariant at the following 14 loci: *Ak*, *Ald*, *Ap1*, *Enol1*, *Fdp*, *Gapd1*, *Gapd2*, *Glo*, *Me*, *Ndpk1*, *Ndpk2*, *PepD2*, *Pk1*, and *Tpi1*. Sample sizes are shown in parentheses.

	Gadopsis bispinosus **---**	**Northern *G. marmoratus* ---**		**----**	**Southern *G. marmoratus* ----**			
	Goulburn B1	**Eastern B2-4**	**MDB M40-59**	**MDB M60-61**	**SESA M24-30**	**SWV M21-23**	**Gippsland M1-5,10**	**Gippsland M1-5,10**
Locus	**(3)**	**(11)**	**(35)**	**(5)**	**(22)**	**(8)**	**(11)**	**(11)**
Acon1	b	b^{77},a	c	c	c^{90},d	c	c	c
Acon2	b	b^{50},a^{44},d	f^{92},c^{6},g	f	e^{71},f	f^{71},e	e	e
Acp	a	b	c	c	c	a	a	a
Acyc	d	d	b	b	b^{95},c^{3},a	c	c^{55},b	c^{55},b
Ada	b	b	b^{94},a	b	b	c	b	b
Adh	a	b	c	c	c	b	b	b
Ap2	a	a	c	a	a^{95},c	b	a	a
Ck	a	a	b	b	b	b	b	b
Enol2	b	b^{82},c	b^{97},a	b	b	b	b	b
Est	b	b	b	b	b^{95},d	b^{87},a	b^{55},c	b^{55},c
Fum	b	b	a	a	b	b	b	b
Got1	a	a	b	b	b	b	b	b
Got2	e	e	d^{98},c^{1},a	d	d^{98},f	b^{63},d	d	d
Gp	a	a	c^{98},b^{2}	c	c	c	c	c
Gpd	a^{50},b	b	b	b	b	b	b	b
Gpi1	c	c^{64},b^{32},d	c^{66},a^{19},b^{9},d	a	c	c	c^{91},b	c^{91},b
Gpi2	b	b	b	b	b	b^{62},a	b	b
Gsr	b	b^{86},c	a	b^{70},a	a	a	a	a
Idh	b	b^{77},a	b	b	b	b	b	b
Ldh	b	b	b	b	b	b	b	b
Mdh1	b	b	c	c	c	a^{94},c	a	a
Mdh2	b	b^{95},a	b	b	b	b	b	b
Mdh3	b	b	b^{97},d	b	b	a^{81},b	a	a
Mpi	f	f	a^{56},d	a	c^{98},b	d	d	d
PepA1	a	a	a	a	a	a	a	a
PepA2	c	c	a	a	a	a	a^{95},b	a^{95},b
PepB	d	d^{75},c	b^{96},c	b	b^{95},c	a	a^{50},b	a^{50},b
PepD1	b	b^{95},c	b^{97},a	b	b	b	b	b
Pgam	b	b	b^{90},a	b	b	b	b	b
6Pgd	c^{83},d	b	c	c	c	b	b	b
Pgk	b	b	b^{96},a	a^{50},b	b	b	b	b
Pgm1	c	c^{82},b	c	c	c	c	c	c
Pgm2	b	b	b	b	b	b	b	b
Pk2	d	d	b	b	b^{88},a^{7},c	b	b	b
Tpi2	c^{83},a	a^{64},c	c	c	c	c	c^{95},b	c^{95},b
Ugpp	d	d^{64},e	a^{98},b	a	a	a	b	b
H_O	0.020	0.042	0.023	0.024	0.014	0.015	0.027	0.027
S.E.	0.011	0.016	0.009	0.017	0.007	0.009	0.019	0.019

Table 8-3. Pairwise genetic comparisons among the seven major genetic groupings (and a distinctive MDB catchment: Tookayerta Creek, Lower Murray) identified for *Gadopsis* species. The lower triangle represents %FD and the upper triangle is Nei's unbiased D. Lineages match lineages in Figure 8-1 and Figure 8-3.

	Gadopsis bispinosus		**Northern *G. marmoratus***			**---- Southern *G. marmoratus* ----**		
Lineage	**Goulburn B1**	**Eastern B2-4**	**MDB M40-59**	**MDB M60-61**	**SESA M24-30**	**SWV M21-23**	**Bass M6-20**	**Gippsland M1-5,10**
B1	-	0.08	0.46	0.44	0.39	0.48	0.43	0.42
B2-4	6	-	0.49	0.46	0.41	0.44	0.39	0.39
M40-90	36	38	-	0.05	0.07	0.24	0.23	0.23
M60-61	34	36	2	-	0.09	0.31	0.26	0.27
M24-30	32	34	6	6	-	0.23	0.17	0.17
M21-23	36	34	18	22	18	-	0.11	0.10
M6-20	32	30	18	20	14	6	-	0.07
M1-5,10	34	32	18	20	14	6	4	-

9. DISCUSSION

9.1. TAXONOMIC CONSIDERATIONS

The disparity between existing taxonomy and the results of this study argues for the re-examination of systematic frameworks underlying all Australian freshwater groups, to ensure that conservation and research are based on sound data. Examination of species boundaries in five groups (excluding the more specific focus in *Mogurnda adspersa*) shows that the actual number of included species should at least double (i.e. two becomes five in *Retropinna*, one becomes two in *Nannoperca australis*, two becomes at least four in *Philypnodon*, and one becomes two in *Gadopsis marmoratus*). The discrepancies in the actual number of species, as revealed by molecular markers, show that the richness and diversity of the fauna is not adequately reflected in conservation and natural resource management (Pfenninger and Schwenk 2007). It is particularly significant given the low species richness of the local fauna, as seven new species from only a handful of the possible study groups would represent a 5-10% increase in the number of obligate freshwater fishes in Australia, and proportionally more (10-20%) for southeastern Australia (cf. Unmack 2001; Allen *et al*. 2002).

It is surprising that cryptic speciation, where distinct but morphologically similar or identical species were classified as a single species (Bickford *et al*. 2007), occurs in a well-studied and densely-populated region of Australia. The large number of other putative single freshwater species occurring across noted biogeographic divides in the same region, and across the remainder of Australia, implies that many additional cryptic species remain to be discovered before a full inventory is available. Prime candidates for further comparative molecular analyses in southern Australia include the mountain galaxias *Galaxias olidus* and dwarf galaxias *Galaxiella pusilla* as southern endemics with distributions complementary to major biogeographic breaks noted herein. Southwestern Western Australia is a hotspot for endemic fauna and flora, including obligate freshwater fishes (Morgan *et al*. 1998). The precedent in the southeast plus preliminary indications of cryptic speciation in the western pygmy perch *Nannoperca vittata* (Unmack *et al*. in review) and western minnow *Galaxias occidentalis* (Watts *et al*. 1995) calls for detailed study in this region.

A need for further work has been suggested in preceding chapters to resolve issues in *Retropinna* and *Philypnodon*, while species complexes are already indicated by phylogeographic studies on numerous other temperate-subtropical/tropical species along the east coast (Musyl and Keenan 1992; Page *et al*. 2004; Wong *et al*. 2004; Thacker *et al*. 2007; Jerry 2008). Thus, complementary

or extended analyses (i.e. beyond the sequence data from single mtDNA gene portions, currently the best molecular data available) are likely to be taxonomically informative. The wide distribution of purple-spotted gudgeons (genus *Mogurnda*) is likely to harbour multiple species (Hurwood and Hughes 1998; Allen and Jenkins 1999; Faulks *et al*. 2008, Adams *et al*. unpublished data). Indeed, precedent in southern Australia and initial studies on temperate-tropical subtropical groups, combined with theoretical prediction (Pfenninger and Schwenk 2007), suggest that northern Australia, where most freshwater species reside, is likely to be even more replete with morphologically-cryptic species. Studies extending into more remote areas of Australia not only have the potential to identify cryptic species, but outright new discoveries (Ivantsoff *et al*. 1991; Pusey and Kennard 2001; Hammer and Walker 2004; Morgan and Gill 2004)

As part of a combined-evidence approach to assess species boundaries, yard sticks of allozyme divergence were used to gauge major genetic groupings in support of species or putative species (taxa), employing a multi-locus approach to assess presumed neutral nuclear genetic characters. One of the challenges associated with molecular identification of cryptic speciation is to provide matching morphological support to allow ready discrimination. In many cases, groups may not have been subject to detailed morphological appraisals and molecular evidence may subsequently guide a search for distinguishing characters (e.g. Crowley and Ivantsoff 1990; Musyl and Keenan 1992; Bertozzi *et al*. 2000). Conflicts in taxonomic interpretation between different authorities are common in species synonymy, and, with the benefit of molecular insight, previously identified forms can be re-erected (e.g. Allen and Jenkins 1999). Ultimately, as may be the case in some of the cryptic species in *Retropinna* and for *Gadopsis marmoratus*, morphological plasticity, conserved features and/or specimen fragility may render species truly cryptic on morphological grounds and subsequently a combined-evidence approach is reduced to molecular and ecological criteria to delineate distribution and occurrence (e.g. Egge and Simons 2006). Unfortunately, two major impediments to the likelihood of morphological data becoming available on these cryptic freshwater species are the diminishing capacity and resources of Australian institutions to undertake biodiversity-related research (Leis *et al*. 2007; Hoese *et al*. 2007).

The occurrence of sporadic hybridisation between similar (e.g. *Nannoperca australis s.s.* × *N.* 'flinders') and more distantly-related species (e.g. *Philypnodon grandiceps* × *P. macrostomus*, *N. australis* × *N. obscura*) continues a trend previously observed among southeastern Australian fishes (Douglas *et al*. 1995; Jerry *et al*. 1999; Bertozzi *et al*. 2000), and is symbolic of the fluid nature of species interactions in freshwater environments (Hubbs 1955; Verspoor and Hammar 1991). It appears that recently-diverged species of freshwater fish are likely to continue to

undergo sporadic introgression should they meet naturally under conditions favourable to hybridization. A predisposition to hybridisation requires some flexibility in the philosophy and interpretation of species boundaries (Georges *et al*. 2002), but the process can at the same time form part of a selection feedback loop to consolidate species boundaries in zones of contact between previously allopatric taxa (e.g. Hoskin 2007).

A limited ability to detect hybridisation and introgression between major genetic groups is one of several reasons why sole use of mtDNA sequence data in species-level studies is problematic (Moritz and Cicero 2004; Will *et al*. 2005; Hickerson *et al*. 2006). Others include the retention of ancestral polymorphism, stochastic lineage sorting, male-biased gene flow, selective sweeps, unrecognised nuclear paralogues, and possibly even gene conversion (Avise 2000; Nichols 2001; Avise 2004; Moritz and Cicero 2004; Tatarenkov and Avise 2007). Indeed, empirical studies of a wide range of animal groups have demonstrated that mtDNA gene trees differ significantly (to the point of appearing incompatible) from their underlying species trees in 20-40% of the cases examined (Funk and Omland 2003). This highlights the fundamental need for multiple genetic markers and bi-parental inheritance in molecular systematics (Richardson *et al*. 1986; Moritz and Cicero 2004). In combination with the ability of allozyme analysis to rapidly and inexpensively provide a large number of co-dominant nuclear markers (Verspoor *et al*. 2005), the technique remains as an important part of the combined-evidence approach for resolving species boundaries.

9.2. GENETIC SUB-STRUCTURE

Southern Australia represents an important hub of genetic diversity and evolutionary potential in freshwater fishes. Population sub-structure was assessed either through detailed population studies or by qualitative analysis of the allozyme data obtained during overview studies. In all species groups examined there was major genetic division between at least two and up eight parts of the range, and significant between-population diversification in all or some of the range. This outcome is especially noteworthy given that (1) allozymes are reasonably 'conservative' genetic markers which underestimate diversity present in the actual genes surveyed (Murphy *et al*. 1996), (2) population studies based on allozymes are prone to Type II statistical errors, when the analysis fails to reject the null hypothesis even though genuine differences exist (Richardson *et al*. 1986), (3) the relatively-small sample sizes in population studies do not permit the detection of subtle sub-structuring (i.e. $\Delta p < \sim 40\%$), with obviously additional limitations again for overview studies. Together these caveats indicate that the degree of sub-structuring detected can only be an underestimate of that which genuinely exists, especially in complex areas where greater spatial resolution and/or sample sizes have been recommended (i.e. coastal range of *Retropinna* and *Philypnodon*, MDB range of *Nannoperca australis* and *Gadopsis bispinosus*, and general range of *G. marmoratus s.l.*).

Patterns of genetic sub-structure showed varied spatial scales across species groups, with partial congruence to regional patterns. High sub-structure was most apparent on the east coast in southern Queensland and northern New South Wales, eastern Tasmania, most of coastal Victoria, and between discrete sections of the Murray-Darling Basin (MDB). While high genetic sub-structure was the norm, some genetic types appear to have greater potential or ability for wide dispersal than others within the same taxon or lineage. Genetically more-homogeneous clusters tended to occur nested among other more distinctive population groups, especially across Bass Strait (all groups examined) and within large parts of the MDB (half of species examined). A typical example of these partially conflicting scales was MDB *Gadopsis marmoratus*, which had a generally widespread homogenous group but with one geographically integrated distinctive population (Tookayerta Creek). Similarly, Mt Emu Creek *Nannoperca obscura* formed an outlying western genetic population in the geographic range of an eastern lineage, and *Philypnodon macrostomus* was overall highly structured but had a homogeneous widespread grouping in the MDB. So, while at least partially corresponding to definable geographic regions, the contemporary geographic distribution of species is not necessarily a good indicator of genetic relatedness, as geographic proximity may mask divergent evolutionary histories (e.g. Antunes *et al*. 2001; McGlashan and Hughes 2001).

9.3. BIOGEOGRAPHIC PATTERNS

The biogeographic focus of this study has been to examine patterns of major genetic structure as continuing evidence for future consideration of underlying processes. Investigation of multiple and largely co-occurring groups has highlighted numerous localised patterns attributable to geographic features, with congruence across one or more species groups. However, an overall incongruence across groups indicates that the formation of genetic breaks operates at many scales and involves a range of different evolutionary processes, including the potential interaction of intrinsic biological traits and extrinsic environmental conditions (cf. Tibbets and Dowling 1996; McGlashan and Hughes 2002; Burridge *et al*. 2008). The formation and preservation of historic patterns in contemporary gene pools rely on the deterministic or stochastic ability of organisms (and their genes) to (1) disperse, (2) colonise areas, and (3) either persist in new areas (i.e. non-extirpation) or integrate into or replace other entities and thus continue a genetic heritage (i.e. nuclear or matrilineal signatures). Only greater replication across additional fishes and other aquatic fauna will help to fully unravel biogeographic patterns and processes in southern Australia. Findings from this study are placed in perspective of existing biogeographic provinces and regions.

At the broadest level, patterns supported freshwater fish biogeographic provinces proposed by Unmack (2001), namely separation provided by the Great Dividing Range, Wilsons Promontory, disconnection at the Murray Mouth and barrier between the northwestern MDB and Lake Eyre Basin into four main provinces: Eastern, Bass, Murray-Darling and Australian Central (Figure 1-2). Patterns have been discussed comparatively in chapters, but as an example the boundary between Eastern and Bass provinces, roughly aligned to Wilsons Promontory, showed species levels splits in *Nannoperca australis s.l.* and *Retropinna semoni s.l.*, the distributional limit for *Philypnodon macrostomus s.l.*, and major genetic divergence (provisional ESUs) in *Gadopsis marmoratus s.l.* Instances of recent historic dispersal across (and within) provinces were nevertheless apparent, including localised points of movement across the Great Dividing Range (e.g. Burnett River and Glenelg River) and coastal movement potentially occurring at times of greater drainage connectivity (coalescence) or flood plumes (e.g. Lower Murray and Millicent Coast river basins, Wilsons Promontory). Human-mediated translocation probably affected at least two groups (*Philypnodon* and *Gadopsis*).

As part of biogeographical analysis, Unmack (2001) grouped river basins into discrete regional clusters based on species distribution patterns, and those for southern Australia (plus a further distinction, Wimmera River Basin) are shown in Figure 1-2. Numerous genetic groups or lineages identified in this study were aligned with these boundaries, and were thus similar to patterns of

diversity and distribution in described taxa. In the Eastern Province biogeographic province of Unmack (2001), the Fitzroy River, Southeast Queensland and Northeast New South Wales regions had matching genetic breaks in one or more groups. Southeast Queensland as a region of high biodiversity could be further split at the Mary River to bolster mounting evidence for faunal distinctiveness in many groups (summarised in Chapter 7). There was only clinal distinction in groups across northeastern and southeastern coastal New South Wales, but the Clarence was distinct within the former. Southeastern Victoria had divergent populations of *Nannoperca* 'flinders', *Gadopsis marmoratus* and *Philypnodon grandiceps*. In contrast regions on either side of Bass Strait were similar, albeit with distinctive populations (multiple groups), especially in the Ansons River, Tasmania and the Glenelg River, Victoria. The presence of four distinct sections within the MDB (akin to biogeographic sub-regions) were well supported across most taxa, but included numerous anomalies such as the Lachlan, Goulburn and eastern Mount Lofty Ranges. Indeed, the Lower Murray was an important hub of genetic diversity, with one or more distinctive populations in all groups examined except *Philypnodon*. This diversity is particularly significant considering all populations represent range outliers of locally or nationally threatened species, notably including native *Mogurnda adspersa*, thought previously extinct from the southern MDB.

9.4. ECOLOGY

The initial aim of this study included a strong focus on contrasting and integrating the intrinsic biological characteristics of a suite of largely co-occurring species. This was achieved to a large degree for traits such as diadromy (*Retropinna*), demersal adults and larvae (*Nannoperca*), habitat specialisation (*Mogurnda* and *Gadopsis*) and salt tolerance (*Philypnodon*) (Appendix 6). However, broad comparisons of the interaction between intrinsic biological characters and extrinsic environmental divisions were confounded by taxonomic complexity, varying spatial scales of genetic units and stochastic evolutionary processes. These factors ensured that a mismatch of major genetic groupings of differing levels of divergence occurred in particular geographic areas and, even within lineages, differing patterns of sub-structure occurred (e.g. divergent local populations *v*. broad homogenous groupings). Cross-group comparisons would succeed better if they were to use locally-gathered ecological information to align with population genetic data, once a sound systematic framework is in place. For example, comparative genetic distinctions within multiple species for the lower Murray region or across the Lower Murray compared to the Millicent Coast River Basin conform well to the hypothesised magnitude of genetic structure at the outset (Appendix 6).

Prior knowledge of native fish ecology in Australia could be undermined by future changes to systematic frameworks in many groups. Importantly, similar physical appearance does not necessarily correspond to ecological similarity. An appropriate historic example concerns MDB *Maccullochella*, where the presence of two species went unrecognised for some time, as did now-obvious ecological differences in habitat/flow requirements, time of spawning and conservation status (Koehn and Harrington 2006; Lintermans 2007; Nicol *et al*. 2007). A similar scenario applies to the two species of *Gadopsis* in the MDB (Sanger 1984), while ecological heterogeneity is suggested in *Nannoperca australis s.s*. represented by separate genetic lineages and habitats in Tasmania and the MDB (Chapter 5). In southern endemic species, regional populations attracting conservation concern were often realised as distinct genetic entities, and may show some ecological divergence that disposes them to anthropogenic change. Pooling data derived from locations spread across the geographic range of presumed single species is likely to conceal any ecological heterogeneity resulting from evolutionary divergence in discrete drainage divisions, groups of river basins or even particular river systems. The ‘take home’ message here is that there can be no substitute for local understanding of species’ biology and status to underpin research, conservation and management, and assessments that by necessity utilise information from other regions must be mindful of the potential implications.

9.5. CONSERVATION

A failure to recognise the full complement of species and conservation units of southern Australian freshwater fishes is very likely to undermine biodiversity conservation, research and natural resource management. Degradation of habitats, given an already-extensive list of local extinctions and imperilled populations, and ongoing habitat and climate change (e.g. Wager and Jackson 1993; McDowall and Fulton 1996; Hammer *et al*. 2007a), highlight the risk of losing distinct evolutionary components and even species before they can even be identified. The natural propensity of freshwater fishes to hybridise suggests that any rapid modifications to aquatic environments are likely to exacerbate the incidence and severity of introgression between sympatric congeners (e.g. Seehausen *et al*. 1997; Fisher *et al*. 2006). Human-mediated translocations, *via* vectors such as interbasin water transfers, stocking, and use of fish as bait, also pose heightened genetic risk in freshwater environments and warrant particular attention in catchment and fisheries management (e.g. Esa *et al*. 2000; Hughes 2003; Ferguson 2004).

The rationale behind characterising evolutionary components within species is to (1) identify higher-level genetic distinctions reflecting long isolation and shared *versus* separate evolutionary heritage and their potential as targets for conservation (i.e. Evolutionarily Significant Units, ESUs), and (2) document smaller scale sub-populations that harbour such low levels of gene flow as to be functionally independent and form the focus of population monitoring and demographic study (i.e. Management Units, MUs, analogous to 'stocks' in fisheries management). Crandall *et al*. (2000) have pointed out that the ESU and MU concepts were originally devised to include morphological and ecological features (i.e. phenotypes likely to result from the expression of non-neutral genetic markers), with the phylogeographic insight provided by neutral markers being but one component of any assessment. However, the combination of current socio-political climates, an urgent need to address human-mediated habitat degradation, and the increased analytical power now provided by molecular datasets is a powerful argument for a two-stage approach. Stage I would involve the designation of provisional ESUs and MUs based on a rapid, low-cost assessment of the genetic affinities among regional populations for mtDNA phylogeography plus multiple nuclear markers (i.e. allozymes), with an initial focus on threatened species or 'common' species showing regional decline. Stage II would involve follow-up comparative studies of the different provisional conservation units assessing biological parameters.

While assessment of ESUs in Australia has been undertaken for terrestrial vertebrates (e.g. Firestone *et al*. 1999; Cooper *et al*. 2000; Scott and Keogh 2007), and some aquatic species including freshwater isopods (Gouws and Stewart 2007), this is the first study to explicitly define 'Moritzean' conservation units in Australian freshwater fishes (*Nannoperca obscura*, *N. australis*

s.l. and *Gadopsis bispinosus*). The requisite nuclear genetic information is also presented here for *Retropinna*, *Philypnodon* species and *G. marmoratus*, ensuring that future supplementary mtDNA (and other) studies are likely to be fruitful. Indeed, several authors have suggested the presence of ESUs based on major sub-structure derived only from partial genetic information (e.g. mtDNA only datasets: Page *et al*. 2004; Faulks *et al*. 2008). Most ESUs herein, both defined and predicted, are geographically distributed at scales ranging from a single river basin to related groups of river basins, an outcome comparable to other small demersal fishes throughout the world (Ling *et al*. 2001; Mesquita *et al*. 2001; Quattro *et al*. 2001; Hedrick *et al*. 2006). The spatial scale for the conservation of evolutionary diversity in obligate freshwater fishes is comparatively restricted when compared to more vagile fauna such as marine vertebrates (e.g. Karl and Bowen 1998; Russello *et al*. 2007), and therefore warrants consideration in regional conservation programs.

The presence of defined or inferred ESUs and MUs highlights conservation implications for several regions. Southeastern Queensland and northern New South Wales represent areas of particularly high biodiversity. Both are also areas of rapid human development and environmental change (see Arthington *et al*. 1983; Hughes *et al*. 1999). Eastern Victoria and Tasmania (namely Gippsland and Ansons River) represent more intact areas, and proactive efforts can help to preserve divergent species and populations. This study adds highly restricted conservation units to a list of distinctive species in the Glenelg River Basin, reinforcing restoration initiatives to combat hydrological, habitat and salinity degradation (e.g. ARI 2003; Honan 2004). Finally, there is increasing evidence that the Lower Murray region, in particular the Lower Lakes and Mount Lofty Range streams, present a significant long-term freshwater refuge and unique environment facilitating genetic preservation and adaptive evolution. Knowledge of this uniqueness comes at a time of unprecedented environmental change. Climatic deficiencies in rainfall exacerbated by major human consumptive use have, in a very short period of time (i.e. 18 months since January 2007), eliminated vast areas of wetland habitat and desiccated stream habitats, and this has in turn decimated distributions and populations of native fishes with specialised environmental requirements (Chapters 4-6).

Molecular genetic appraisals of biodiversity and conservation units need to have utility beyond documenting distinctive elements subsequent to or at the same time as human-induced extirpation, or for guiding the maintenance of species *ex situ* (i.e. as with Lower Murray *Nannoperca obscura* and *Mogurnda adspersa*). Instead conservation and natural resource management need to acknowledge newly-recognised species and conservation units and urgently provide cross-jurisdictional action aimed at protection and restoration of habitats.

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11. APPENDICES

APPENDIX 1. RETROPINNA

Allozyme profiles at the 37 variable loci for the 122 *Retropinna* surveyed in the overview study. Individuals are identified by site code plus a unique number (e.g. 1-1 = fish #1 from site 1). A dash indicates no genotype was assignable and reflects either poor activity or mobility overlap with another locus in this taxon.

Fish #	Taxon	*Acon1*	*Acon2*	*Acyc*	*Ada*	*Ak1*	*Ca*	*Ck*	*Enol1*	*Enol2*	*Est1*	*Est2*	*Fdp*	*Fum*	*G6pd*	*Glo*	*Got1*	*Got2*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Ldh1*	*Ldh2*	*Mdh*	*Me1*	*Me2*	*Mpi*	*PepA1*	*PepA2*	*PepB*	*PepD*	*6Pgd*	*Pgk*	*Pgm*	*Pk2*	*Tpi2*	*Ugpp*
1-1	CEQ	c	b	f	d	b	c	b	d	a	c	a	c	a	a	b	b	c	b	e	b	d	b	b	b	b	d	c	d	e	b	d	c	b	b	a	d	c
1-2	"	c	b	f	d	b	c	b	d	a	c	a	c	a	a	b	bc	eg	bf	e	b	d	b	b	b	b	de	c	d	e	b	bd	c	b	b	a	d	c
1-3	"	c	b	f	d	b	c	b	d	a	c	a	c	a	a	b	b	ce	b	e	b	d	b	b	b	b	d	c	d	e	b	bd	c	b	b	a	d	c
1-4	"	c	b	f	d	b	c	b	d	a	c	a	c	a	a	b	b	ce	b	e	b	d	b	b	b	b	de	c	d	e	b	bd	c	b	b	a	d	c
3-1	"	c	b	f	d	b	c	b	d	a	c	a	c	b	a	b	b	h	b	e	b	d	b	b	b	b	bd	c	d	de	b	d	cd	b	b	a	d	c
3-2	"	c	b	f	d	b	c	b	d	a	c	a	c	ab	a	b	b	h	b	e	b	d	b	b	b	b	d	c	cd	de	b	d	c	b	b	a	d	c
3-3	"	-	b	f	d	b	c	b	d	a	c	a	c	b	a	b	b	gh	b	e	b	d	b	b	b	b	d	c	c	d	b	d	c	b	b	a	d	c
4-1	"	c	b	f	d	b	c	b	d	a	c	a	c	a	a	b	b	ag	b	e	b	d	b	b	b	b	d	c	d	eg	b	d	cd	b	b	a	d	bc
4-2	"	c	b	f	d	b	c	b	d	a	c	a	c	ab	a	b	b	g	b	e	b	d	b	b	b	b	d	c	d	de	b	d	d	b	b	a	d	b
4-3	"	c	b	f	d	b	c	b	d	a	c	a	c	ab	a	b	b	dh	b	be	b	d	b	b	b	b	d	c	d	e	b	d	cd	b	b	a	d	c
2-1	SEQ	-	b	ab	f	b	c	b	c	a	c	a	c	a	a	d	c	d	b	e	a	d	a	b	b	b	f	d	d	de	b	d	c	b	b	a	d	c
2-2	"	-	b	ab	f	b	c	b	c	a	c	a	c	a	-	d	c	d	b	e	a	d	a	b	b	b	f	d	d	-	b	d	-	b	b	a	d	-
2-3	"	-	b	ab	fg	b	c	b	c	a	c	a	c	a	a	d	c	d	b	e	a	d	a	b	b	b	f	d	d	de	b	d	c	b	b	a	d	c
2-4	"	-	b	b	f	b	bc	b	c	a	c	a	c	a	a	d	c	d	bf	e	a	d	a	b	b	b	f	d	d	de	b	d	c	b	b	a	d	c
4-4	"	-	b	ab	df	b	c	b	c	a	c	a	c	a	a	bd	c	d	b	e	a	d	a	b	b	b	f	d	d	de	b	d	c	b	b	a	d	bc
4-5	"	-	b	b	d	b	c	b	cd	a	c	a	c	a	a	d	c	d	b	ef	a	d	a	b	b	b	f	d	d	d	ab	d	c	b	b	a	d	c
4-6	"	-	b	b	f	b	c	b	c	a	c	a	c	a	a	d	c	d	b	e	a	d	a	b	b	b	f	d	d	d	b	d	c	b	b	a	d	c
5-1	"	-	b	e	d	b	c	b	c	a	c	a	d	a	a	b	c	d	b	e	a	d	ac	b	b	b	d	d	d	d	b	d	c	b	e	a	d	c

Fish #	Taxon	*Acon1*	*Acon2*	*Acyc*	*Ada*	*Ak1*	*Ca*	*Ck*	*Enol1*	*Enol2*	*Est1*	*Est2*	*Fdp*	*Fum*	*G6pd*	*Glo*	*Got1*	*Got2*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Ldh1*	*Ldh2*	*Mdh*	*Me1*	*Me2*	*Mpi*	*PepA1*	*PepA2*	*PepB*	*PepD*	*6Pgd*	*Pgk*	*Pgm*	*Pk2*	*Tpi2*	*Ugpp*
5-2	“	-	b	e	d	b	c	b	c	a	c	a	d	a	a	b	c	d	ab	e	a	d	a	b	b	b	d	d	d	d	b	ad	c	b	e	a	d	c
5-3	“	-	b	e	d	b	c	b	c	a	c	a	d	a	a	b	c	d	ab	e	a	d	a	b	b	b	d	df	d	d	b	d	c	b	e	a	d	c
5-4	“	-	b	e	d	b	c	b	c	a	c	a	d	a	a	b	c	d	a	e	a	bd	a	b	b	b	d	d	d	d	b	d	c	b	e	a	d	c
6-1	“	-	b	e	d	b	c	b	c	a	c	a	c	a	a	b	c	d	b	e	a	d	a	b	b	b	g	d	d	d	b	d	c	b	b	a	d	c
6-2	“	-	b	e	d	b	c	b	c	a	c	a	c	a	a	b	c	d	b	e	a	d	a	b	b	b	g	d	d	d	b	d	c	b	be	a	d	c
7-1	“	-	b	e	d	b	c	b	c	a	c	a	c	a	a	b	c	d	b	e	a	d	a	b	b	b	g	d	d	d	b	d	c	b	b	a	d	c
7-2	“	-	b	e	d	b	c	b	c	a	c	a	c	a	a	b	c	d	b	e	a	d	a	b	b	b	g	d	d	d	b	d	c	b	be	a	d	c
8-1	“	-	b	e	d	b	c	b	c	a	c	a	c	a	a	b	c	d	b	e	b	d	a	b	b	b	d	d	d	d	b	d	c	b	be	a	d	c
8-2	“	-	b	e	d	b	c	b	c	a	c	a	c	a	a	b	c	d	b	e	b	d	a	b	b	b	d	d	d	d	b	d	c	b	b	a	d	c
9-1	“	-	b	be	d	b	c	b	c	a	c	a	c	a	a	b	c	d	b	e	a	d	a	b	b	c	dg	d	d	ad	b	d	c	b	e	a	d	ac
9-2	“	-	b	be	d	b	c	b	c	a	cd	a	c	a	a	b	c	d	b	e	a	d	a	b	b	bc	d	d	d	df	b	d	c	b	e	a	d	ac
10-1	“	-	b	e	d	b	c	b	c	a	c	a	c	-	a	b	ce	d	b	e	a	d	a	b	b	bf	d	d	d	d	b	d	c	-	e	a	-	c
10-2	“	-	b	e	d	b	c	b	c	a	c	a	c	-	a	b	c	d	bg	e	a	d	a	b	b	b	d	d	d	d	b	d	c	-	e	a	-	c
11-1	“	-	b	e	d	b	c	b	c	a	cd	a	c	a	a	b	c	d	bg	ef	a	bd	a	b	ab	d	d	d	d	d	b	d	c	b	e	a	d	c
11-2	“	-	b	e	d	b	c	b	c	a	c	a	c	a	a	b	c	d	b	e	a	d	a	b	b	bd	d	d	d	d	b	d	c	b	e	a	d	c
11-3	“	-	b	e	d	b	c	b	c	a	c	a	c	a	a	b	c	d	bg	ef	a	d	a	b	b	cd	d	d	d	d	b	d	c	b	e	a	d	c
12-1	“	-	b	be	d	b	c	b	c	a	c	a	c	a	a	b	c	d	b	e	a	d	a	-	b	b	d	d	d	d	b	e	c	b	e	a	d	c
13-1	“	-	b	be	d	b	c	b	ac	a	c	a	c	a	a	b	c	d	b	e	a	d	a	b	b	c	d	d	d	d	b	de	c	-	f	a	d	bc
12-2	SEC	c	b	e	d	a	b	b	c	a	e	a	c	b	a	b	b	g	e	bc	d	d	b	b	b	e	b	ce	b	d	df	di	c	b	a	b	d	b
12-3	“	c	bc	be	d	a	b	b	c	a	e	a	c	b	a	bc	b	g	e	ab	de	d	b	b	b	ce	bc	e	b	d	df	i	c	b	a	b	d	b
12-4	“	c	b	e	d	a	b	b	c	a	e	a	c	b	a	b	b	g	e	ac	d	d	b	b	b	ce	b	ce	b	d	f	i	c	b	a	b	d	b
13-2	“	c	b	e	d	a	b	b	bc	c	e	a	c	b	a	b	b	d	e	b	d	d	b	b	b	c	b	c	b	d	df	d	c	b	b	b	d	b
13-3	“	c	b	be	d	a	b	b	bc	c	e	a	c	b	a	b	b	d	e	b	d	d	b	b	b	c	b	c	b	bd	d	d	c	b	b	b	d	ab
13-4	“	c	b	e	d	a	b	b	c	c	e	a	c	b	a	b	b	d	e	b	d	d	b	b	b	e	bc	c	b	d	df	d	c	b	b	b	d	b
13-5	“	c	b	e	d	a	b	b	c	c	e	a	c	b	a	b	b	d	e	b	d	d	b	b	b	ce	b	c	b	d	f	d	c	b	b	b	d	b
13-6	“	c	b	e	d	a	b	b	c	c	e	a	c	b	a	b	b	d	e	b	d	d	b	b	b	ce	bc	c	b	d	d	d	d	b	b	b	d	b
14-1	“	c	b	be	d	a	b	b	c	a	e	a	c	b	a	ab	b	g	c	b	d	d	b	b	ab	e	c	c	b	d	dg	d	c	b	b	b	d	b
14-2	“	c	b	be	bd	a	b	b	c	a	e	a	c	b	a	ab	b	dg	ce	b	d	d	b	b	b	eg	c	c	b	d	dg	d	c	b	a	b	d	b
15-1	“	c	b	ef	d	a	b	b	c	a	e	a	c	b	a	b	b	d	b	c	d	d	b	b	b	ce	bc	c	b	d	d	i	c	b	b	b	d	b

Fish #	Taxon	*Acon1*	*Acon2*	*Acyc*	*Ada*	*Ak1*	*Ca*	*Ck*	*Enol1*	*Enol2*	*Est1*	*Est2*	*Fdp*	*Fum*	*G6pd*	*Glo*	*Got1*	*Got2*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Ldh1*	*Ldh2*	*Mdh*	*Me1*	*Me2*	*Mpi*	*PepA1*	*PepA2*	*PepB*	*PepD*	*6Pgd*	*Pgk*	*Pgm*	*Pk2*	*Tpi2*	*Ugpp*
15-2	“	c	b	f	d	a	b	b	c	a	e	a	c	b	a	b	b	dg	b	bc	d	d	b	b	b	e	c	c	b	d	d	hi	c	b	b	b	d	b
16-1	“	a	b	bf	bd	a	b	b	c	a	be	a	cd	b	a	bc	b	d	e	b	d	d	b	b	b	g	b	c	b	d	d	i	c	b	b	b	d	ab
16-2	“	ac	b	ef	bd	a	b	b	c	a	e	a	c	b	a	b	b	d	e	c	d	d	b	b	b	g	b	c	b	d	d	di	c	b	b	b	d	b
17-1	“	c	b	e	f	a	b	b	c	a	de	a	c	b	a	bc	b	d	e	bc	d	d	b	b	b	ce	c	c	b	d	d	d	c	b	be	b	d	b
17-2	“	c	b	be	f	a	b	b	c	a	d	a	c	b	a	c	b	d	e	b	d	d	b	bc	b	e	c	c	b	d	d	d	c	b	b	b	d	b
18-1	“	c	b	ef	d	a	b	b	c	a	e	a	c	b	a	b	b	dg	e	c	d	d	b	b	b	e	ab	c	b	d	d	d	c	b	b	b	d	b
18-2	“	c	b	f	d	a	b	b	c	a	e	a	c	b	a	bc	b	d	e	c	d	d	b	b	b	eg	b	c	b	d	d	di	c	b	b	b	d	b
19-1	“	c	b	ef	d	a	b	b	c	a	be	a	c	b	a	b	b	g	e	c	d	d	b	b	b	ce	ab	c	b	d	d	d	c	b	b	b	cd	b
19-2	“	c	b	ef	d	a	b	b	c	a	be	a	c	b	a	b	b	g	e	cd	d	d	b	b	b	ce	b	c	b	d	d	d	c	b	b	b	cd	b
20-1	“	c	b	ef	b	a	b	b	c	a	be	a	c	b	a	b	b	dg	e	c	d	ad	b	b	b	ab	b	c	b	d	d	di	c	b	b	b	d	b
20-2	“	c	b	ef	d	a	b	b	c	a	e	a	c	b	a	b	b	d	e	c	e	d	b	b	b	bg	b	c	b	d	d	di	c	b	b	b	ad	b
21-1	“	c	b	e	d	a	b	b	c	a	a	a	c	b	a	c	b	bd	e	b	d	d	b	b	b	e	b	c	b	d	d	d	c	b	b	b	d	b
21-2	“	c	b	e	d	a	b	b	c	a	a	a	c	b	a	c	b	dg	e	b	d	d	b	b	b	ce	b	c	b	d	d	d	c	b	b	b	d	b
22-1	“	c	b	be	d	a	b	b	c	ab	b	a	d	b	a	b	b	d	e	b	e	d	b	b	b	ce	a	c	b	d	d	d	c	b	e	b	d	ab
22-2	“	c	b	ef	d	a	b	b	c	a	ab	a	d	bc	a	c	b	d	e	b	e	d	b	b	b	c	a	c	b	d	d	i	c	b	e	b	d	b
23-1	“	c	b	e	d	a	b	b	c	ab	ab	a	d	b	a	c	b	d	e	b	d	d	b	b	b	cg	a	c	b	d	d	d	c	b	e	b	d	b
23-2	“	-	ab	e	d	a	b	b	c	b	ab	a	d	b	a	c	b	d	e	b	d	d	b	ab	b	c	a	c	b	d	d	d	c	b	e	b	d	b
24-1	“	c	b	e	d	a	b	b	c	a	b	a	d	b	a	c	b	d	e	b	d	d	b	b	b	c	ab	c	b	d	d	d	c	b	c	b	d	b
24-2	“	c	b	e	d	a	b	b	c	a	b	a	d	b	a	bc	b	d	e	b	d	d	b	b	b	c	a	c	b	d	d	d	c	b	ce	b	d	b
25-1	MTV	c	c	f	d	b	b	b	d	a	e	a	c	b	a	bc	b	df	e	b	c	d	b	a	b	b	c	c	b	d	bd	j	b	b	b	b	d	c
25-2	“	c	c	f	d	b	b	b	d	a	e	a	c	b	a	b	b	d	e	b	c	d	b	a	b	b	c	c	b	d	bd	cj	b	b	b	b	d	c
26-1	“	c	c	f	d	b	b	b	de	a	e	a	c	b	a	b	b	d	h	b	c	cd	b	ab	b	b	c	c	b	d	d	j	b	b	b	b	c	c
26-2	“	c	c	f	d	b	b	b	de	a	e	a	c	b	a	b	b	d	h	b	c	d	b	ab	b	b	c	c	b	d	d	j	b	b	b	b	c	c
27-1	“	c	c	f	d	b	ab	b	de	a	e	a	c	b	a	b	b	df	e	b	c	d	b	b	b	b	c	c	b	d	d	h	b	b	b	b	d	c
27-2	“	c	c	f	d	b	b	b	de	a	-	a	c	b	a	bc	b	d	e	b	c	d	b	b	b	b	c	c	b	d	d	eh	b	b	b	b	d	c
28-1	“	c	c	f	d	b	c	b	e	a	be	a	c	b	a	c	b	d	e	b	c	d	b	b	b	b	bc	c	b	d	d	fj	b	b	b	b	d	c
28-2	“	c	c	f	d	b	bc	b	e	a	e	a	c	b	a	bc	b	d	e	b	c	d	b	b	b	b	c	c	b	d	d	j	b	b	b	b	d	c
29-1	“	c	c	f	c	b	bc	b	d	a	e	a	c	b	a	b	b	d	e	b	c	d	b	b	b	b	c	c	b	d	d	ej	b	b	b	b	d	c
29-2	“	c	c	f	ce	b	b	b	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	b	b	b	c	c	b	d	d	gj	b	bc	b	b	d	c

Fish #	Taxon	*Acon1*	*Acon2*	*Acyc*	*Ada*	*Ak1*	*Ca*	*Ck*	*Enol1*	*Enol2*	*Est1*	*Est2*	*Fdp*	*Fum*	*G6pd*	*Glo*	*Got1*	*Got2*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Ldh1*	*Ldh2*	*Mdh*	*Me1*	*Me2*	*Mpi*	*PepA1*	*PepA2*	*PepB*	*PepD*	*6Pgd*	*Pgk*	*Pgm*	*Pk2*	*Tpi2*	*Ugpp*
30-1	“	c	c	f	df	b	c	b	de	a	e	a	c	b	a	bc	b	d	e	b	c	d	b	b	c	b	c	c	b	d	d	d	b	b	b	b	de	c
30-2	“	c	c	f	-	b	b	b	d	a	e	a	c	b	a	bc	b	d	e	b	c	d	b	b	bc	ab	c	c	b	d	d	dg	b	b	b	b	d	c
31-1	“	c	c	f	d	b	b	b	de	a	e	a	c	b	a	b	b	f	eh	b	d	d	b	a	b	b	c	c	b	d	b	j	b	b	b	b	d	bc
31-2	“	c	c	f	cd	b	b	b	d	a	e	a	c	b	a	bc	b	f	eh	b	d	d	b	a	b	b	c	c	b	d	b	j	-	b	b	b	d	c
32-1	“	c	c	f	df	b	c	b	d	a	e	a	c	bc	a	c	b	d	de	b	c	d	b	b	c	a	c	c	b	d	b	dg	b	b	b	b	de	c
32-2	“	c	c	f	d	b	c	b	d	a	e	a	c	b	a	c	b	d	de	b	c	d	b	b	c	a	c	c	b	d	d	dg	b	b	b	b	de	c
33-1	“	c	c	f	d	b	b	a	d	a	be	a	c	b	a	b	b	df	eh	b	cd	d	b	a	b	a	c	bc	b	d	d	cj	b	b	b	b	d	c
33-2	“	c	c	f	d	b	b	b	d	a	be	a	c	b	a	b	b	d	e	be	d	d	b	a	b	a	c	c	b	d	bd	cj	b	b	b	b	d	c
34-1	“	c	c	f	d	b	c	b	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	b	c	ab	c	c	b	d	bd	d	a	b	b	b	d	c
34-2	“	c	c	f	d	b	cd	b	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	b	c	b	c	c	b	d	d	d	a	-	b	b	d	c
35-1	“	d	c	f	d	b	c	b	d	a	e	a	c	b	a	c	b	d	e	e	c	d	b	b	bc	ab	b	c	b	d	d	df	c	b	b	b	d	c
35-2	“	d	c	f	d	b	c	b	d	a	be	a	c	b	a	c	b	d	e	e	c	d	b	b	b	b	b	c	b	d	d	bd	c	b	b	b	bd	c
37-1	“	c	c	f	d	b	b	b	d	a	e	a	c	b	a	b	b	f	e	b	cd	d	b	a	b	b	c	c	b	d	bd	fj	b	b	b	b	d	bc
37-2	“	c	c	f	d	b	b	ab	d	a	e	a	c	b	a	bc	b	f	e	b	d	d	b	a	b	b	c	c	b	d	b	fj	b	b	bd	b	d	c
38-1	“	c	c	f	d	b	b	b	d	a	e	a	c	b	a	bc	b	f	e	b	d	d	b	a	b	b	c	c	b	d	bd	j	b	b	bd	b	d	c
38-2	“	bc	c	f	d	b	b	b	d	a	e	a	c	b	a	bc	b	f	e	b	d	d	b	a	b	b	c	bc	b	bd	b	gj	b	b	b	b	d	c
39-1	“	c	c	f	d	b	b	b	d	a	e	a	c	b	a	c	b	d	e	b	d	d	b	a	b	b	c	c	b	d	bd	j	b	b	d	b	d	c
39-2	“	c	c	f	d	b	b	b	d	a	e	a	c	b	a	bc	b	f	e	b	d	d	b	a	b	b	c	c	b	d	d	j	b	b	b	b	d	c
40-1	“	c	c	f	d	b	b	ab	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	a	b	b	b	b	b	d	bd	j	b	b	b	b	d	c
40-2	“	c	c	f	d	b	b	ab	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	a	b	b	b	b	b	d	bd	j	b	b	b	b	d	c
41-1	“	c	c	f	d	b	b	b	d	a	e	ab	c	b	a	b	b	df	h	b	c	d	b	a	b	b	c	c	b	d	bd	j	b	b	bd	b	d	c
41-2	“	c	c	f	d	b	b	ab	d	a	e	a	c	b	a	b	b	f	eh	b	cd	d	b	a	b	b	bc	c	b	d	b	ij	b	b	b	b	d	c
42-1	“	c	c	f	df	b	c	b	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	b	c	ab	c	c	b	d	bd	d	b	b	b	b	de	ac
42-2	“	c	c	cf	cd	b	c	b	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	b	c	b	c	c	b	d	bd	d	b	bc	b	b	d	ac
45-1	“	c	c	f	df	b	c	b	d	a	e	a	c	b	a	c	b	d	de	b	c	d	b	b	c	ab	c	c	b	d	b	d	b	bc	b	b	d	c
45-2	“	c	c	bf	ad	b	c	b	d	a	e	a	c	b	ab	c	b	d	e	b	c	d	b	b	c	ab	c	c	b	d	bd	d	b	c	b	b	de	c
46-1	“	c	c	f	d	b	c	b	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	b	c	a	c	c	c	d	d	d	b	bc	b	b	de	c
46-2	“	c	c	f	d	b	c	b	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	b	c	a	c	c	bc	d	bd	d	b	bc	b	b	d	c
47-1	“	bc	c	f	d	b	bc	b	d	a	e	a	c	b	a	c	ab	d	e	b	c	d	b	b	c	ab	c	c	ab	d	bc	d	b	bc	b	b	d	c

Fish #	Taxon	*Acon1*	*Acon2*	*Acyc*	*Ada*	*Ak1*	*Ca*	*Ck*	*Enol1*	*Enol2*	*Est1*	*Est2*	*Fdp*	*Fum*	*G6pd*	*Glo*	*Got1*	*Got2*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Ldh1*	*Ldh2*	*Mdh*	*Me1*	*Me2*	*Mpi*	*PepA1*	*PepA2*	*PepB*	*PepD*	*6Pgd*	*Pgk*	*Pgm*	*Pk2*	*Tpi2*	*Ugpp*
47-2	“	c	c	f	d	b	bc	b	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	b	c	b	c	c	ab	d	c	d	b	c	b	b	d	c
48-1	“	c	c	df	d	b	c	b	d	a	e	a	bc	b	a	c	b	d	de	b	c	d	b	b	c	ab	c	c	b	d	b	d	b	c	b	b	d	c
48-2	“	c	c	f	d	b	c	b	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	b	c	b	c	c	b	d	b	d	b	c	b	b	d	ac
49-1	“	c	c	f	d	b	d	b	d	a	e	a	a	b	a	c	b	d	de	b	c	d	b	b	c	b	c	c	b	d	b	d	b	c	b	b	d	ac
49-2	“	c	c	f	df	b	c	b	d	a	e	a	a	b	a	c	b	d	de	b	c	d	b	b	c	b	c	c	b	d	b	d	b	c	b	b	d	a
51-1	“	-	c	f	d	b	b	b	d	a	e	a	c	b	a	bc	b	d	eh	b	c	d	b	b	b	b	c	c	b	d	b	dj	-	b	b	b	d	bc
51-2	“	c	c	f	d	b	b	b	d	a	e	a	c	b	a	bc	b	d	e	b	c	d	b	a	bc	b	c	c	b	d	b	d	-	b	b	b	d	b
56-1	“	c	c	f	d	b	b	b	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	a	b	ab	c	ac	b	d	b	d	ab	b	b	b	d	c
56-2	“	c	c	f	d	b	b	b	d	a	e	a	c	b	a	c	b	d	e	b	c	d	b	a	bc	b	c	c	b	d	b	d	b	b	b	b	d	c
58-1	“	c	c	f	d	b	bd	b	d	a	e	a	c	b	a	bc	b	d	e	b	c	d	b	ab	ab	b	c	c	b	cd	b	dj	b	b	b	b	d	c
58-2	“	c	c	f	d	b	bc	b	d	a	e	a	c	b	a	b	b	d	e	b	c	d	b	ab	b	b	c	c	b	d	b	d	-	b	b	b	de	c
60-1	COO	d	c	g	d	c	c	b	d	a	e	a	c	b	a	c	d	d	i	b	c	d	b	b	c	a	c	c	bc	d	be	a	b	ab	e	b	d	b
60-2	“	de	c	g	d	c	c	b	d	a	e	a	c	b	a	c	bd	d	i	b	c	d	b	b	c	a	c	c	c	d	be	a	b	b	be	b	d	b
61-1	“	d	c	g	d	c	c	b	d	a	ce	a	c	b	-	c	bd	d	i	b	c	d	b	b	c	a	cd	c	c	d	b	a	b	ab	e	b	d	b
61-2	“	d	c	g	d	c	cd	b	d	a	e	a	c	b	a	c	d	d	i	b	cf	d	b	bc	c	a	c	c	c	d	b	a	b	a	e	b	d	b
62-1	“	bd	c	g	d	c	c	b	d	a	ce	a	c	b	a	c	d	d	i	b	c	d	b	bc	c	a	c	c	c	d	be	a	b	b	be	b	d	ab
62-2	“	d	c	g	d	c	cd	b	d	a	e	a	c	b	a	c	bd	d	i	b	c	d	b	b	c	a	c	c	bc	d	bg	a	b	b	be	b	d	b

APPENDIX 2. NANNOPERCA OBSCURA

Allele frequencies at 52 loci for the allozyme overview study. For polymorphic loci, the frequencies of all but the rarer/rarest alleles are expressed as percentages and shown as superscripts (allowing the frequency of each rare allele to be calculated by subtraction from 100%). A dash indicates the locus was not scoreable in this population. Sample sizes at each site are shown in brackets. Invariant loci: *Ak*, *Ald1*, *Ck*, *Enol2*, *Fdp*, *Gapd1*, *Gdh*, *Glo*, *Idh1*, *Ldh1*, *Ldh2*, *Mdh*, *Ndpk*, and *Pk2*.

Locus	5 (3)	10 (3)	14 (6)	17 (5)	Na1 (2)	Na7 (2)	Na8 (2)	Na14 (2)	Na17 (2)
Acon1	c	c	c	c	b	b	a^{75},b	a	b^{83},a
Acon2	b	b	b	b	c	c	c	c	c^{50},a^{33},d
Acon3	b	b	b	b	-	-	-	a	-
Ada	c	c	c^{83},d	c	d^{75},b	d^{75},a	d	d	d
Adh	b	b	b^{92},a^{8}	b	-	-	-	b	b
Ald2	a	a^{83},b	a	a	-	-	-	a	-
Ap	a	a	a	a	b	b	b	b	b
Ca	b	b	b	b	a	a^{50},b	a	a	b
Enol1	b	b	b^{92},a	b	b	b	b	b	b
Est1	a	a	a	a	b	b	b	b	b
Est2	c^{67},d	d	d	d	b	b	a^{75},b	b	a^{83},b
Fum	a	a	a	a	b	b	b	b	b
G6pd	b	b	b	b	a^{75},c	a^{75},c	a	a	a
Gapd2	a	a	a	b	a	a	a	a	a
Gda	a	a	a	a	a	a	a^{75},b	b	a
Got1	b	b	b	b	a	a	a	a	a
Got2	d	d	d	d	b	b	b^{75},a	b	b^{50},a^{33},c
Gpi1	e	e	e	e	f	a^{75},f	f	f	df^{33},bc^{17}
Gpi2	a	a	a	a	b	b	b	b	b
Gsr	b	a^{83},b	a^{92},b	b	b	b	b	b	b
Idh2	a	a	a	a	b	b^{75},a	b	b	a^{50},b
Me1	b	b	b	b	b^{75},a	b	b	b	b
Me2	c	c	a^{50},c	a	b^{75},a	a	b	b	b^{50},d
Mpi	c	c	c	c	b	b^{75},a	b	b	b
PepA1	c^{83},b	b	b^{92},c	b	a	a	a	a	a
PepA2	b	b	b	b	c	c	c	c	c
PepB	c	d	d	d	b	a^{75},b	b	b	b
PepD1	b	b	b	b	a	a	a	a	a
PepD2	b	b	b	b	b	b	b	b	a^{67},b
Pgam1	a	a	a	a	b	b	b	b	b
Pgam2	b	b	b	b	b^{75},a	b^{75},a	b	b	b
6Pgd	d	d^{67},b	b	b	b^{50},cd^{25}	b^{75},d	b	b	b
Pgk	c	c	c	c	a^{75},b	a	a^{75},b	a	a
Pgm1	a^{67},b	a	a	a	a	b^{75},a	a	a	a
Pgm2	b	b	b	b	a	a	a	a	a^{83},c
Pk1	a	a	a	a	-	-	-	b	b
Sordh	b	b	b	b	a	a	a	a	a
Tpi	b	b	b	b	a	a	a	a	a

APPENDIX 3. NANNOPERCA AUSTRALIS

Allele frequencies at 23 variable loci for the 57 sites surveyed. For polymorphic loci, the frequencies of all but the rarer/rarest alleles are expressed as percentages and shown as superscripts (allowing the frequency of each rare allele to be calculated by subtraction from 100%). Alleles not separated by a comma shared the same frequency. A dash indicates this locus was not scorable in these individuals, due either to an overlap with other loci (*Acon2*, *Acon3*) or low activity (*Gsr*).

Site	Lineage	*Acon1*	*Acon2*	*Acon3*	*Ada*	*Ca*	*Ck*	*Fum*	*G6pd*	*Got2*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Me1*	*Me2*	*Mpi*	*PepA2*	*PepB*	*PepD2*	*Pgk*	*Pgm1*	H_O	S.E.
1	MDB	f	-	-	e	b	a	b	b	b	g	d	-	c	c^{94},a	d	c	f	c^{94},e	d	c	b^{50},d	b	e	0.022	0.018
2	"	f^{89},e	-	-	e	b	a	b	b	b	e^{72},g	d	c	c	a	d	c	d^{94},f	e	d	c	d	b	e	0.014	0.009
3	"	e^{67},f	-	-	e	b	a	b	b	b	e^{42},g^{33},k	d	-	c	a	d	c	d	e	d	c	d^{83},b	b	e	0.030	0.018
4	"	f^{95},e	-	-	e^{95},f	b	a	b	b	b^{82},d	g^{55},k^{27},e	d^{95},f	-	c	a^{86},c	d^{95},e	c	d^{68},f	e^{82},c	d^{95},a	c	d^{82},b	b	e	0.061	0.021
5	"	f^{95},e	-	-	e	b	a	b	b	b	k^{55},g	d^{80},f	-	c	a	d	c	d	e^{70},c	a^{75},d	c	b^{70},d	b	e	0.042	0.017
6	"	f	-	-	e	b	a	b	b	b	g^{72},k	d	c	c	a	d	c	d	c^{94},e	d^{56},a	c	d^{56},b	b	e	0.034	0.018
7	"	f	-	-	e	b	a	b	b	b^{55},a	f^{70},k	d	-	c	c	d	c	d	c^{70},a	d	c	d	b	e^{85},d	0.040	0.020
8	"	f^{81}, b^{15},e	-	-	e	b^{96},a	a	b	b	b^{58}, d^{30},a	k^{50},g^{35},de f^{4},h^{3}	d^{96},f	c	c	a^{50},c	d	c	d^{65},f	e^{81},c	d	c	b^{50},d	b	e^{96},c	0.080	0.028
9	"	f^{75}, b^{13},e	-	-	e	b	a	b	b	b^{81}, d^{13},a	k^{47},g^{34},e^{13} ,dm	d	-	c	a^{69},c	d	c	d^{59},f	e^{78}, c^{19},a	d^{91}, a^{6},b	c	d^{72}, b^{22},e	b	e^{97},d	0.072	0.026
13	"	f	-	-	e	b	a	b	b^{95},c	b	k^{85},g	d	c	c	c	d	c	f^{80},g^{15},d	e^{55},c^{35},b	d	c	b^{50},d	b	e	0.043	0.021
14	"	f^{60},c	-	-	e	b	a	b	b^{70},c	d^{60},b	k	d	c^{75},a	c	a^{60}, c^{25},b	d	c	f	e^{55}, c^{40},b	d	c	d^{85},b	b	e	0.080	0.031
15	"	f	-	-	e	b^{56},a	a	b	b	a^{80},b	k^{95},g	d^{95},e	-	c	c	d	c	f^{95},d	e	d	d	b^{70},d	b	e	0.034	0.016
16	"	f	-	-	e	b	a	b	b	b^{95},a	g^{55},k^{35},al^{5}	d^{80},c	c	c^{95},b	a^{80},c	d	c	d^{80},f	e^{80}, c^{15},d	d	c	b^{65}, d^{25},e	b	e	0.054	0.021
17	"	f	-	-	e	b^{60},a	a	b	b^{95},c	b^{90},a	k^{90}, hm	d	c	c^{70},a	a^{85}, b^{10},c	d^{95},e	c	d^{80}, b^{15},e	e	d	c	d	b	e^{95},f	0.054	0.021
18	"	f	-	-	e	b^{60},a	a	b	b^{95},c	b^{95},a	f^{90},k	d	c	c	a	d	c	f^{55},d	e^{75},c	d	c	b^{50},d^{35}, a	b	e^{80},f	0.072	0.028

Site	Lineage	*Acon1*	*Acon2*	*Acon3*	*Ada*	*Ca*	*Ck*	*Fum*	*G6pd*	*Got2*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Me1*	*Me2*	*Mpi*	*PepA2*	*PepB*	*PepD2*	*Pgk*	*Pgm1*	H_O	S.E.
19	"	f^{89},g	-	-	e	a	a	b	b	b^{90},a	k^{55},f	d	c	c	a	d	c	f^{50}, d^{45},b	e^{80},c	d	c^{75}, d^{20},a	b^{45}, d^{45},a	b^{94},c	e^{85}, f^{10},d	0.066	0.024
20	"	f	-	-	e	b	a	b	b	a^{50},b	k	d	c	c	a^{95},c	d	c	f^{55},d	g^{80},c	d	c^{80},b	b^{60},d	b	e	0.052	0.023
21	"	f	-	-	e	b	a	b	b	b	k	d	-	a	c	d	c	f	e	d	c	b	b	e	0.000	0.000
10	SEC/Tas	b^{60},f	-	-	e	a	a	b	b^{55}, a^{40},c	b	k	d	c	c	c	d	c	d^{85},a	e	d	c	d	b	e	0.028	0.017
12	"	f^{59}, b^{33},c	-	-	e	a^{83},b	a	b	a^{50},b	b	k	d	c	c	c	d	c	d^{75},b	e^{75}, d^{17},c	d	c	d	b^{92},a	e	0.058	0.024
22	"	b^{90}, c^{5},f	-	-	e	a	a	b	b^{90},c	b	k	d	-	c	c	d	c	d	e	d	c	d^{95},e	b	e^{90},c	0.016	0.008
23	"	b^{94},f	-	-	e	a	a	b	b^{78},c	b	k	d	c	c	c^{94},d	d	c	d	e	d	c^{94},d	d	b	e	0.017	0.010
24	"	b^{88},f	-	-	e	a	a	b	b	b	k	d	-	c	c	d	c	d	e	d	c	d	b	e	0.006	0.006
25	"	b^{90},f	-	-	e	a	a	b	b	b	k	d	c	c	c	d	c	d	e	d	c	d	b	e^{90},c	0.009	0.006
26	"	b	-	-	e^{78},a	a^{94},b	a	b^{94},c	b	b^{94},f	k^{56},h^{22}, i^{17},m	d^{72},c	-	c	c	d^{94},e	c	d^{89},b	e^{78},f	d	c^{94},b	d^{89},f	b^{94},a	e^{89}, c^{6},g	0.069	0.021
27	"	b^{75},f	-	-	e^{85}, d^{10},b	a	a	b	b	b	k^{85}, m^{10},h	d^{85},c	c	c	c	d	c	d	e	d	c	d	b	e^{75},c	0.035	0.016
28	"	b^{60},f	-	-	e	a	a	b	b^{50}, c^{45},a	b	k^{95},m	d	c	c	c	d^{95},c	c	d^{90},f	e	d^{95},c	c	d^{95},f	b	e^{95},c	0.035	0.015
29	"	b	-	-	e	a	a	b	b^{81},c	b	k^{94},j	d	c	c	c	d^{94},a	c	d	e	d	c^{94},b	d	b^{94},a	e^{94},a	0.022	0.010
31	"	b^{65}, f^{30},a	-	-	a^{90},e	a	a	b	b^{85}, c^{10},a	b	k	d	c	c^{95},b	c	d^{85},a	c^{95},b	d^{50},b^{30},a ^{15},f	e	d	c	d^{95},e	b	e^{95},h	0.059	0.024
32	"	f^{60},b	-	-	a^{50}, e^{45},f	a	a	b	b^{60},c	b	k	d	c	c	c	d^{95},a	c^{95},b	d^{75},b	e	d	c	d^{95},e	b	e	0.057	0.024
33	"	b^{70},f	-	-	e^{95},a	a	a	b	b	b^{95},a	k	d^{95},c	c	c	c	d	c	d	e	d	c	d^{75},c	b^{90}, ad	e	0.030	0.015
34	"	f^{85}, b^{10},d	-	-	e^{75}, d^{15},a	a^{60},b	a	b	b^{95},c	b	c^{80},k	d	c	c^{60},a	c^{95},a	d	c	b^{75},d	e^{80},c	d	a^{70},c	d	b	f^{65},e	0.091	0.028

Site	Lineage	*Acon1*	*Acon2*	*Acon3*	*Ada*	*Ca*	*Ck*	*Fum*	*G6pd*	*Got2*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Me1*	*Me2*	*Mpi*	*PepA2*	*PepB*	*PepD2*	*Pgk*	*Pgm1*	H_O	S.E.
35	"	b^{45},f^{45},c	-	-	e^{60}, f^{30},ad	a	a	b	b^{95},c	b	k^{85}, a^{10},c	d	c	c	c	d	c	d^{80},b	e^{95},c	d^{95},c	c	c^{65},d	b	e^{85},c	0.063	0.024
36	"	b^{65},f	-	-	e	a	a	b^{85},a	b	b	k^{95},b	d	c	c	c	d	c	d^{90},b	e	d	a^{85},c	c^{50},d	b	e	0.035	0.016
37	"	b^{85},f	-	-	e	a	a	b^{85},a	b	b	k^{95},b	d	-	c	c	d	c	d^{95},b	e	d	a^{85},c	d^{55},f	b	e	0.036	0.016
38	"	b^{43}, f^{36},c	-	-	e	a	a	b	b	b	k^{50}, c^{29},f	d	-	c^{79},a	c	d	c	b^{79},d	c^{50},e	d	a^{86},c	d^{93},f	b	e	0.067	0.026
39	"	b^{70},f	-	-	e	a	a	b	b^{95},a	b^{95},c	k^{55},f^{25}, ac	d	c	c	c^{65},a	d	c	b^{65},d	c^{70},e	d	c^{95},a	c^{65},d	b^{95},f	e	0.087	0.031
40	"	f^{71},b	-	-	e	a	a	b	b	b	k^{79}, j^{14},f	d^{93},b	-	c	c^{64},a	d	c	d^{86},e	e^{86},c	d	c	c^{57},f	b	e^{93},d	0.051	0.019
41	"	f^{75},b	-	-	e	a	a	b	b^{88}, ac^{6}	b	k^{94},f	d	c	c	c	d	c^{81},b	d^{75}, b^{19},f	e	d	c	c^{69},d	b	e^{94},f	0.049	0.020
42	"	f	-	-	e^{95},c	a	a	b	b^{75},c	b	k	d	c	c	c	d	c^{90},b	d^{95},b	e	d	c	d	b^{90},d	e	0.024	0.013
43	"	f^{90},b	-	-	e	a^{80},b	a	b	b	b	k	d	c	c	c	d	c^{95},a	d	e	d	c	d^{85},f	b^{80},d	e^{85},i	0.037	0.015
44	"	f^{75},b	-	-	e	a	a	b	b^{75},c	b	k	d	c	c	c	d	c	d^{95},b	e^{95},c	d	c	d	b^{55},d	e	0.028	0.014
52	"	f	-	-	e	a	a	b	b	b	k	d	c	c^{95},d	c^{80},d	a^{65},d	c	d	e^{95},c	d	c	d	b	e^{90},d	0.033	0.018
59	"	b^{55},f	-	-	e	a^{95},b	a	b	b	b	k	d^{95},a	c	c	c	d	c	d^{95},f	e	d	c	d	b	e^{90},c	0.022	0.012
60	"	b	-	-	e	a	a	b	b	b	k	d	c	c	c^{95},a	b	c	d	e	d	c	d	b	e	0.002	0.002
61	"	b	-	-	e	a	a	b	b	b	k	d	-	c	c	d	c	d	e	d	c	d	b	e	0.000	0.000
62	"	b^{89}, a^{6},f	-	-	e	a	a	b	b	b	k	d	-	c	c	d	c	d	e	d	c	d^{94},b	b	e	0.007	0.005
63	"	b^{88},f	-	-	e	a	a	b	b	b	k	d	-	c	c	d^{75},b	c	d	e	d	c	d^{81},b	b	e^{94},c	0.022	0.013
46	Hybrid	b^{50},f	b^{44}, a^{33},c	-	e^{70},g	a	a^{70},b	b^{55},c	b	b	k	d	d^{55},c	c	c	d^{65},b	c	d^{55},g	h^{55},e	d	c^{55},b	d^{95},f	b^{60},g	e	0.110	0.031
47	'flindersi'	f^{94},b	b	c^{81},d	g	a	b	c	b	b	k	d	d	c	c	b	c	g^{88},h	h	d	b	d	g	e	0.016	0.010
54	Victoria	f	b	c	g	a	b	c	b	b^{95},e	k	d	d	c	c	b	c	c^{95},d	h^{95},i	d	b	d^{85},f	g	e^{75},g	0.023	0.012
55	"	f^{75},b	b	c	g	a	b	c	b	b	k	d^{95},b	d^{95},e	c	c	b	c	g	h^{95},i	d	b	d^{95},f	g^{90}, eh^{5}	e^{85}, g^{10},b	0.025	0.010

Site	Lineage	*Acon1*	*Acon2*	*Acon3*	*Ada*	*Ca*	*Ck*	*Fum*	*G6pd*	*Got2*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Me1*	*Me2*	*Mpi*	*PepA2*	*PepB*	*PepD2*	*Pgk*	*Pgm1*	H_O	S.E.
56	"	f	b	c	g	a	b	c	b	b	k	d	d	c	c	b	c	g	h	d	b	d	g	e^{60},c	0.013	0.013
57	"	f	b	c	g^{90}, df^{5}	a	b	c^{95},d	b	b^{90},a	k	d	d	c	c	b	c	g^{60}, f^{35},e	h	d	b^{85},c	d	g	e	0.029	0.015
58	"	f	b	b^{90},a	g	a	b	c	b	b	k	d	d	c	c	b	c	i	h	d	c	d	g	e	0.004	0.004
64	"	f	b	c	g	a	b	c	b	b	k	d	c	c	c	b	c	c	h	d	b	d	g	e	0.000	0.000
65	'flindersi' Tas	f	b	b	g	a	b	c	b	b	k	d	c^{95},b	c	c	b	c	c	h	d	c	d	g	e	0.002	0.002

APPENDIX 4. PHILYPNODON

Allozyme profiles at the 45 variable loci for the 269 *Philypnodon* surveyed in the overview study. Individuals are identified by site code plus a unique number (e.g. G1-1 = fish #1 from site G1). Prefixes for site codes indicate species group (G = *Philypnodon grandiceps*, M = *Philypnodon macrostomus*, H = *P. grandiceps* × *P. macrostomus* hybrid). Taxon abbreviations for *P. grandiceps* are: FC = Fitzroy/Clarence, SEQ = SEQ complex, south = southern; for *P. macrostomus*: Mary, SEMD = SEQ/MDB. A dash indicates no genotype was assignable and reflects either poor activity or mobility overlap with another locus.

Fish #	Taxon	*Acon1*	*Acp1*	*Ada*	*Adh1*	*Adh2*	*Ald1*	*Ald2*	*Ap*	*Ca*	*Enol1*	*Enol2*	*Est*	*Fdp*	*Fum*	*Gda*	*Glo*	*Got1*	*Got2*	*Gp*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Me*	*Mpi*	*PepA1*	*PepA2*	*PepB1*	*PepB2*	*PepD1*	*PepD2*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk1*	*Pk2*	*Tpi1*	*Tpi2*	*Ugpp*
G1-1	FC	f	b	d	b	c	a	b	b	e	a	a	a	c	b	a	a	d	b	b	g	h	b	e	b	d	c	b	c	f	c	e	f	-	c	d	a	d	d	d	d	-	b	b	d	a
G2-1	"	f	b	d	b	c	a	b	b	e	a	a	a	c	b	a	a	d	b	b	g	hj	b	e	b	d	c	b	c	e	c	e	f	-	c	bd	a	d	f	d	e	a	b	b	d	a
G2-2	"	f	b	d	b	c	a	b	b	e	a	a	a	c	b	a	a	d	b	b	g	j	b	e	b	d	c	b	c	e	c	e	f	-	c	bd	a	d	f	d	e	a	b	b	d	a
G3-1	"	df	b	d	bc	c	a	b	b	e	a	a	a	c	b	a	a	d	b	b	g	h	b	e	b	d	c	b	c	e	ac	ce	f	-	c	d	a	d	f	d	h	a	b	b	d	a
G3-2	"	f	b	d	bc	c	a	b	b	ce	a	a	a	c	b	a	a	d	b	b	g	h	b	e	b	d	c	b	c	e	ac	ce	f	-	c	d	a	d	f	d	h	a	b	b	d	a
G4-1	South	d	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	ef	a	e	c	-	c	dg	a	d	f	d	e	a	b	b	d	a
G4-2	"	d	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	ef	a	e	c	-	c	d	a	d	f	d	e	a	b	b	d	a
G4-3	"	d	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	a	e	c	-	c	dg	a	d	f	d	e	a	b	b	d	a
G4-4	"	d	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	a	e	c	-	c	d	a	d	f	d	e	a	b	b	d	a
G4-5	"	d	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	a	e	c	-	c	dg	a	d	f	d	e	a	b	b	d	a
G5-1	"	d	b	c	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	ab	e	ce	-	c	d	a	d	d	d	e	a	ab	b	cd	a
G5-2	"	d	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	ab	e	ce	-	c	dg	a	d	d	d	e	a	b	b	d	a
G6-1	SEQ	fg	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	e	b	c	f	-	c	g	a	d	f	d	g	a	b	b	d	a
G6-2	"	fg	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	e	b	c	f	-	c	g	a	d	f	d	e	a	b	b	d	a
G6-3	"	fg	b	cd	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	b	c	f	-	c	g	a	d	f	d	eg	a	b	b	d	a
G6-4	"	g	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	e	b	c	f	-	c	g	a	d	f	d	eg	a	b	b	d	a
G6-5	"	g	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	e	b	c	f	-	c	g	a	d	f	d	e	a	b	b	d	a
G7-1	"	d	b	b	b	c	ab	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	a	ac	ef	b	c	f	-	c	d	a	d	d	d	e	a	bc	b	d	a
G7-2	"	d	b	b	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	a	c	f	b	c	f	-	c	d	a	d	d	d	e	a	bc	b	d	a

Fish #	Taxon	*Acon1*	*Acp1*	*Ada*	*Adh1*	*Adh2*	*Ald1*	*Ald2*	*Ap*	*Ca*	*Enol1*	*Enol2*	*Est*	*Fdp*	*Fum*	*Gda*	*Glo*	*Got1*	*Got2*	*Gp*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Me*	*Mpi*	*PepA1*	*PepA2*	*PepB1*	*PepB2*	*PepD1*	*PepD2*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk1*	*Pk2*	*Tpi1*	*Tpi2*	*Ugpp*
G7-3	“	d	b	b	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	a	c	e	b	c	f	-	c	d	a	d	d	d	e	a	b	b	d	a
G7-4	“	d	b	b	b	c	ab	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	a	c	ef	b	c	f	-	c	d	a	d	d	d	e	a	b	b	d	a
G7-5	“	d	b	b	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	a	c	ef	b	c	f	-	c	d	a	d	d	d	e	a	bc	b	cd	a
G8-1	“	fi	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	ae	b	d	c	b	c	f	e	e	ce	-	c	e	b	d	d	bd	e	a	b	b	d	a
G8-2	“	i	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	ae	b	d	c	b	c	f	e	e	e	-	c	e	b	d	d	d	e	a	b	b	d	a
G8-3	“	f	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	e	e	e	-	c	e	b	d	d	d	e	a	b	b	d	a
G8-4	“	i	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	ae	b	d	c	b	c	f	e	e	e	-	c	e	ab	d	d	d	e	a	b	b	d	a
G8-5	“	i	b	d	bc	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	hi	b	ae	b	d	c	b	c	f	e	e	ce	-	c	eg	b	d	d	bd	e	a	b	b	d	a
G9-1	Maroon	e	a	d	c	c	a	b	b	e	b	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	e	c	-	c	e	b	d	d	b	g	a	b	b	d	a
G9-2	“	e	a	d	bc	c	a	b	b	e	b	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	e	ce	-	c	e	b	d	d	b	eg	a	b	b	d	a
G9-3	“	e	a	d	c	c	a	b	b	e	b	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	e	c	-	c	e	b	d	d	b	g	a	b	b	d	a
G9-4	“	e	a	d	c	c	a	b	b	e	b	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	e	c	-	c	e	b	d	d	b	g	a	b	b	d	a
G9-5	“	e	a	d	c	c	a	b	b	e	b	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	e	c	-	c	e	b	d	d	b	g	a	b	b	d	a
G10-1	SEQ	f	b	cd	b	c	a	b	b	e	a	a	a	c	b	b	b	d	b	a	d	h	b	e	b	d	c	b	c	f	c	ce	c	-	c	g	a	d	d	bd	e	a	b	b	d	a
G10-2	“	f	b	c	b	c	a	b	b	e	a	a	a	c	b	b	b	d	b	a	d	hi	b	e	b	d	c	b	c	f	c	de	c	-	c	f	a	d	d	bd	e	a	b	b	d	a
G10-3	“	f	b	c	b	c	a	b	b	e	a	a	a	c	b	b	b	d	b	a	dg	h	b	e	b	d	c	b	c	f	c	e	c	-	c	fg	a	d	d	bd	e	a	b	b	d	a
G10-4	“	f	b	c	b	c	a	b	b	e	a	a	a	c	b	b	b	d	b	a	d	h	b	e	b	d	c	b	c	f	c	e	c	-	c	g	a	d	d	b	e	a	b	b	d	a
G10-5	“	f	b	cd	b	c	a	b	b	e	a	a	a	c	b	b	b	d	b	a	d	hi	b	e	b	d	c	b	c	f	c	e	c	-	c	fg	a	d	d	bd	e	a	b	b	d	a
G11-1	South	bf	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	cd	c	b	c	f	cf	e	c	-	c	g	a	d	d	d	f	a	b	b	d	a
G11-2	“	f	b	d	b	c	a	b	b	c	a	a	a	c	b	-	b	d	b	a	g	hi	b	e	b	c	ce	b	c	f	c	e	c	-	c	d	a	d	d	d	f	a	b	b	d	a
G11-3	“	fg	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	c	c	b	c	f	c	e	c	-	cd	g	a	d	d	bd	f	a	bc	b	d	a
G11-4	“	f	b	d	bc	c	a	b	b	ce	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	ce	b	c	f	c	e	c	-	cd	d	a	d	d	d	f	a	b	b	d	a
G11-5	“	fg	b	d	b	c	a	b	b	ce	a	a	a	c	b	a	b	d	b	a	g	fi	b	e	b	cd	e	b	c	f	c	ce	c	-	c	d	a	d	d	d	f	a	b	b	d	a
G12-1	“	f	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	hi	b	e	b	d	c	b	c	f	f	e	c	-	c	g	a	bd	d	df	e	a	b	b	d	a
G12-2	“	fg	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	cf	ef	c	-	c	fg	a	ad	d	d	e	a	b	b	d	a

Fish #	Taxon	*Acon1*	*Acp1*	*Ada*	*Adh1*	*Adh2*	*Ald1*	*Ald2*	*Ap*	*Ca*	*Enol1*	*Enol2*	*Est*	*Fdp*	*Fum*	*Gda*	*Glo*	*Got1*	*Got2*	*Gp*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Me*	*Mpi*	*PepA1*	*PepA2*	*PepB1*	*PepB2*	*PepD1*	*PepD2*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk1*	*Pk2*	*Tpi1*	*Tpi2*	*Ugpp*
G12-3	“	dg	b	d	bc	c	a	b	b	ce	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	f	e	c	-	c	d	a	d	d	d	e	a	b	b	d	a
G12-4	“	dg	b	d	c	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	cf	ef	c	-	c	dg	a	bd	d	d	e	a	b	b	d	a
G12-5	“	fg	b	cd	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	fi	-	e	b	d	c	b	c	f	f	e	c	-	c	g	a	d	d	d	e	a	b	b	d	a
G13-1	FC	d	b	c	b	c	a	b	b	e	a	a	a	c	b	ab	a	d	b	a	g	h	b	e	b	d	e	b	c	f	c	c	f	-	d	d	a	d	d	d	e	a	b	b	d	a
G13-2	“	d	b	ad	b	c	a	b	b	e	a	a	a	c	b	ab	a	d	b	a	g	h	b	e	b	d	e	b	c	f	c	c	f	-	d	d	a	d	d	d	e	a	b	b	d	a
G13-3	“	d	b	ad	b	c	a	b	b	de	a	ac	a	c	b	a	a	d	b	a	g	h	b	e	b	d	e	b	c	f	c	c	f	-	d	d	a	d	d	d	e	a	b	b	d	a
G13-4	“	d	b	cd	b	c	a	b	b	e	a	a	a	c	b	a	a	d	b	a	g	h	b	e	b	d	e	b	c	f	c	c	f	-	d	d	a	d	d	d	e	a	b	b	d	a
G13-5	“	d	b	c	b	c	a	b	b	e	a	a	a	c	b	a	a	d	b	a	g	h	b	e	b	d	e	b	c	f	c	c	f	-	d	d	a	d	d	d	e	a	b	b	d	a
G14-1	South	g	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	g	b	e	b	d	c	b	c	f	cf	e	c	-	c	g	a	d	d	d	e	a	b	b	d	a
G14-2	“	g	b	cd	b	c	a	b	b	ce	a	a	a	c	b	a	b	d	b	a	g	fg	b	e	b	d	c	b	c	f	c	e	ce	-	c	g	a	d	d	d	e	-	b	b	d	a
G14-3	“	g	b	d	b	c	a	b	b	ce	a	a	a	c	b	a	b	d	b	a	g	g	b	e	b	d	c	b	c	fg	c	e	e	-	c	g	a	d	d	d	e	a	b	b	d	a
G15-1	“	g	b	d	b	c	a	b	b	ce	a	a	a	c	b	a	b	d	b	a	g	gi	b	e	b	d	c	b	c	f	d	e	c	-	c	g	a	d	d	d	ef	a	b	b	d	a
G15-2	“	g	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	b	c	f	cf	e	ae	-	c	g	a	d	d	d	e	a	b	b	d	a
G16-1	“	g	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	gh	b	e	b	d	c	b	c	f	cf	e	c	-	c	g	a	d	d	d	de	a	b	b	d	a
G16-2	“	g	b	d	bc	c	a	b	b	ce	a	a	a	c	b	a	b	d	b	a	g	fi	b	e	b	d	c	b	c	f	f	e	c	-	c	dg	a	d	d	d	e	a	c	b	d	a
G17-1	“	f	b	d	b	c	a	b	b	ce	ab	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	ef	c	-	c	d	a	d	d	d	eg	a	b	b	d	a
G17-2	“	g	b	d	b	c	a	b	b	ce	b	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	ef	c	-	c	d	a	d	d	d	g	a	b	b	d	a
G17-3	“	g	b	d	b	c	a	b	b	e	ab	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	ef	c	-	c	d	a	d	d	d	eg	a	b	b	d	a
G17-4	“	g	b	d	b	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	d	a	d	d	d	eg	a	b	b	d	a
G18-1	“	g	b	d	bc	c	a	b	b	c	a	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	cf	ef	c	-	c	dg	a	d	d	d	e	b	bc	b	d	a
G18-2	“	g	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	e	c	-	c	g	a	d	d	d	e	a	c	b	d	a
G18-3	“	g	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	ab	b	b	d	a
G19-1	“	g	b	d	bc	c	a	b	b	e	a	a	a	c	b	a	b	d	b	a	g	h	b	a	b	d	c	b	c	f	cd	e	c	-	c	g	a	d	d	d	ef	a	b	b	d	a
G19-2	“	g	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	eh	b	e	b	d	c	b	c	ef	df	e	c	-	c	dg	a	d	d	d	e	b	bc	b	d	a
G19-3	“	g	b	d	bc	c	a	b	b	ce	a	a	a	c	b	a	b	d	b	a	g	eh	b	e	b	d	c	b	c	ef	c	de	c	-	c	g	a	d	d	d	e	ab	b	b	d	a

Fish #	Taxon	*Acon1*	*Acp1*	*Ada*	*Adh1*	*Adh2*	*Ald1*	*Ald2*	*Ap*	*Ca*	*Enol1*	*Enol2*	*Est*	*Fdp*	*Fum*	*Gda*	*Glo*	*Got1*	*Got2*	*Gp*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Me*	*Mpi*	*PepA1*	*PepA2*	*PepB1*	*PepB2*	*PepD1*	*PepD2*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk1*	*Pk2*	*Tpi1*	*Tpi2*	*Ugpp*
G20-1	“	g	b	d	ab	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	ef	e	-	c	dg	a	d	d	d	e	a	bc	b	d	a
G20-2	“	g	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	ef	ce	-	c	g	a	d	d	d	e	a	bc	b	d	a
G20-3	“	g	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	ef	e	-	c	g	a	d	d	d	e	a	bc	b	d	a
G21-1	“	g	b	d	bc	c	a	b	b	c	a	a	a	c	b	b	b	d	b	a	g	hi	b	e	b	d	c	b	c	f	bc	f	c	-	c	dg	a	d	d	d	e	b	b	b	d	a
G21-2	“	g	b	df	c	c	a	b	b	c	a	a	a	c	b	b	b	d	b	a	g	hi	b	ce	b	d	c	b	c	f	bc	f	c	-	c	g	a	d	d	d	e	b	bc	b	d	a
G21-3	“	g	b	df	c	c	a	b	b	c	a	a	a	c	b	ab	b	d	b	a	g	i	b	c	b	d	c	b	c	f	bc	f	c	-	c	d	a	d	d	d	e	b	b	b	d	a
G22-1	“	g	b	d	a	c	a	b	b	c	a	a	a	c	b	b	b	d	b	-	g	h	b	e	b	d	c	b	c	f	bc	ef	c	-	c	dg	a	d	d	d	e	b	bc	b	d	a
G22-2	“	g	b	d	c	c	a	b	b	c	a	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	bc	b	d	a
G22-3	“	g	b	d	c	c	a	b	b	c	a	a	a	c	b	-	b	d	b	a	g	hi	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	b	b	d	a
G23-1	“	g	b	d	ac	c	a	b	b	c	ac	a	a	c	b	b	b	d	b	a	g	h	b	c	b	d	c	b	c	f	bc	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G23-2	“	g	b	d	ac	c	a	b	b	c	a	a	a	c	b	b	b	d	b	a	g	h	b	c	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	bc	b	d	a
G24-1	“	-	b	d	c	c	a	b	b	c	a	a	a	c	b	b	b	d	b	a	g	h	b	c	b	d	c	b	c	f	bc	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G24-2	“	g	b	d	c	c	a	b	b	c	a	a	a	c	b	b	b	d	b	a	g	h	b	c	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	eg	b	c	b	d	a
G24-3	“	g	b	d	c	c	a	b	b	c	a	a	a	c	b	-	b	d	b	a	g	h	b	c	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G24-4	“	g	b	d	c	c	a	b	b	c	a	a	a	c	b	b	b	d	b	a	g	h	b	c	b	d	c	b	c	f	bc	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G25-1	“	g	b	d	c	c	a	b	b	c	a	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G25-2	“	g	b	d	c	c	a	b	b	c	ac	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G26-1	“	g	b	d	c	c	a	b	b	c	c	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G26-2	“	g	b	d	c	c	a	b	b	c	c	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G27-1	“	g	b	d	c	c	a	b	b	c	c	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G28-1	“	g	b	d	c	c	a	b	b	c	ac	a	a	c	b	ab	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	bc	c	b	d	a
G28-2	“	g	b	d	c	c	a	b	b	c	ac	a	a	c	b	ab	b	d	b	a	g	eh	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	ab	c	b	d	a
G29-1	“	g	b	d	bc	c	a	b	b	c	c	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G30-1	“	g	b	d	c	c	a	b	b	c	c	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	bc	b	d	a
G30-2	“	g	b	d	c	c	a	b	b	c	c	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a

Fish #	Taxon	*Acon1*	*Acp1*	*Ada*	*Adh1*	*Adh2*	*Ald1*	*Ald2*	*Ap*	*Ca*	*Enol1*	*Enol2*	*Est*	*Fdp*	*Fum*	*Gda*	*Glo*	*Got1*	*Got2*	*Gp*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Me*	*Mpi*	*PepA1*	*PepA2*	*PepB1*	*PepB2*	*PepD1*	*PepD2*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk1*	*Pk2*	*Tpi1*	*Tpi2*	*Ugpp*
G31-1	“	h	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	hi	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	bd	d	d	f	a	c	b	d	a
G31-2	“	gh	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	d	d	d	f	a	b	b	d	a
G32-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	d	c	-	c	g	a	d	d	d	e	ab	c	b	d	a
G32-2	“	g	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	ef	a	c	b	d	a
G33-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	d	d	d	ef	a	bc	b	d	a
G33-2	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	e	b	e	b	d	c	b	c	ef	c	df	c	-	c	g	a	d	d	d	ef	a	bc	b	d	a
G34-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	f	c	d	c	-	c	g	a	d	d	d	f	a	bc	b	d	a
G35-1	“	g	b	d	c	c	a	b	b	c	ac	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G35-2	“	g	b	d	c	c	a	b	b	c	ac	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	e	b	c	b	d	a
G37-1	“	gh	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	b	c	e	c	e	c	-	c	g	a	d	d	d	f	a	bc	b	d	a
G37-2	“	gh	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	e	bc	e	c	-	c	g	a	d	d	d	f	a	bc	b	d	a
G37-3	“	gh	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	f	c	e	c	-	c	g	a	d	d	d	f	a	bc	b	d	a
G38-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	ef	c	de	c	-	c	g	a	d	d	d	ef	a	c	b	d	a
G38-2	“	h	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	f	c	d	c	-	c	g	a	d	d	d	e	a	bc	b	d	a
G39-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	d	d	d	e	a	bc	b	d	a
G39-2	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	e	b	e	b	d	c	b	c	e	c	d	c	-	c	g	a	d	d	d	ef	a	bc	b	d	a
G39-3	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	d	d	d	ef	a	b	b	d	a
G40-1	“	gh	b	d	c	c	a	b	b	c	a	a	a	c	b	ab	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	ef	c	-	c	g	a	d	d	d	ef	ab	c	b	d	a
G40-2	“	g	b	d	c	c	a	b	b	c	a	a	a	c	b	b	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	f	c	-	c	g	a	d	d	d	ef	b	c	b	d	a
G40-3	“	g	b	d	c	c	a	b	b	c	ac	a	a	c	b	ab	b	d	b	a	g	hi	b	e	b	d	c	b	c	ef	c	df	c	-	c	g	a	d	d	d	ef	b	c	b	d	a
G40-4	“	gh	b	d	c	c	a	b	b	c	a	a	a	c	b	ab	b	d	b	a	g	h	b	e	b	d	c	b	c	f	c	df	c	-	c	g	a	d	d	d	ef	ab	c	b	d	a
G41-1	“	gh	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	bd	d	d	e	a	bc	b	d	a
G41-2	“	gh	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	e	b	e	b	d	c	b	c	e	c	de	c	-	c	g	a	d	d	d	ef	a	bc	b	d	a
G42-1	“	gh	b	d	c	c	a	b	b	c	a	a	a	c	b	ab	b	d	b	a	g	ei	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	d	d	d	e	ab	bc	b	d	a
G42-2	“	gj	b	d	c	c	a	b	b	c	a	a	a	c	b	ab	b	d	b	a	g	hi	b	e	b	d	c	b	c	ef	c	f	c	-	c	g	a	bd	d	d	e	ab	c	b	d	a

Fish #	Taxon	*Acon1*	*Acp1*	*Ada*	*Adh1*	*Adh2*	*Ald1*	*Ald2*	*Ap*	*Ca*	*Enol1*	*Enol2*	*Est*	*Fdp*	*Fum*	*Gda*	*Glo*	*Got1*	*Got2*	*Gp*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Me*	*Mpi*	*PepA1*	*PepA2*	*PepB1*	*PepB2*	*PepD1*	*PepD2*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk1*	*Pk2*	*Tpi1*	*Tpi2*	*Ugpp*
G43-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	e	c	d	c	-	c	g	a	d	d	d	f	a	c	b	d	a
G43-2	“	h	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	f	c	d	c	-	c	g	a	d	d	d	f	a	b	b	d	a
G44-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	e	c	d	c	-	c	g	a	d	d	d	ef	a	b	b	d	a
G44-2	“	gh	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	e	b	e	b	d	c	b	c	e	c	d	c	-	c	g	a	d	d	d	ef	a	b	b	d	a
G45-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	e	b	e	b	d	c	b	c	e	c	de	c	-	c	g	a	d	d	d	ef	a	bc	b	d	a
G46-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	d	d	d	ef	a	c	b	d	a
G47-1	“	h	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	d	d	d	e	a	c	b	d	a
G48-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	f	c	df	c	-	c	g	a	bd	d	d	f	a	c	b	d	a
G49-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	e	b	e	b	d	c	b	c	e	c	d	c	-	c	g	a	d	d	d	ef	a	bc	b	d	a
G49-2	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	d	d	d	ef	a	c	b	d	a
G50-1	“	gh	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	eh	b	e	b	d	c	b	c	e	c	de	c	-	c	g	a	bd	d	d	f	a	bc	b	d	a
G50-2	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	bd	d	d	ef	a	c	b	d	a
G51-1	“	gh	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	e	b	e	b	d	c	b	c	e	c	de	c	-	c	g	a	b	d	d	f	a	bc	b	d	a
G51-2	“	g	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	b	c	ef	c	e	c	-	c	g	a	bd	d	d	f	a	b	b	d	a
G51-3	“	g	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	bd	d	d	f	a	b	b	d	a
G51-4	“	gh	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	e	c	de	c	-	c	g	a	b	d	d	ef	a	c	b	d	a
G52-1	“	gh	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	e	b	e	b	d	c	b	c	e	c	d	c	-	c	g	a	d	d	d	ef	a	b	b	d	a
G52-2	“	h	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	e	b	e	b	d	c	b	c	e	c	d	c	-	c	g	a	d	d	d	f	a	b	b	d	a
G53-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	b	c	f	c	d	c	-	c	g	a	d	d	d	ef	a	b	b	d	a
G53-2	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	b	c	f	c	d	c	-	c	g	a	d	d	d	f	a	bc	b	d	a
G54-1	“	h	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	i	b	e	b	d	c	b	c	ef	c	de	c	-	c	g	a	d	d	d	e	a	b	b	d	a
G54-2	“	gh	b	d	b	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	eh	b	e	b	d	c	b	c	e	c	de	c	-	c	g	a	d	d	d	e	a	c	b	d	a
G54-3	“	gh	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	ef	c	de	c	-	c	g	a	d	d	d	f	a	bc	b	d	a
G55-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	e	b	e	b	d	c	b	c	e	c	de	c	-	c	g	a	bd	d	d	ef	a	c	b	d	a
G55-2	“	gh	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	ei	b	e	b	d	c	b	c	e	c	d	c	-	c	g	a	d	d	d	ef	a	b	b	d	a

Fish #	Taxon	*Acon1*	*Acp1*	*Ada*	*Adh1*	*Adh2*	*Ald1*	*Ald2*	*Ap*	*Ca*	*Enol1*	*Enol2*	*Est*	*Fdp*	*Fum*	*Gda*	*Glo*	*Got1*	*Got2*	*Gp*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Me*	*Mpi*	*PepA1*	*PepA2*	*PepB1*	*PepB2*	*PepD1*	*PepD2*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk1*	*Pk2*	*Tpi1*	*Tpi2*	*Ugpp*
G56-1	“	h	b	d	c	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	hi	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	bd	d	d	ef	a	bc	b	d	a
G56-2	“	gh	b	d	bc	c	a	b	b	c	a	a	a	c	b	a	b	d	b	a	g	eh	b	e	b	d	c	b	c	ef	c	d	c	-	c	g	a	bd	d	d	e	a	c	b	d	a
H1-1	Hybrid	dh	b	d	b	bc	a	ab	b	cd	a	a	ab	cd	b	a	b	ad	bc	ab	eg	di	-	e	ab	bd	ac	b	cd	be	bc	cd	c	-	bc	fg	a	ab	d	d	cf	a	bc	ab	bd	ab
M1-1	SEMD	ae	b	c	b	b	a	a	b	ad	a	a	b	d	b	-	b	a	c	b	f	d	c	f	a	b	a	b	d	b	b	c	b	a	b	c	a	a	c	d	c	a	b	a	b	b
M1-2	“	e	b	c	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	f	d	c	f	a	b	a	b	d	b	b	c	b	-	b	c	a	a	ac	d	c	a	b	a	b	b
M1-3	“	de	b	cg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	f	d	c	f	a	b	a	b	d	b	b	c	b	a	b	c	a	a	c	d	c	a	b	a	b	b
M1-4	SEMD	e	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	f	d	c	f	a	b	a	b	d	b	b	c	b	a	b	c	a	a	c	d	c	a	b	a	b	b
M1-5	“	de	b	-	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	f	d	c	f	a	b	a	b	d	b	b	c	b	-	b	c	a	a	c	d	c	a	b	a	b	b
M2-1	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	d	b	e	d	c	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M2-2	“	d	b	cd	b	b	a	a	b	d	a	a	b	d	b	-	b	a	d	b	e	d	cd	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M2-3	“	d	b	cg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	d	b	e	d	cd	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M2-4	“	d	b	cg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	d	b	e	d	cd	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M2-5	“	d	b	cg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	d	b	e	d	c	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M3-1	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	d	b	e	d	c	d	a	b	a	b	d	b	b	c	b	-	b	f	a	a	c	d	c	a	b	a	b	b
M3-2	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	c	d	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M3-3	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	cd	b	e	d	c	d	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M3-4	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	d	b	e	d	c	d	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M3-5	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	cd	b	e	d	c	d	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M4-1	Burrum	d	b	c	b	b	a	a	b	d	a	b	b	d	b	-	b	a	a	b	b	d	c	df	a	b	a	b	c	bd	b	c	b	a	b	f	a	a	c	d	cd	a	b	a	b	b
M4-2	“	d	b	d	b	b	a	a	b	d	a	ab	b	d	b	-	b	a	a	b	b	ad	c	d	a	b	a	b	c	b	b	c	b	a	b	f	a	a	c	d	cd	a	b	a	b	b
M4-3	“	d	b	cd	b	b	a	a	b	d	a	a	b	d	b	-	b	a	a	b	b	d	c	df	a	b	a	b	c	d	b	c	b	a	b	f	a	a	c	d	cd	a	b	a	b	b
M4-4	“	d	b	cd	b	b	a	a	b	d	a	ab	b	d	b	-	b	a	a	b	b	d	c	df	a	b	a	b	c	d	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M4-5	“	d	b	c	b	b	a	a	b	d	a	ab	b	d	b	-	b	a	a	b	b	d	c	df	a	b	a	b	c	bd	b	c	bd	a	b	f	a	a	c	d	cd	a	b	a	b	b
M5-1	“	d	b	cd	b	b	a	a	b	d	a	b	b	d	b	-	b	a	c	a	b	bd	c	d	a	b	a	b	c	b	b	c	d	a	b	a	a	a	c	d	c	a	b	a	a	b
M5-2	“	d	b	ce	b	b	a	a	b	d	a	b	b	d	b	-	b	a	c	a	b	d	c	d	a	b	a	b	c	b	b	c	d	a	b	a	a	a	c	d	c	a	b	a	ab	b

Fish #	Taxon	*Acon1*	*Acp1*	*Ada*	*Adh1*	*Adh2*	*Ald1*	*Ald2*	*Ap*	*Ca*	*Enol1*	*Enol2*	*Est*	*Fdp*	*Fum*	*Gda*	*Glo*	*Got1*	*Got2*	*Gp*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Me*	*Mpi*	*PepA1*	*PepA2*	*PepB1*	*PepB2*	*PepD1*	*PepD2*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk1*	*Pk2*	*Tpi1*	*Tpi2*	*Ugpp*
M5-3	“	d	b	ce	b	b	a	a	b	d	a	b	b	d	b	-	b	a	c	a	b	bd	c	d	a	b	a	b	c	b	b	c	d	a	b	a	a	a	c	d	c	a	b	a	b	b
M5-4	“	d	b	c	b	b	a	a	b	d	a	b	b	d	b	-	b	a	c	a	b	b	c	d	a	b	a	b	c	b	b	c	d	a	b	a	a	a	c	d	c	a	b	a	b	b
M5-5	“	d	b	d	b	b	a	a	b	d	a	b	b	d	b	-	b	a	c	a	b	b	c	d	a	b	a	b	c	d	b	c	d	a	b	a	a	a	c	d	c	a	b	a	a	b
M6-1	Mary	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	c	d	b	a	b	a	b	c	b	b	c	b	b	b	af	a	b	c	d	f	a	b	a	b	b
M6-2	“	d	b	de	b	b	a	a	b	d	a	b	b	d	b	-	b	a	c	b	e	c	d	b	a	b	a	b	c	b	b	c	b	b	b	af	a	b	c	d	f	a	b	a	b	b
M6-3	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	c	d	b	a	b	a	b	c	b	b	c	b	b	b	f	a	b	c	d	f	a	b	a	b	b
M6-4	“	d	b	d	b	b	a	a	b	d	a	b	b	d	b	-	b	a	c	b	e	c	d	b	a	b	a	b	c	b	b	c	b	b	b	f	a	b	c	d	f	a	b	a	b	b
M6-5	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	c	d	b	a	b	a	b	c	b	b	c	b	b	b	af	a	b	c	d	f	a	b	a	b	b
M7-1	SEC	bd	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	f	d	c	f	a	b	a	b	b	b	b	c	b	c	b	c	a	a	c	d	d	a	b	a	b	b
M7-2	“	b	b	c	b	b	a	a	b	d	a	a	b	d	b	-	b	a	cd	a	f	d	c	f	a	b	a	b	bd	b	b	c	b	cd	b	c	a	a	bc	d	bd	a	b	a	b	b
M7-3	“	d	b	cd	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	f	d	c	f	a	b	a	b	b	b	b	c	b	c	b	c	a	a	c	d	d	a	b	a	b	b
M7-4	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	f	d	c	f	a	b	a	b	b	b	b	c	b	cd	b	c	a	a	bc	d	d	a	b	a	b	b
M7-5	“	bd	b	c	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	f	d	c	f	a	b	a	b	b	b	b	c	b	c	b	c	a	a	bc	d	d	a	b	a	b	b
M8-1	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	b	c	b	c	b	c	a	a	c	d	b	a	b	a	b	b
M8-2	“	e	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	bd	bc	b	c	b	cd	b	c	a	a	c	d	b	a	b	a	b	b
M8-3	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	cd	f	a	b	a	b	b	bc	b	c	b	cd	b	c	a	a	c	d	b	a	b	a	b	b
M9-1	“	e	b	bd	b	b	a	a	b	d	a	a	b	d	ab	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	c	c	b	ac	b	c	a	a	c	d	cd	a	b	a	b	b
M9-2	“	e	b	b	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	bd	c	f	a	b	a	b	d	bc	c	c	b	cd	b	cf	a	a	bc	d	d	a	b	a	b	b
M9-3	“	d	b	bd	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	bd	b	bc	bc	b	d	b	c	a	a	c	d	cd	a	b	a	b	b
M9-4	“	e	b	bd	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	bc	c	b	ac	b	cf	a	a	c	d	bd	a	b	a	b	b
M9-5	“	be	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	b	c	b	cd	b	c	a	a	c	d	bd	a	b	a	b	b
M10-1	“	de	b	df	b	b	a	a	b	d	a	a	b	b	b	-	b	a	c	a	g	d	c	f	a	b	a	b	d	b	b	c	b	cd	b	cf	a	a	c	d	b	a	b	a	b	b
M10-2	“	d	b	df	b	b	a	a	b	d	a	a	b	bd	b	-	b	a	c	a	g	d	c	f	a	b	a	b	d	b	b	c	b	cd	b	cf	a	a	c	d	-	a	b	a	b	b
M10-3	“	e	b	df	b	b	a	a	b	d	a	a	b	bd	b	-	b	a	c	a	g	d	c	f	a	b	a	b	d	b	bc	c	b	d	b	f	a	a	c	df	bc	a	b	a	b	b
M10-4	“	de	b	df	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	g	d	c	f	a	b	a	b	d	b	b	c	b	d	b	cf	a	a	c	d	bd	a	b	a	b	b

Fish #	Taxon	*Acon1*	*Acp1*	*Ada*	*Adh1*	*Adh2*	*Ald1*	*Ald2*	*Ap*	*Ca*	*Enol1*	*Enol2*	*Est*	*Fdp*	*Fum*	*Gda*	*Glo*	*Got1*	*Got2*	*Gp*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Me*	*Mpi*	*PepA1*	*PepA2*	*PepB1*	*PepB2*	*PepD1*	*PepD2*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk1*	*Pk2*	*Tpi1*	*Tpi2*	*Ugpp*
M10-5	“	e	b	d	b	b	a	a	b	d	a	a	b	b	b	-	b	a	c	a	g	d	c	f	a	b	a	b	d	b	b	c	b	d	b	cf	a	a	c	d	c	a	b	a	b	b
M11-1	“	d	b	b	b	b	a	a	b	d	a	a	b	d	a	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	b	bc	b	ac	b	c	a	a	c	d	b	a	b	a	b	b
M11-2	“	de	b	b	b	b	a	a	b	d	a	a	b	d	ab	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	ab	bc	b	cd	b	c	a	a	c	d	bd	a	b	a	b	b
M11-3	“	de	b	b	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	ab	c	b	ac	b	cf	a	a	c	d	ad	a	b	a	b	b
M11-4	“	d	b	bd	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	a	bc	b	cd	ab	c	a	a	c	d	bd	a	b	a	b	b
M11-5	“	d	b	b	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	ce	d	a	f	a	b	a	b	b	b	b	c	b	c	b	f	a	a	c	d	d	a	b	a	b	b
M12-1	“	de	b	b	b	b	a	a	b	d	a	a	b	d	ab	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	bc	bc	b	c	b	c	a	a	c	d	bd	a	b	a	b	b
M12-2	“	e	b	ab	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	b	c	b	c	b	c	a	a	c	d	bd	a	b	a	b	b
M12-3	“	de	b	ab	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	bd	bc	b	c	b	c	b	c	a	a	c	d	ad	a	b	a	b	b
M12-4	“	de	b	b	b	b	a	a	b	d	a	a	b	d	ab	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	b	ac	b	c	b	cf	a	a	c	d	b	a	b	a	b	b
M12-5	“	d	b	bd	b	b	a	a	b	bd	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	b	c	b	d	b	c	a	ac	c	d	bd	a	b	a	b	b
M13-1	“	de	b	bd	b	b	a	a	b	d	a	a	b	ad	b	-	b	a	c	a	e	d	c	f	a	b	ab	b	b	b	b	c	b	ac	b	cf	a	a	c	d	b	a	b	a	b	b
M14-1	“	de	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	dg	c	fg	a	b	a	b	d	b	b	c	b	d	b	cf	a	a	c	d	d	a	b	a	b	b
M14-2	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	ab	c	a	e	d	c	fg	a	b	a	b	d	b	b	c	b	d	b	f	a	a	c	d	c	a	b	a	b	b
M15-1	“	d	b	b	b	b	a	a	b	d	a	a	b	d	b	-	b	ab	c	a	e	d	c	fg	a	a	a	b	bd	ab	b	c	b	c	b	c	a	a	c	cd	b	a	b	ac	b	b
M16-1	“	de	b	b	b	ab	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	bd	c	b	c	b	c	b	cf	a	a	ce	d	bd	a	b	a	b	b
M16-2	“	de	b	bd	b	a	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	be	a	b	b	c	b	c	b	d	b	cf	a	a	e	d	d	a	b	a	b	b
M16-3	“	de	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	ac	c	a	ae	d	c	f	a	be	a	b	d	bc	b	c	b	c	b	f	a	a	bc	d	d	a	b	a	b	b
M16-4	“	d	b	bd	b	b	a	a	b	d	a	a	b	d	b	-	b	ac	c	a	e	d	c	f	a	b	a	b	d	bc	b	c	b	c	b	f	a	a	ce	d	bd	a	b	a	b	b
M16-5	“	de	b	bd	b	a	a	a	b	d	a	a	b	d	ab	-	b	a	c	a	e	d	c	f	a	b	a	b	b	c	b	c	b	c	b	f	a	a	e	d	bd	a	b	a	b	b
M17-1	“	d	b	cd	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	b	b	b	c	b	ad	b	c	a	a	bc	d	d	a	b	a	b	b
M17-2	“	d	b	c	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	bd	c	b	c	b	ad	b	cf	a	a	bc	ad	d	a	b	a	b	b
M17-3	“	de	b	cd	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	g	c	f	a	b	a	b	d	b	b	c	b	d	b	f	a	a	c	d	d	a	b	a	b	b
M17-4	“	d	b	c	b	b	a	a	b	d	a	a	b	d	ab	-	b	a	c	a	e	dg	c	f	a	b	ad	b	b	bc	b	c	b	ac	b	c	a	a	bc	d	b	a	b	a	b	b
M17-5	“	de	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	dg	c	f	a	b	a	b	b	bc	b	c	b	d	b	c	a	a	bc	ad	bd	a	b	a	b	b

Fish #	Taxon	*Acon1*	*Acp1*	*Ada*	*Adh1*	*Adh2*	*Ald1*	*Ald2*	*Ap*	*Ca*	*Enol1*	*Enol2*	*Est*	*Fdp*	*Fum*	*Gda*	*Glo*	*Got1*	*Got2*	*Gp*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Me*	*Mpi*	*PepA1*	*PepA2*	*PepB1*	*PepB2*	*PepD1*	*PepD2*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk1*	*Pk2*	*Tpi1*	*Tpi2*	*Ugpp*
M18-1	“	de	b	cd	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	d	b	b	c	b	c	b	c	a	a	bc	d	d	a	b	a	b	b
M18-2	“	e	b	c	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	d	b	b	c	b	c	b	c	a	a	be	d	d	a	b	a	b	b
M18-3	“	de	b	cd	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	bd	b	b	c	b	c	b	c	a	a	e	d	d	a	b	a	b	b
M18-4	“	d	b	cd	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	d	b	b	c	b	cd	b	c	a	a	be	d	d	a	b	a	b	b
M19-1	“	d	b	df	b	b	a	a	b	d	a	a	b	d	ab	-	b	b	c	a	e	d	-	f	a	b	a	b	b	b	bc	c	b	d	b	f	a	a	b	ad	d	a	b	a	b	b
M19-2	“	d	b	d	b	b	a	a	b	d	a	a	b	d	ab	-	b	b	c	a	e	d	c	f	a	b	a	b	bd	b	b	c	b	d	b	f	a	a	b	ad	d	a	b	a	b	b
M19-3	“	d	b	bc	b	b	a	a	b	d	a	a	b	d	b	-	b	b	c	a	e	d	c	f	a	b	a	b	bd	b	b	c	b	d	b	f	a	a	b	a	d	a	b	a	b	b
M19-4	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	a	e	d	c	f	a	b	a	b	d	b	b	c	b	d	b	f	ac	a	be	a	d	a	b	a	b	b
M19-5	“	d	b	cd	b	b	a	a	b	d	a	a	b	d	b	-	b	b	c	a	e	d	c	f	a	b	a	b	b	b	b	c	b	ad	b	f	ac	a	be	a	d	a	b	a	b	b
M20-1	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	b	c	a	e	d	c	f	a	b	a	b	bd	b	b	c	b	d	b	f	c	a	b	d	d	a	b	a	b	b
M20-2	“	d	b	cd	b	b	a	a	b	d	a	a	b	d	b	-	b	b	c	a	e	d	c	f	a	b	a	b	bd	b	b	c	b	d	b	f	c	a	b	d	d	a	b	a	b	b
M21-1	“	d	b	c	b	b	a	a	b	d	a	a	b	d	b	-	b	b	c	a	e	d	c	f	a	b	a	b	d	b	b	c	b	d	b	f	c	a	b	d	d	a	b	a	b	b
M21-2	“	d	b	c	b	b	a	a	b	d	a	a	-	d	b	-	b	b	c	a	e	d	c	f	a	b	a	b	d	b	b	c	b	d	b	f	c	a	b	ad	d	a	b	a	b	b
M21-3	“	cd	b	c	b	b	a	a	b	d	a	a	b	d	b	-	b	b	c	a	e	d	c	f	a	b	a	b	d	b	b	c	b	d	b	f	c	a	b	d	d	a	b	a	b	b
M22-1	SEMD	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M22-2	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M22-3	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M23-1	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	cd	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M23-2	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M23-3	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	cd	f	a	b	a	ab	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M23-4	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M24-1	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M24-2	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	cd	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	c	c	a	b	a	b	b
M25-1	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	-	a	b	a	b	b
M25-2	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b

Fish #	Taxon	*Acon1*	*Acp1*	*Ada*	*Adh1*	*Adh2*	*Ald1*	*Ald2*	*Ap*	*Ca*	*Enol1*	*Enol2*	*Est*	*Fdp*	*Fum*	*Gda*	*Glo*	*Got1*	*Got2*	*Gp*	*Gpi1*	*Gpi2*	*Gsr*	*Idh1*	*Idh2*	*Mdh1*	*Mdh2*	*Mdh3*	*Me*	*Mpi*	*PepA1*	*PepA2*	*PepB1*	*PepB2*	*PepD1*	*PepD2*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk1*	*Pk2*	*Tpi1*	*Tpi2*	*Ugpp*
M25-3	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M26-1	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M27-1	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M27-2	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M27-3	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M28-1	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M28-2	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M29-1	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M29-2	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M30-1	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	de	c	a	b	a	b	b
M31-1	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M31-2	“	d	b	g	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M31-3	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M31-4	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M31-5	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M32-1	“	d	b	dg	b	b	a	a	ab	d	a	a	b	cd	b	-	b	a	c	b	e	d	cd	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M32-2	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M33-1	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	c	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M33-2	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M33-3	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M34-1	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M34-2	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M34-3	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M34-4	“	d	b	dg	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	d	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b
M34-5	“	d	b	d	b	b	a	a	b	d	a	a	b	d	b	-	b	a	c	b	e	d	cd	f	a	b	a	b	d	b	b	c	b	a	b	f	a	a	c	d	c	a	b	a	b	b

APPENDIX 5. GADOPSIS

Allozyme profiles at the 36 variable loci for 137 *Gadopsis* surveyed in the overview study. Individuals are identified by site code plus a unique number (e.g. M1-1 = fish #1 from site M1; M = *G. Marmoratus*, B = *G. bispinosus*). A dash indicates no genotype was assignable and reflects either poor activity or mobility overlap with another locus.

Fish #	Lineage	*Acon1*	*Acon2*	*Acp*	*Acyc*	*Ada*	*Adh1*	*Ap2*	*Ck*	*Enol2*	*Est*	*Fum*	*Got1*	*Got2*	*Gp*	*Gpd*	*Gpi1*	*Gpi2*	*Gsr*	*Idh*	*Ldh*	*Mdh1*	*Mdh2*	*Mdh3*	*Mpi*	*PepA1*	*PepA2*	*PepB*	*PepD1*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk2*	*Tpi2*	*Ugpp*
B1-1	Goulburn	b	b	a	d	b	a	a	a	b	b	b	a	e	a	b	c	b	b	b	b	b	b	b	f	a	c	d	b	b	cd	b	c	b	d	ac	d
B1-2	"	b	b	a	d	b	a	a	a	b	b	b	a	e	a	a	c	b	b	b	b	b	b	b	f	a	c	d	b	b	c	b	c	b	d	c	d
B1-3	"	b	-	a	d	b	a	a	a	b	b	b	a	e	a	ab	c	b	b	b	b	b	b	b	f	a	c	d	b	b	c	b	c	b	d	c	d
B2-1	Eastern	b	b	b	d	b	b	a	a	b	b	b	a	e	a	b	bc	b	b	b	b	b	b	b	f	a	c	d	b	b	b	b	c	b	d	a	e
B2-2	"	b	b	b	d	b	b	a	a	b	b	b	a	e	a	b	c	b	b	b	b	b	b	b	f	a	c	d	b	b	b	b	c	b	d	a	e
B2-3	"	b	ab	b	d	b	b	a	a	b	b	b	a	e	a	b	bc	b	b	b	b	b	b	b	f	a	c	d	b	b	b	b	c	b	d	a	e
B2-4	"	b	ab	b	d	b	b	a	a	b	b	b	a	e	a	b	cd	b	b	b	b	b	b	b	f	a	c	d	b	b	b	b	c	b	d	a	e
B3-1	"	b	-	b	d	b	b	a	a	b	b	b	a	e	a	b	b	b	bc	b	b	b	b	b	f	a	c	c	b	b	b	b	c	b	d	a	d
B3-2	"	b	-	b	d	b	b	a	a	b	b	b	a	e	a	b	b	b	b	b	b	b	b	b	f	a	c	c	b	b	b	b	c	b	d	a	d
B3-3	"	b	-	b	d	b	b	a	a	b	b	b	-	e	a	-	bc	b	c	b	b	b	b	b	f	a	c	-	bc	b	-	b	c	b	d	a	d
B4-1	"	ab	ab	b	d	b	b	a	a	bc	b	b	a	e	a	b	c	b	b	a	b	b	b	b	f	a	c	d	b	b	b	b	bc	b	d	c	d
B4-2	"	ab	ab	b	d	b	b	a	a	c	b	b	a	e	a	b	c	b	b	b	b	b	b	b	f	a	c	d	b	b	b	b	bc	b	d	c	d
B4-3	"	a	a	b	d	b	b	a	a	bc	b	b	a	e	a	b	c	b	b	ab	b	b	ab	b	f	a	c	cd	b	b	b	b	b	b	d	c	d
B4-4	"	ab	ad	b	d	b	b	a	a	b	b	b	a	e	a	b	c	b	b	a	b	b	b	b	f	a	c	d	b	b	b	b	c	b	d	c	d
M1-1	Gippsland	c	e	a	c	b	b	a	b	b	bc	b	b	d	c	-	c	b	a	b	b	a	b	a	d	a	a	a	b	b	b	b	c	b	b	c	b
M1-2	"	c	e	a	c	b	b	a	b	b	bc	b	b	d	c	b	c	b	a	b	b	a	b	a	d	a	a	a	b	b	b	b	c	b	b	c	b
M2-1	"	c	e	a	c	b	b	a	b	b	bc	b	b	d	c	b	c	b	a	b	b	a	b	a	d	a	a	a	b	b	b	b	c	b	b	c	b
M2-2	"	c	e	a	c	b	b	a	b	b	bc	b	b	d	c	b	c	b	a	b	b	a	b	a	d	a	a	a	b	b	b	b	c	b	b	c	b
M3-1	"	c	e	a	c	b	b	a	b	b	bc	b	b	d	c	b	c	b	a	b	b	a	b	a	d	a	a	a	b	b	b	b	c	b	b	c	b
M4-1	"	c	e	a	c	b	b	a	b	b	bc	b	b	d	c	b	c	b	a	b	b	a	b	a	d	a	ab	ab	b	b	b	b	c	b	b	c	b

Fish #	Lineage	*Acon1*	*Acon2*	*Acp*	*Acyc*	*Ada*	*Adh1*	*Ap2*	*Ck*	*Enol2*	*Est*	*Fum*	*Got1*	*Got2*	*Gp*	*Gpd*	*Gpi1*	*Gpi2*	*Gsr*	*Idh*	*Ldh*	*Mdh1*	*Mdh2*	*Mdh3*	*Mpi*	*PepA1*	*PepA2*	*PepB*	*PepD1*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk2*	*Tpi2*	*Ugpp*
M4-2	“	c	e	a	b	b	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	a	b	a	d	a	a	b	b	b	b	b	c	b	b	c	b
M5-1	“	c	e	a	b	b	b	a	b	b	bc	b	b	d	c	b	bc	b	a	b	b	a	b	a	d	a	a	b	b	b	b	b	c	b	b	c	b
M5-2	“	c	e	a	b	b	b	a	b	b	bc	b	b	d	c	b	c	b	a	b	b	a	b	a	d	a	a	b	b	b	b	b	c	b	b	bc	b
M6-1	Bass	b	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	b	a	b	b	b	b	b	c	a	b	c	b
M6-2	“	b	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	bc	c	b	b	d	b	a	b	b	b	b	b	c	a	b	c	b
M7-1	“	b	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	a	b	b	b	b	c	ab	b	d	b
M8-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	a	b	b	b	b	c	b	b	c	b
M8-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	a	b	b	b	b	c	b	b	c	b
M9-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	ab	a	b	b	b	b	b	cd	b	b	d	b
M9-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	b	b	b	b	b	d	ab	b	d	b
M10-1	Gippsland	c	e	a	b	b	b	a	b	b	bc	b	b	d	c	b	bc	b	a	b	b	a	b	a	d	a	a	b	b	b	b	b	c	b	b	c	b
M10-2	“	c	e	a	b	b	b	a	b	b	bc	b	b	d	c	b	c	b	a	b	b	a	b	a	d	a	a	b	b	b	b	b	c	b	b	c	b
M11-1	Bass	bc	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	a	b	b	b	b	c	ab	b	c	b
M11-2	“	bc	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	a	b	b	b	b	c	ab	b	ac	b
M12-1	“	b	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	b	b	b	b	b	c	a	b	ac	b
M12-2	“	b	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	b	b	b	b	b	c	a	b	a	b
M13-1	“	bc	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	ab	b	b	b	b	c	a	b	ac	b
M13-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	ab	ab	b	b	b	b	c	a	b	c	b
M14-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	b	b	b	b	b	c	a	b	c	b
M14-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	b	b	b	b	b	c	a	b	c	b
M15-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	ab	b	b	b	b	c	a	b	c	b
M15-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	a	b	b	b	b	c	a	b	c	b
M16-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	b	b	b	b	b	ac	a	b	c	b
M16-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	b	b	b	b	b	c	a	b	c	b
M17-1	“	c	-	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	b	b	b	b	b	c	a	b	c	b
M17-2	“	c	-	-	c	c	b	a	-	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	b	b	b	-	b	c	a	b	-	b
M18-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	b	b	b	b	b	c	a	b	c	b

Fish #	Lineage	*Acon1*	*Acon2*	*Acp*	*Acyc*	*Ada*	*Adh1*	*Ap2*	*Ck*	*Enol2*	*Est*	*Fum*	*Got1*	*Got2*	*Gp*	*Gpd*	*Gpi1*	*Gpi2*	*Gsr*	*Idh*	*Ldh*	*Mdh1*	*Mdh2*	*Mdh3*	*Mpi*	*PepA1*	*PepA2*	*PepB*	*PepD1*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk2*	*Tpi2*	*Ugpp*
M18-2	“	c	e	-	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	b	b	b	b	c	a	b	c	b
M19-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	ab	c	b	b	e	a	a	a	b	b	b	b	c	ab	b	c	b
M19-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	e	a	a	a	b	b	b	b	c	ab	b	c	b
M20-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ac	e	a	a	a	b	b	b	b	c	a	b	c	b
M20-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	e	a	a	a	b	b	b	b	c	a	b	c	b
M21-1	SWV	c	f	a	c	c	b	b	b	b	b	b	b	b	c	b	c	b	a	b	b	a	b	a	d	a	a	a	b	b	b	b	c	b	b	c	a
M21-2	“	c	f	a	c	c	b	b	b	b	b	b	b	b	c	b	c	b	a	b	b	a	b	a	d	a	a	a	b	b	b	b	c	b	b	c	a
M21-3	“	c	f	a	c	c	b	b	b	b	b	b	b	b	c	b	c	b	a	b	b	a	b	a	d	a	a	a	b	b	b	b	c	b	b	c	a
M22-1	“	c	f	a	c	c	b	b	b	b	b	b	b	b	c	b	c	b	a	b	b	ac	b	a	d	a	a	a	b	b	b	b	c	b	b	c	a
M22-2	“	c	f	a	c	c	b	b	b	b	b	b	b	b	c	b	c	b	a	b	b	a	b	a	d	a	a	a	b	b	b	b	c	b	b	c	a
M23-1	“	c	e	a	c	c	b	b	b	b	ab	b	b	d	c	b	c	a	a	b	b	a	b	ab	d	a	a	a	b	b	b	b	c	b	b	c	a
M23-2	“	c	e	a	c	c	b	b	b	b	ab	b	b	d	c	b	c	a	a	b	b	a	b	ab	d	a	a	a	b	b	b	b	c	b	b	c	a
M23-3	“	c	-	a	c	c	b	b	b	b	b	b	b	d	c	b	c	a	a	b	b	a	b	ab	d	a	a	a	b	b	b	b	c	b	b	c	a
M24-1	SESA	c	e	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M24-2	“	c	e	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M24-3	“	c	e	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M24-4	“	c	e	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M25-1	“	c	-	c	b	b	c	c	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	bc	c	a
M25-2	“	c	-	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	bc	b	b	-	b	c	b	bc	c	a
M26-1	“	c	e	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	bc	a	a	b	b	b	c	b	c	b	b	c	a
M26-2	“	c	e	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M27-1	“	c	-	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	-	b	c	b	b	c	a
M28-1	“	cd	e	c	b	b	c	a	b	b	d	b	b	df	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M28-2	“	cd	e	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	-	c	a
M29-1	“	cd	e	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	ab	c	a
M29-2	“	cd	e	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	bc	b	b	c	b	c	b	ab	c	a
M30-1	“	-	e	c	ab	b	c	a	b	b	b	b	b	d	c	-	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	ab	c	a

Fish #	Lineage	*Acon1*	*Acon2*	*Acp*	*Acyc*	*Ada*	*Adh1*	*Ap2*	*Ck*	*Enol2*	*Est*	*Fum*	*Got1*	*Got2*	*Gp*	*Gpd*	*Gpi1*	*Gpi2*	*Gsr*	*Idh*	*Ldh*	*Mdh1*	*Mdh2*	*Mdh3*	*Mpi*	*PepA1*	*PepA2*	*PepB*	*PepD1*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk2*	*Tpi2*	*Ugpp*
M31-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	ab	b	b	b	b	c	b	b	c	b
M31-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	ab	bc	ab	b	b	c	b	b	c	b
M32-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	a	b	b	ab	b	c	b	b	c	b
M32-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	a	b	b	b	b	c	b	b	c	b
M33-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	d	d	b	a	b	b	b	b	b	c	b	b	c	b
M34-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	b	b	b	b	b	c	a	b	c	b
M35-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	a	b	b	b	b	c	ab	b	c	bc
M36-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	a	bc	b	b	b	c	b	b	c	c
M36-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	a	bc	b	b	b	c	b	b	ac	c
M37-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	b	a	b	b	b	b	b	c	b	b	c	b
M37-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	a	b	b	b	b	c	ab	b	c	b
M38-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	a	b	b	b	b	c	ab	b	c	b
M38-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	ab	a	ab	b	b	b	b	c	b	b	c	b
M39-1	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	a	b	b	b	b	c	b	b	c	b
M39-2	“	c	e	a	c	c	b	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	ab	d	a	a	a	b	b	b	b	c	b	b	c	b
M40-1	MDB	c	-	c	b	b	c	c	b	b	b	a	b	d	bc	b	c	b	a	b	b	c	b	b	a	a	a	b	b	b	c	b	c	b	b	c	a
M40-2	“	c	-	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	a	a	a	b	b	b	c	b	c	b	b	c	a
M41-1	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	a	a	a	b	b	ab	c	b	c	b	b	c	a
M41-2	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	a	a	a	b	b	b	c	b	c	b	b	c	a
M42-1	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	a	a	a	b	b	ab	c	b	c	b	b	c	a
M42-2	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	a	a	a	b	b	ab	c	b	c	b	b	c	a
M43-1	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	a	a	a	b	b	b	c	b	c	b	b	c	a
M43-2	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	a	a	a	b	b	b	c	b	c	b	b	c	a
M44-1	“	c	c	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	b	b	b	c	b	c	b	b	c	a
M44-2	“	c	c	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	b	b	b	c	b	c	b	b	c	a
M45-1	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	a	b	a	b	b	c	b	b	d	a	a	b	b	b	c	b	c	b	b	c	a
M45-2	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	a	b	a	b	b	c	b	b	d	a	a	b	b	b	c	b	c	b	b	c	a

Fish #	Lineage	*Acon1*	*Acon2*	*Acp*	*Acyc*	*Ada*	*Adh1*	*Ap2*	*Ck*	*Enol2*	*Est*	*Fum*	*Got1*	*Got2*	*Gp*	*Gpd*	*Gpi1*	*Gpi2*	*Gsr*	*Idh*	*Ldh*	*Mdh1*	*Mdh2*	*Mdh3*	*Mpi*	*PepA1*	*PepA2*	*PepB*	*PepD1*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk2*	*Tpi2*	*Ugpp*
M46-1	“	c	fg	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	a	a	a	b	b	b	c	b	c	b	b	c	a
M47-1	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	ad	a	a	b	b	b	c	b	c	b	b	c	a
M48-1	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	cd	b	a	b	b	c	b	b	d	a	a	b	b	b	c	b	c	b	b	c	a
M48-2	“	c	f	c	b	b	c	c	b	b	b	a	b	ad	c	b	cd	b	a	b	b	c	b	b	d	a	a	b	b	b	c	ab	c	b	b	c	a
M48-3	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	b	b	ab	c	b	c	b	b	c	a
M49-1	“	c	f	c	b	b	c	c	b	a	b	a	b	cd	c	b	c	b	a	b	b	c	b	b	ad	a	a	b	b	ab	c	b	c	b	b	c	a
M49-2	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	d	b	a	b	b	c	b	b	d	a	a	b	b	b	c	b	c	b	b	c	a
M50-1	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	bc	b	a	b	b	c	b	b	ad	a	a	b	b	b	c	ab	c	b	b	c	a
M50-2	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	b	b	a	b	b	c	b	bd	ad	a	a	b	b	b	c	ab	c	b	b	c	a
M51-1	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	bc	b	a	b	b	c	b	b	ad	a	a	b	b	b	c	b	c	b	b	c	a
M51-2	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	bc	b	a	b	b	c	b	b	d	a	a	b	b	b	c	b	c	b	b	c	a
M52-1	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	d	a	a	bc	b	b	c	b	c	b	b	c	a
M52-2	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	c	b	a	b	b	c	b	b	a	a	a	c	b	b	c	b	c	b	b	c	a
M53-1	SESA	c	f	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M53-2	“	c	f	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M54-1	“	c	f	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M55-1	“	c	e	c	bc	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	-	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M56-1	“	c	f	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M56-2	“	c	f	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M56-3	“	c	-	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M56-4	“	c	-	c	b	b	c	a	b	b	b	b	b	d	c	b	c	b	a	b	b	c	b	b	c	a	a	b	b	b	c	b	c	b	b	c	a
M57-1	MDB	c	f	c	b	a	c	c	b	b	b	a	b	d	c	b	ac	b	a	b	b	c	b	b	ad	a	a	b	ab	ab	c	b	c	b	b	c	ab
M57-2	“	c	f	c	b	b	-	-	-	-	b	a	b	d	-	-	-	b	a	-	b	-	b	b	ad	a	a	b	b	-	c	b	c	b	b	c	-
M57-3	“	c	f	c	b	ab	-	-	-	-	b	a	b	d	-	-	-	b	a	-	b	c	b	b	ad	a	a	b	b	-	c	b	c	b	b	c	-
M57-4	“	c	f	c	b	ab	-	-	-	-	b	a	b	d	-	-	-	b	a	-	b	-	b	b	ad	a	a	b	ab	-	c	b	c	b	b	c	-
M58-1	“	c	f	c	b	b	c	c	b	b	b	a	b	d	c	b	a	b	a	b	b	c	b	bd	a	a	a	b	b	b	c	b	c	b	b	c	a
M58-2	“	c	f	c	b	b	-	-	-	-	b	a	b	d	-	-	-	b	a	-	b	c	b	b	a	a	a	b	b	-	c	b	c	b	b	c	-

Fish #	Lineage	*Acon1*	*Acon2*	*Acp*	*Acyc*	*Ada*	*Adh1*	*Ap2*	*Ck*	*Enol2*	*Est*	*Fum*	*Got1*	*Got2*	*Gp*	*Gpd*	*Gpi1*	*Gpi2*	*Gsr*	*Idh*	*Ldh*	*Mdh1*	*Mdh2*	*Mdh3*	*Mpi*	*PepA1*	*PepA2*	*PepB*	*PepD1*	*Pgam*	*6Pgd*	*Pgk*	*Pgm1*	*Pgm2*	*Pk2*	*Tpi2*	*Ugpp*
M58-3	“	c	f	c	b	b	-	-	-	-	b	a	b	d	-	-	-	b	a	-	b	c	b	b	a	a	a	b	b	-	c	b	c	b	b	c	-
M58-4	“	c	f	c	b	b	-	-	-	-	b	a	b	d	-	-	-	b	a	-	b	c	b	b	a	a	a	b	b	-	c	b	c	b	b	c	-
M59-1	“	c	-	c	b	b	c	c	b	b	b	a	b	d	c	b	a	b	a	b	b	c	b	b	ad	a	a	b	b	b	c	b	c	b	b	c	a
M59-2	“	c	-	c	b	b	c	c	b	b	b	a	b	d	c	b	a	b	a	b	b	c	b	b	ad	a	a	b	b	b	c	b	c	b	b	c	a
M60-1	“	c	f	c	b	b	c	a	b	b	b	a	b	d	c	b	a	b	b	b	b	c	b	b	a	a	a	b	b	b	c	a	c	b	b	c	a
M61-1	“	c	f	c	b	b	c	a	b	b	b	a	b	d	c	b	a	b	b	b	b	c	b	b	a	a	a	b	b	b	c	ab	c	b	b	c	a
M61-2	“	c	f	c	b	b	c	a	b	b	b	a	b	d	c	b	a	b	ab	b	b	c	b	b	a	a	a	b	b	b	c	ab	c	b	b	c	a
M61-3	“	c	f	c	b	b	c	a	b	b	b	a	b	d	c	b	a	b	ab	b	b	c	b	b	a	a	a	b	b	b	c	ab	c	b	b	c	a
M61-4	“	c	f	c	b	b	c	a	b	b	b	a	b	d	c	b	a	b	ab	b	b	c	b	b	a	a	a	b	b	b	c	b	c	b	b	c	a

APPENDIX 6. COMPARATIVE DATA

Summary of predicted and realised genetic divergence and variation within study groups, with a focus on the Lower Murray region. (*) limited analysis only, ([#]) based on distribution gap between populations, (-) data not relevant to species distribution, ([A]) some historic admixture.

	Nannoperca australis	***Nannoperca obscura***	***Gadopsis bispinosus***	***Gadopsis marmoratus***	***Mogurnda adspersa***	***Philypnodon grandiceps***	***Philypnodon macrostomus***	***Retropinna semoni***
Hypothesised genetic structure	Strong	Medium-strong	Strong	Medium-strong	Medium	Low-medium	Medium	Low
Realised allozyme divergence	Mostly strong	Medium-strong	Strong	Mix of strong & low	Medium	Mix of strong & low	Mix of strong & low	Mix of strong & medium
Major genetic groups	2	1	1	2	1*	2 to ≥ 4	2-4	5
Lineages	4	4	2	5	1*	11	8	≥ 11
Lower Murray v. Millicent Coast	Lineages Nei D = 0.11	Lineages Nei D = 0.03	-	Lineages Nei D = 0.09	-	Similar sites Nei D = 0.01	Lineages/taxa[#] Nei D = 0.16	Lineages[A] Nei D = 0.21
Heterozygosity (H_O)	High	Low	High	Medium	Low-medium	High	Low-high	High
MDB	0.041 ± 0.020	0.008 ± 0.005	0.037 ± 0.015	0.023 ± 0.009	0.023 ± 0.014	0.063 ± 0.021	0.017 ± 0.012	0.080 ± 0.026
Lower Murray	0.048 ± 0.019	0.008 ± 0.005	-	0.024 ± 0.017	0.041 ± 0.018	0.061 ± 0.020	0.014 ± 0.010	0.107 ± 0.030

APPENDIX 7. OTHER PUBLICATIONS SUBMITTED DURING CANDIDATURE

Hammer, M. P., and Walker, K. F. (2004). A catalogue of South Australian freshwater fishes including new records, range extensions and translocations. *Transactions of the Royal Society of South Australia* **128**, 85-97.

Hammer, M. (2006). Range extensions for four estuarine gobies (Pisces: Gobiidae) in southern Australia: historically overlooked native taxa or recent arrivals? *Transactions of the Royal Society of South Australia* **130**, 187-196.

Transactions of the Royal Society of S. Aust. (2004), **128**(2), 85-97.

A CATALOGUE OF SOUTH AUSTRALIAN FRESHWATER FISHES, INCLUDING NEW RECORDS, RANGE EXTENSIONS AND TRANSLOCATIONS

by M. P. HAMMER*† & K. F. WALKER*

Summary

HAMMER, M. P. & WALKER, K. F. (2004) A catalogue of South Australian freshwater fishes, including new records, range extensions and translocations. *Trans. R. Soc. S. Aust.* **128**(2), 85-97, 30 November, 2004.

Published data, recent surveys and studies of museum specimens are combined to provide a list of 84 fishes for South Australia in five drainage divisions. The list includes 58 native species (44 restricted to freshwater) and 26 alien species. Seven endemics are recognised, namely *Chlamydogobius eremius* (Zeitz), *Chlamydogobius gloveri* Larson, *Craterocephalus dalhousiensis* Ivanstoff & Glover, *Craterocephalus eyresii* (Steindachner), *Craterocephalus gloveri* Crowley & Ivanstoff, *Mogurnda thermophila* Allen & Jenkins and *Neosilurus gloveri* Allen & Feinberg. New records are reported for *Craterocephalus stercusmuscarum ?stercus-muscarum* (Günther), *Galaxias truttaceus* Valenciennes and *Neochanna cleaveri* (Scott), and a terapontid of uncertain status also is noted. Range extensions are reported for *Nannoperca obscura* (Klunzinger), *Nannoperca australis* Günther and an undescribed species of *Hypseleotris*, and the presence of *Galaxias olidus* Günther and *Galaxias brevipinnis* Günther in particular regions is confirmed. Possible extirpations are reported for *Ambassis agassizii* Steindachner, *Gadopsis marmoratus* Richardson, *Galaxias rostratus* Klunzinger, *Maccullochella macquariensis* (Cuvier), *Macquaria australasica* Cuvier, *Mogurnda adspersa* (Castelnau), *Neochanna cleaveri* and *Prototroctes maraena* Günther. There is need for further evaluations of fish distributions, better systematic frameworks, clarifications of conservation status, reviews of the introduction and impacts of alien species and development of protective measures for fish species and communities and their ecosystems.

KEY WORDS: Freshwater fishes, conservation, management, taxonomy

Introduction

Despite a generally dry landscape, South Australia harbours a diverse array of aquatic habitats including artesian mound springs, swamps, lakes, episodic streams and the River Murray and associated wetlands. These habitats, and the effects of biogeographical isolation (e.g. Unmack 2001), sustain a corresponding diversity of freshwater biota. The term "fresh water" here includes inland saline waters (≥3000 mg L^{-1}), as these are common in the state (e.g. Williams 1967; EPA 1998; Hammer 2002a).

Freshwater fishes in South Australia display a variety of physical forms and life histories. The dwarf galaxias *Galaxiella pusilla* is remarkable for its ability to survive dry periods in seasonal swamps, where it takes refuge in swamp-crayfish burrows (*Geocharax*: Beck 1985). Large species like the Murray-Darling golden perch *Macquaria ambigua ambigua* may cover long distances (for example, a tagged fish is known to have travelled 2300 km along the Murray and Darling rivers: Reynolds 1983), whereas small species like the southern pygmy perch *Nannoperca australis* are much less vagile (Hammer[1]). Other species need to move between fresh water and marine habitats, although even diadromous species like the galaxiids *Galaxias maculatus* and *G. brevipinnis* may occur in 'landlocked' populations (Pierce *et al.* 1985; Hammer 2002a; SKM 2002). In addition, there are euryhaline species like the small-mouthed hardyhead *Atherinosoma microstoma*, found in fresh or salt water (Molsher *et al.* 1994; Hammer 2002a).

This catalogue lists 84 species in the freshwater fish fauna of South Australia. It updates earlier work (Waite 1923; Scott *et al.* 1974; Sim 2000), corrects and amends records of species and their distributions, and is designed to assist in research and planning for management and conservation.

Methods

Drainage divisions

Five of the 13 principal drainage divisions in Australia (AWRC 1976) occur wholly or partly in South Australia, and provide a biogeographic framework (Fig. 1):

- South East Coast (SEC), including the Millicent Coast and Glenelg River (part) river basins,
- Murray Darling (MD), part of the Lower Murray River Basin,
- South Australian Gulf (SAG), the only division contained wholly within the state (the shared

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[1] HAMMER, M. (2001) Molecular systematics and conservation biology of the southern pygmy perch Nannoperca australis (Günther, 1861) (Teleostei: Percichthyidae) in south-eastern Australia. Unpub. BSc(Hons) Thesis, Department of Environmental Biology, The University of Adelaide.

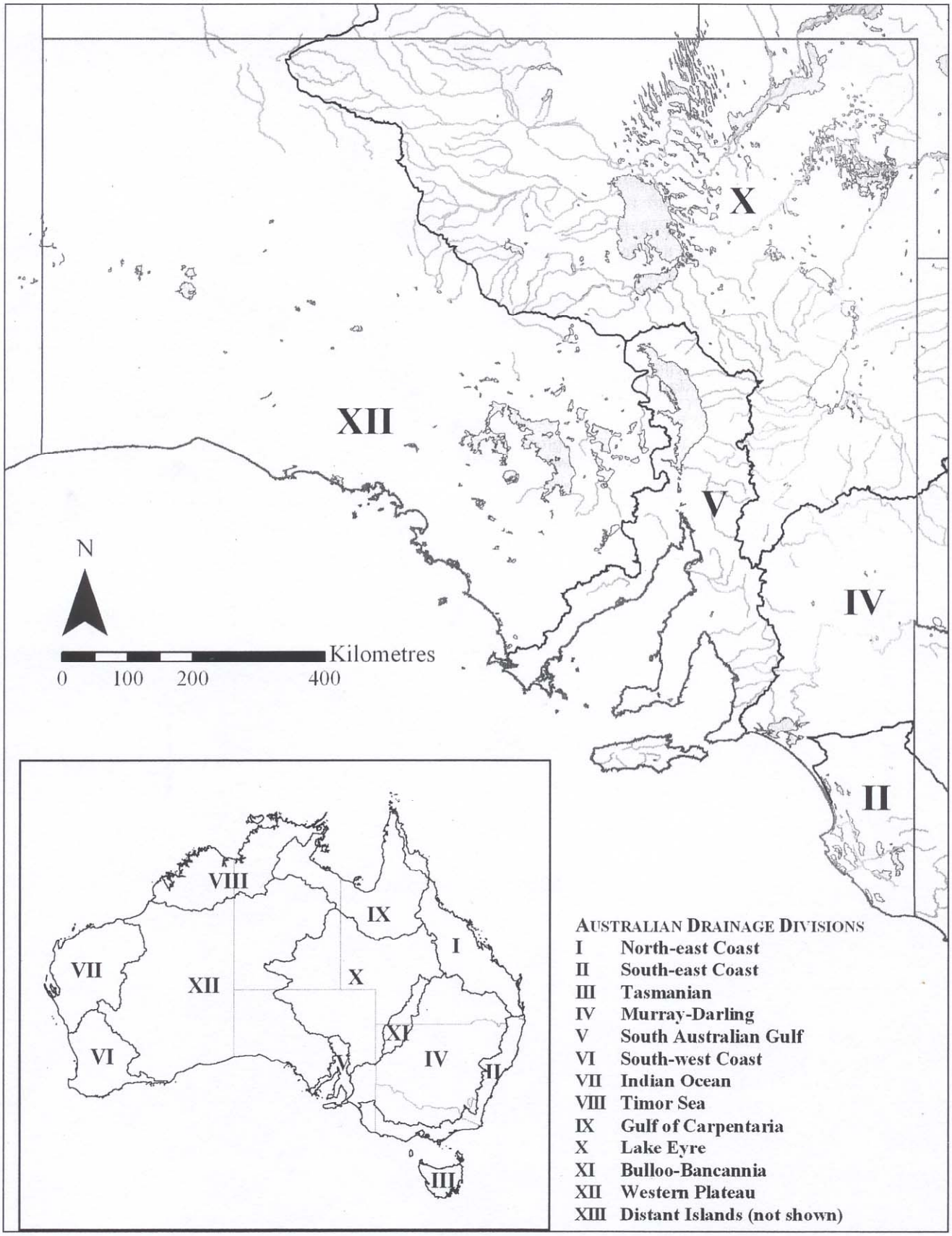

Fig. 1. Drainage divisions in Australia and South Australia (AWRC 1976).

boundary with MD is west of the Murray Mouth, but SAG includes the coastal streams of Fleurieu Peninsula),
- Lake Eyre (LE), draining toward lakes Eyre and Frome, and
- Western Plateau (WP), containing sparse coastal lakes and some ephemeral waters.

Records of species were obtained from the literature and examination of specimens at the South Australian Museum, Adelaide (SAMA), including material from recent collections by the senior author. Information on rare or doubtful species was scrutinised with special care.

Nomenclature

The systematic framework and nomenclature employed here follow Eschmeyer (1998) and subsequent updates (see Californian Academy of Sciences on-line "Catalogue of Fishes", March 2003[2]), except that the lamprey families Geotriidae and Mordaciidae replace Petromyzontidae (Strahan 1980), subspecific status is recognised for *M. a. ambigua* (after Musyl & Keenan 1992) and six informal taxa and a species complex are recognised. The informal taxa include dwarf flathead gudgeon *Philypnodon* sp. (Larson & Hoese 1996), Lake Eyre golden perch *Macquaria* sp. (Musyl & Keenan 1992) and western chanda perch, an undescribed species referred to in earlier literature as "*Ambassis muelleri* Klunzinger" (syn. *A. agassizii*), but lacking a formal name since "*A. muelleri*" was invalidated by Allen *et al.* (2002). The carp gudgeon genus *Hypseleotris* awaits a formal review but, following Allen *et al.* (2002), this catalogue recognizes Midgley's carp gudgeon *H.* "sp. 1" *sensu* Hoese *et al.* (1980) and Murray-Darling carp gudgeon *H.* "sp. 3" *sensu* Unmack (2000). In addition, a species complex of hybrids and possible semi-clonal hybridogenic forms are recognised (Bertozzi *et al.* 2000), including Lake's carp gudgeon *H.* "sp. 2" *sensu* Hoese *et al.* (1980). Following Allen and Jenkins (1999), prior records of northern purple-spotted gudgeon *Mogurnda mogurnda* (Richardson) in South Australia should be referred to Dalhousie purple-spotted gudgeon *M. thermophila* or Flinders Ranges purple-spotted gudgeon *M. clivicola* (these were described from within the range of *M. mogurnda*).

Criteria for inclusion

A "freshwater" species here includes obligate freshwater and diadromous species and select euryhaline taxa known to complete their lifecycle in fresh water. "Alien" species include exotic species (not native to Australia) and native Australian species translocated outside their natural range. Alien fishes in natural waterways are regarded as *established* species if their populations are self-sustaining or if they are continually stocked, and as *introduced* species if records are few and isolated or confined to artificial waterbodies (and potentially could become established). The latter include interstate translocations within drainage divisions.

[2] http://www.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

Results

Native fish richness

A total of 58 native freshwater fish species in 15 families is recorded for South Australia (Table 1). All are shared with other states, except for seven endemics in isolated areas of LE. *Mogurnda clivicola* may be another endemic, as only small populations of uncertain affinity occur outside the state (Allen & Jenkins 1999; Wager & Unmack 2000).

Forty-four native species are confined to fresh water. One of these, Australian smelt *Retropinna semoni*, may occasionally occur in the Coorong (Eckert & Robinson 1990), but is not strictly diadromous. Four euryhaline taxa meet the aforementioned criteria of "freshwater" species, namely *A. microstoma*, flathead gudgeon *Philypnodon grandiceps*, western bluespot goby *Pseudogobius olorum* and lagoon goby *Tasmanogobius lasti* (e.g. Wedderburn & Hammer 2003). Thirteen of the 44 obligate freshwater species occur in more than one division, and none is common to all. Most obligate freshwater species occur in LE (24) and MD (24, plus 11 diadromous and euryhaline taxa). Diadromous and euryhaline species generally occur in more than one division. Remarkably, three diadromous species are recorded for WP, although data there are sparse (Table 1).

New records for South Australia

Fly-specked hardyhead *Craterocephalus stercusmuscarum* ?*stercusmuscarum* (Günther)

This taxon was identified in samples collected from the northern Flinders Ranges in 1994-95 (SAMA F7331, F9002, F9078). It is distinguished from the Lake Eyre hardyhead *Craterocephalus eyresii* (Steindachner), which occurs in the same region but not the same habitats, by fewer transverse scale rows (7-8 cf. 11-14 in *C. eyresii*) and dark lateral banding (Ivanstoff *et al.* 1987; Crowley & Ivanstoff 1990a). Subspecific identification is tentative owing to taxonomic problems and the isolated nature of the population (the nearest known conspecifics are from Aramac Springs in the remote

TABLE 1. *Native freshwater fishes in drainage divisions of South Australia.* [X = recorded; E = presumed extinct; ? = uncertain status], #Endemic to South Australia, †See text.

Family	Taxon	Common name	Division				
			SEC	MD	SAG	LE	WP
Geotriidae	*Geotria australis* Grey, 1851	Pouched lamprey	X	X	X		
Mordaciidae	*Mordacia mordax* (Richardson, 1846)	Shortheaded lamprey	X	X	X		
Anguillidae	*Anguilla australis* Richardson, 1841	Shortfinned eel	X	X	X		
Plotosidae	*Neosiluroides cooperensis* Allen & Feinberg, 1998	Cooper catfish				X	
	Neosilurus gloveri Allen & Feinberg, 1998#	Dalhousie catfish				X	
	Neosilurus hyrtlii Steindachner, 1867	Hyrtl's catfish				X	
	Porochilus argenteus (Zietz, 1896)	Silver tandan				X	
	Tandanus tandanus Mitchell, 1838	Freshwater catfish		X			
Clupeidae	*Nematalosa erebi* (Günther, 1868)	Bony herring		X	?	X	
Retropinnidae	*Prototroctes maraena* Günther 1864	Australian grayling	E				
	Retropinna semoni (Weber, 1895)	Australian smelt		X		X	
Galaxiidae	*Galaxias brevipinnis* Günther, 1866	Climbing galaxias		X	X		
	Galaxias maculatus (Jenyns, 1842)	Common galaxias	X	X	X		
	Galaxias olidus Günther, 1866	Mountain galaxias	X	X	X		
	Galaxias rostratus Klunzinger, 1872	Murray galaxias		E			
	Galaxias truttaceus Valenciennes, 1846	Spotted galaxias	X				
	Galaxiella pusilla (Mack, 1936)	Dwarf galaxias	X				
	Neochanna cleaveri (Scott, 1934)	Tasmanian mudfish	X				
Melanotaeniidae	*Melanotaenia fluviatilis* (Castelnau, 1878)	Murray rainbowfish	?	X			
	Melanotaenia splendida tatei (Zietz, 1896)	Desert rainbowfish				X	
Atherinidae	*Atherinosoma microstoma* (Günther, 1861)	Small-mouthed hardyhead	X	X	X		X
	Craterocephalus dalhousiensis Ivanstoff & Glover, 1974#	Dalhousie hardyhead				X	
	Craterocephalus eyresii (Steindachner, 1883)#	Lake Eyre hardyhead			X	X	?
	Craterocephalus fluviatilis McCulloch, 1912	Murray hardyhead		X			
	Craterocephalus gloveri Crowley & Ivanstoff, 1990#	Glover's hardyhead				X	
	Craterocephalus stercusmuscarum fulvus Ivanstoff, Crowley & Allen, 1987	Unspecked hardyhead		X			
	Craterocephalus stercusmuscarum ?stercusmuscarum (Günther, 1867)	Fly-specked hardyhead				X	
Ambassidae	*Ambassis agassizii* Steindachner, 1867	Chanda perch		E			
	Ambassis sp. (undescribed)†	Western chanda perch				X	
Percichthyidae	*Gadopsis marmoratus* Richardson, 1848	River blackfish	X	X	E		
	Maccullochella macquariensis (Cuvier, 1829)	Trout cod		E			
	Maccullochella peelii peelii (Mitchell, 1838)	Murray cod		X			
	Macquaria ambigua ambigua (Richardson, 1845)	Murray-Darling golden perch		X			
	Macquaria australasica Cuvier, 1830	Macquarie perch		E			
	Macquaria colonorum (Günther, 1863)	Estuary perch	X	X			
	Macquaria sp. (undescribed)†	Lake Eyre golden perch				X	
	Nannoperca australis Günther, 1861	Southern pygmy perch	X	X	X		
	Nannoperca obscura (Klunzinger, 1872)	Yarra pygmy perch	X	X			

Family	Taxon	Common name	Division				
			SEC	MD	SAG	LE	WP
	Nannoperca variegata Kuiter & Allen, 1986	Variegated pygmy perch	X				
Terapontidae	*Amniataba percoides* (Günther, 1864)	Banded grunter				X	
	Bidyanus bidyanus (Mitchell, 1838)	Silver perch		X			
	Bidyanus welchi (McCulloch & Waite, 1917)	Welch's grunter				X	
	Leiopotherapon unicolor (Günther, 1859)	Spangled grunter		X	?	X	
	Scortum barcoo (McCulloch & Waite, 1917)	Barcoo grunter				X	
Pseudaphritidae	*Pseudaphritis urvillii* (Valenciennes, 1832)	Congolli	X	X	X		X
Eleotridae	*Hypseleotris klunzingeri* (Ogilby, 1898)	Western carp gudgeon		X		X	
	Hypseleotris sp. 1 (undescribed)†	Midgley's carp gudgeon		X		X	
	Hypseleotris sp. 3 (undescribed)†	Murray Darling carp gudgeon		X	X		
	Hypseleotris spp. (species complex)†	Hybrid forms (e.g. Lake's carp gudgeon)		X		X	
	Mogurnda adspersa (Castelnau, 1878)	Southern purple-spotted gudgeon		E	E		
	Mogurnda clivicola Allen & Jenkins, 1999	Flinders Ranges purple-spotted gudgeon				X	
	Mogurnda thermophila Allen & Jenkins, 1999#	Dalhousie purple-spotted gudgeon				X	
	Philypnodon grandiceps (Krefft, 1864)	Flathead gudgeon	X	X	X		
	Philypnodon sp. (undescribed)†	Dwarf flathead gudgeon		X	?		
Gobiidae	*Chlamydogobius eremius* (Zeitz, 1896)#	Desert goby				X	
	Chlamydogobius gloveri Larson 1995#	Dalhousie goby				X	
	Pseudogobius olorum (Sauvage, 1880)	Western bluespot goby	X	X	X		X
	Tasmanogobius lasti Hoese, 1991	Lagoon goby	X	X	X		
Totals (Grand Total 58)			**19**	**35**	**16**	**24**	**3**

upper reaches of Cooper Creek, Queensland). A molecular revision of *Craterocephalus* in progress indicates that sub-species within the *C. stercusmuscarum* species complex remain confused (P. Unmack, Arizona State University, pers. comm.) and further morphological and molecular analyses are required.

Spotted galaxias
Galaxias truttaceus Valenciennes

This species was first reported in 1999 from karstic springs in coastal south-eastern South Australia (e.g. Ewens Ponds: Hammer *et al.* 2000; SAMA F9217, F10111) representing a minor westward range extension into South Australia. Another single specimen from the same area occurred among specimens of *G. maculatus* collected in 1979 (SAMA F10109). Note that a prior report of *G. truttaceus* from SAG (Scott *et al.* 1974) was based on misidentified specimens (SAMA F3094, F3188).

Tasmanian mudfish
Neochanna cleaveri (Scott)

This species is known in South Australia only from a single specimen collected from Bool Lagoon in 1974, and previously registered as *G. maculatus* (SAMA F4919). Recent surveys have failed to locate others (Hammer 2002a). The new record is noteworthy as the species is cryptic, with an ability to survive extended dry periods by burrowing into mud or hiding under rocks and wood, and otherwise is native to Tasmania and Victoria (Fulton 1986; Koehn & Raadik 1991).

A possible new terapontid

A form of grunter (Terapontidae) resembling a deep-bodied Welch's grunter *Bidyanus welchi* or a hybrid *B. welchi* x Barcoo grunter *Scortum barcoo* is known from Coongie Lakes (J. Puckridge, University of Adelaide, pers. comm. 2001). This form is listed as the 'Cooper grunter' by Sim (2000). It was also reported near Goyder Lagoon on the lower Warburton River in 2002 (Costelloe *et al.* 2003).

Range extensions

Surveys in the Mount Lofty Ranges (Hammer[1]) have provided three new drainage division records, namely a genetically distinct sub-population of *Nannoperca australis* from the Inman River Catchment (SAG), *Hypseleotris* sp. 3 from the same location, and Yarra pygmy perch *Nannoperca obscura* from Lake Alexandrina (MD). The review uncovered other, previously misidentified specimens of *N. obscura* in the museum collection dating from 1915 (SAMA F572), suggesting the species is native.

The presence of mountain galaxias *Galaxias olidus* (a species complex presently under systematic review: Raadik 2001) recently was confirmed from the South Australian section of SEC (Mosquito Creek: Hammer 2002a). Despite its inclusion in a south east regional list by Glover (1983), no specimens of the species were previously known. In addition, Glover mistakenly referred to the Mosquito Creek population as *G. maculatus*. The presence of *G. brevipinnis* in MD is also confirmed (SAMA F153: Angas River, 1914; previously registered as *G. maculatus*), a record predating the Snowy Mountains Hydroelectric Scheme which appears to be the source of *G. brevipinnis* in the upper Murray catchment (Waters *et al.* 2002).

A report of *R. semoni* from SAG (SKM 2002) is suspect because voucher specimens are not available and no other records exist for the division (e.g. McDowall 1979; Unmack 2001). Other SAG reports of bony herring *Nematalosa erebi* and spangled grunter *Leiopotherapon unicolor* in the Lake Torrens catchment, and western carp gudgeon *Hypseleotris klunzingeri* as native to the Broughton River (Pierce *et al.* 2001) are also discounted in the absence of voucher specimens or other data. There is an uncertain report of fish resembling *C. eyresii* in the remote, isolated Durkin Swamp (WP), following exceptional rainfall (Ehmann & Tynan 1997).

Finke goby *Chlamydogobius japalpa* Larson, Finke hardyhead *Craterocephalus centralis* Crowley & Ivanstoff and Finke purple-spotted gudgeon *Mogurnda larapintae* (Zeitz) potentially could colonise the ephemeral, lower reaches of the Finke River in South Australia, following floods from the headwaters in the Northern Territory, but they have not been formally recorded.

Alien species

There are records of 26 alien species in South Australia (Tables 2-3), although two may prove to be natives (*Philypnodon* sp. from the Onkaparinga River (SAG) (SAMA F10087, April 2002), and Murray rainbowfish *Melanotaenia fluviatilis* from SEC (SAMA F2409, dated 1903)). Most alien species records are for SAG (20 species, including 13 established alien species). There are high numbers also for MD and SEC, but few in the remote LE and WP (Table 2).

Fourteen alien species are established in South Australia. These include seven exotic taxa and seven translocated native taxa. Another 12 alien species have been introduced, but are not established or present only in artificial waterways (Tables 2-3). These include barramundi *Lates calcarifer* in the River Torrens and Australian bass *Macquaria*

TABLE 2. *Alien fishes in fresh water environments in drainage divisions of South Australia.* [X = continually introduced and/or established; I = introduced, few records; A = introduced to artificial habitats (e.g. farm dams, sewage treatment ponds); ? = uncertain status], †See text.

Family	Taxon	Common name	Division				
			SEC	MD	SAG	LE	WP
EXOTIC SPECIES							
Cyprinidae	*Carassius auratus* (Linnaeus, 1758)	Goldfish	X	X	X	X	A
	Cyprinus carpio Linnaeus, 1758	Common carp	I	X	X	A	
	Tinca tinca (Linnaeus, 1758)	Tench	X	X	X		
Cobitidae	*Misgurnus anguillicaudatus* (Cantor, 1842)	Oriental weatherloach		I			
Salmonidae	*Oncorhynchus mykiss* (Walbaum, 1792)	Rainbow trout	I	X	X		
	Salmo salar Linnaeus, 1758	Atlantic salmon		I			
	Salmo trutta Linnaeus, 1758	Brown trout	I	X	X		
	Salvelinus fontinalis (Mitchell, 1814)	Brook trout			I		
Poeciliidae	*Gambusia holbrooki* Girard, 1859	Gambusia	X	X	X	X	I
Percidae	*Perca fluviatilis* Linnaeus, 1758	European perch	X	X	X	I	
TRANSLOCATED AUSTRALIAN NATIVE SPECIES							
Plotosidae	*Tandanus tandanus* (Mitchell, 1838)	Freshwater catfish	I	A	X		
Galaxiidae	*Galaxiella pusilla* (Mack, 1936)	Dwarf galaxias			I?		
Melanotaeniidae	*Melanotaenia fluviatilis* (Castelnau, 1878)	Murray rainbowfish	I?		X		
Centropomidae	*Lates calcarifer* (Bloch, 1790)	Barramundi			I		
Ambassidae	*Ambassis agassizii* Steindachner, 1867	Chanda perch		A			
Percichthyidae	*Gadopsis marmoratus* Richardson, 1848	River blackfish			A		
	Maccullochella peelii peelii (Mitchell, 1838)	Murray cod	I	A	X	I	
	Macquaria ambigua ambigua (Richardson, 1845)	Murray-Darling golden perch	I	A	X	A	
	Macquaria novemaculeata (Steindachner, 1866)	Australian bass		I			
	Nannoperca australis Günther, 1861	Southern pygmy perch			A		
Terapontidae	*Bidyanus bidyanus* (Mitchell, 1838)	Silver perch	I	A	A	A	
Eleotridae	*Hypseleotris* sp. 1 (undescribed)†	Midgley's carp gudgeon			X		
	Hypseleotris sp. 3 (undescribed)†	Murray Darling carp gudgeon	X				
	Mogurnda adspersa (Castelnau, 1878)	Southern purple-spotted gudgeon		A	A		
	Oxyeleotris lineolata (Steindachner, 1867)	Sleepy cod		I			
	Philypnodon sp. (undescribed)†	Dwarf flathead gudgeon			X?		
Totals (Grand Total 26)			**13**	**17**	**20**	**7**	**2**
Total established (Grand Total 14)			**5**	**7**	**13**	**2**	**0**

TABLE 3. *Distributions annotations for selected freshwater fishes in South Australia (in support of Tables 1-2).* [Record types: 1 = range extension or new state record; 2 = restricted presence; 3 = established alien species; 4 = introduced; 5 = erroneous report], SA = South Australia; Vic. = Victoria; SAMA = South Australian Museum, Adelaide; AM = Australian Museum, Sydney; SMNS = Staatliches Museum für Naturkunde, Stuttgart, Germany

Species	Record Type	Details	Source
SOUTH EAST COAST DRAINAGE DIVISION			
Galaxias truttaceus	1	Lower south east SA, springs and coastal creeks.	Hammer (2002a) = SAMA F9217; F10109 (1979)
Neochanna cleaveri	1	Specimen from Bool Lagoon labelled as *Galaxias maculatus*.	SAMA F4919 (1974)
Galaxias olidus	2	Mosquito Creek. Referred to as *Galaxias maculatus* by Glover (1983).	Hammer (2002a) = SAMA F10121
Geotria australis	2	Photographed in Ewens Ponds. Collected from Glenelg R. in SA.	Kuiter (1983); SAMA F1046 (1928)
Macquaria colonorum	2	Glenelg R. in SA.	SAMA F1704 (1932); Hammer (2002a)
Mordacia mordax	2	Near Port MacDonnell. Other SAMA records from western Vic.	F10103 (1982)
Prototroctes maraena	2	Photographed in Ewens Ponds. No recent records.	Kuiter (1983); Hammer (2002a)
Tasmanogobius lasti	2	Some freshwater lakes (e.g. L. Bonney).	Hammer (2002a)
Hypseleotris sp. 3	3	No records <1980; now widespread in Millicent Coast Basin.	Hammer (2002a)
Tinca tinca	3	Naracoorte Creek, L. Cockatoo.	Hammer (2002a); SAMA F10144
Cyprinus carpio	4	One record from Bool Lagoon (1995), unconfirmed report for Valley L.	SAMA F7700 (1995); Hammer (2002a)
Oncorhynchus mykiss and *Salmo trutta*	4	Mosquito Creek, Ewens Ponds. Previously stocked and/or farm escapees. No recent reports.	Glover (1983); Hammer (2002)
Tandanus tandanus	4	Lucindale (1936). Stocked with *Bidyanus bidyanus, Maccullochella peelii peelii, Macquaria ambigua ambigua.*	SAMA F1918 (1936); Atkins *et al.* (1988); Hammer (2002a)
Melanotaenia fluviatilis	4	Freshwater L. near Kingston and Robe with *Atherinosoma microstoma* and *Gadopsis marmoratus*. Could be native.	SAMA F2409 (1903). Concurrent collections = SAMA F1901, 1368
Craterocephalus stercusmuscarum	5	Mis-identified *Atherinosoma microstoma.*	AM IB7303, 7304 cf. Glover (1983)
Hypseleotris klunzingeri	5	Probably *Hypseleotris* sp. 3. as above.	Hammer (2002) cf. Atkins *et al.* (1988)
Philypnodon sp.	5	Absent in region.	Hammer (2002a) cf. Larson & Hoese (1996)
Retropinna semoni	5	Absent between Glenelg R. and the Murray.	Hammer (2002a)
MURRAY DARLING DRAINAGE DIVISION			
Nannoperca obscura	1	L. Alexandrina (2001). SAMA specimens date from 1915.	Hammer[1] = SAMA F10008 (2001); SAMA F572 (1915)
Anguilla australis	2	Occasional R. Murray records, also streams near L. Alexandrina. At western-most range for larval migration (inc. K.I. also, see SAG).	e.g. SAMA F3712 (1972), F7798 (1996)
Galaxias brevipinnis	2	Large adult from Angas R.	SAMA F153 (1914)
Galaxias rostratus	2	Syntypes (R. Murray SA) SMNS, some redeposited with AMS. Another specimen from Murray Bridge needs verification.	SMNS 1597 (1868), ?1696 (1869); AM I19743; McDowall and Frankenberg (1981)
Leiopotherapon unicolor	2	Coorong and L. Alexandrina in 1976 after floods.	SAMA F4152, F4247
Macquaria australasica	2	Upper SA section of R. Murray (historically rare).	Zeitz (1902); SAMA F456 (1917), F497 (1918)
Macquaria colonorum	2	Lower Murray, L. Albert, Alexandrina and Coorong.	Eckert & Robinson (1990); Sim *et al.* (2000)
Maccullochella macquariensis	2	Early record from Purnong on R. Murray. Range extension from upstream populations (e.g. Mildura in 1940-50s).	SAMA 1672 (1932); Cadwallader (1977)
Tasmanogobius lasti	2	L. Albert and Alexandrina (freshwater), apparently spawns in these regions.	Sim *et al.* (2000); Wedderburn & Hammer (2003)

Species	Record Type	Details	Source
Tinca tinca	3	Once common. Occasionally recorded (e.g. Angas R.).	SAMA F10102 (1999); Sim *et al.* (2000)
Ambassis agassizii and *Mogurnda adspersa*	4	Transferred from Darling R. basin in Queensland to Murray Bridge Army Range, to be later released to R. Murray.	Pierce (1997)
Macquaria novemaculeata	4	Near Loxton, per professional fisher.	SAMA F7169 (1992); Pierce (1992)
Misgurnus anguillicaudatus	4	Unconfirmed report for R. Murray at Long Island, Murray Bridge (1980s). Now spreading downstream from Vic.	Wedderburn[4]; Koster *et al.* (2002)
Oxyeleotris lineolata	4	Two R. Murray records. Museum specimen from Kroehns Landing (near Nildotte). Another displayed at Swan Reach Hotel, September 2003, caught near Nildottie per professional fisher.	SAMA F10143 (1995)
Salmo salar	4	Three R. Murray specimens caught near Renmark by professional fishers.	SAMA F7284, F7504, F7505 (1993)
SOUTH AUSTRALIAN GULF DIVISION			
Hypseleotris sp. 3	1	Inman R. catchment. Presumed native as sympatric with *Nannoperca australis*.	Hammer[1]
Nannoperca australis	1	Inman R. catchment, a genetically distinct population of Murray lineage.	Hammer[1]
Anguilla australis	2	South coast catchments of Kangaroo Island.	SAMA F4718, F5175 (1980's)
Craterocephalus eyresii	2	L. Torrens catchment; L. Torrens when full, springs, Willochra Creek.	e.g. SAMA F3176 (1961), F9153 (1996)
Gadopsis marmoratus	2	Historically an edible fish of the Onkaparinga and Torrens rivers. Presence on Kangaroo Island (location unknown) needs verification.	Zietz (1902); SAMA F6467 (1987)
Mogurnda adspersa	2	Historic records for Torrens, Onkaparinga rivers. No reports for >50 years.	SAMA F517, F518 (pre 1917)
Tasmanogobius lasti	2	Lower reaches of Kangaroo Island streams.	Hoese (1991); Hammer pers. obs. 2003
Macquaria ambigua ambigua	3	Introductions do occur (e.g. Clarendon Weir, Broughton R.).	SKM 2002; Bochow (2003)
Maccullochella peelii peelii	3	Regularly stocked into Broughton R.	e.g. Bochow (2003)
Melanotaenia fluviatilis and *Hypseleotris* sp. 1	3	R. Torrens, common in lower reaches.	e.g. SAMA F9277 (1999), F9279 (1999); Hammer pers. obs. 2000-2003.
Philypnodon sp. nov.	3	First record from Onkaparinga R., 2002. Could be native.	SAMA F10087 (2002)
Tandanus tandanus	3	Torrens, Wakefield rivers.	SAMA F9086 (1997); Hicks & Sheldon (1998)
Tinca tinca	3	Few catchments (e.g. Onkaparinga).	SKM (2002); Hammer pers. obs. 1998
Gadopsis marmoratus and *Nannoperca australis*	4	Refuge population in dams at Warrawong Sanctuary since 1980's (trib. Onkaparinga R.).	Hammer pers. obs. 1999-2002
Galaxiella pusilla	4	Listed without detail.	Carter & Pierce[5]
Lates calcarifer	4	Netted from R. Torrens (Torrens L.), April 2002 (376 mm total length).	SAMA F00000 [registry number pending]
Mogurnda adspersa	4	Stocked into Thorndon Park Reservoir (since dried).	Anon. (1996)
Salvelinus fontinalis	4	Previously stocked into Sixth Creek, Torrens Catchment.	Scott *et al.* (1974)

[4] WEDDERBURN, S. (2000) Habitat and conservation status of small fish in the Lower River Murray, and a comparison of the western carp gudgeon (*Hypseleotris klunzingeri*) and gambusia (*Gambusia holbrooki*) as larval mosquito predators. Unpub. BSc (Hons) Thesis, Department of Environmental Biology, University of Adelaide, Adelaide.

[5] CARTER, J. & PIERCE, B. (undated) Freshwater fishes of the Mount Lofty Ranges. Department for Environment and Natural Resources, Adelaide. (unpub.). 18 p.

Species	Record Type	Details	Source
LAKE EYRE DRAINAGE DIVISION			
Craterocephalus stercusmuscarum	1	Specimens from the North Flinders Ranges previously identified as *C. eyresii*. Taxonomic status requires further investigation.	SAMA F7331, F9002, F9078 (1994/95)
?stercusmuscarum			Glover (1985); Hammer pers. obs. 2002
Ammiataba percoides	2	First record Neales R. (1984). Common there in 2002.	
Carassius auratus	3	Coongie Lakes/ Cooper Creek.	SAMA F6199 (1986); Reid & Puckridge (1990)
Cyprinus carpio	4	Leigh Creek retention dam. Poisoning attempted, but still present in 1999 (Hammer pers. obs.)	Pierce *et al.* (2001)
Maccullochella peelii peelii	4	Cooper Creek near Innamincka. Population small, may not be viable.	Pierce (1990)
Macquaria ambigua ambigua and *Bidyanus bidyanus*	4	Stocked into Clayton Bore.	Wager & Unmack (2000)
Perca fluviatilis	4	Introduced to Moro George, Flinders Ranges. Now probably absent.	Glover (1980); Pierce *et al.* 2001
WESTERN PROVINCE DRAINAGE DIVISION			
Pseudogobius olorum	2	Davenport Creek near Ceduna and Laura Bay.	SAMA F5496 (1981), F7405 (1982)
Atherinosoma microstoma	2	Several regional records (e.g. spring at L. Hamilton; L. Newland).	SAMA 2615 (1947), F4789 (1984)
Pseudaphritis urvillii	2	Streaky Bay (not strictly freshwater habitat but included), most westerly record.	SAMA F1388 (1929)
Gambusia holbrooki	3	Spring at L. Hamilton (extant?).	SAMA F10056 (1947)
Carassius auratus	4	Dams, reservoirs at Woomera (with *Gambusia holbrooki*).	Glover (1979)

novemaculeata, sleepy cod *Oxyeleotris lineolata* and Atlantic salmon *Salmo salar* in the River Murray. Gambusia *Gambusia holbrooki* and goldfish *Carassius auratus* were recorded in all drainage divisions.

Four large native MD species (silver perch *Bidyanus bidyanus*, Murray cod *Maccullochella peelii peelii*, freshwater catfish *Tandanus tandanus*, *M. a. ambigua*) are spawned in commercial hatcheries in other states and are commonly introduced to South Australia (Tables 2-3), including undocumented stockings in farm dams in MD and SAG.

Translocations in drainage divisions within South Australia are not considered in detail here, but have reportedly included transportation of *M. clivicola* in the Flinders Ranges region and fish from Cooper Creek to a retention dam at Leigh Creek (see Pierce *et al.* 2001).

Extirpations and species decline

Museum records are not necessarily a true indication of range and abundance, but indications from all sources combined are that there have been significant declines in the range of several species. Records for some species may represent occasional stray individuals on the fringe of their geographic range, but these could not be distinguished from established species due to a paucity of detailed historic surveys and/or temporal replication.

There is historical evidence (Table 3) that Murray galaxias *Galaxias rostratus*, trout cod *Maccullochella macquariensis* and Macquarie perch *Macquaria australasica* formerly occurred in MD in South Australia. *Ambassis agassizii* was last recorded from the Marne River mouth (MD) in 1983 (Lloyd & Walker 1986), and state-wide extirpation appears confirmed for the southern purple-spotted gudgeon *Mogurnda adspersa* (last record in MD 1973: SAMA F3727; no sightings in SAG for >50 years). The river blackfish *Gadopsis marmoratus* can be considered extirpated from SAG (it may persist on Kangaroo Island, but the record is dubious: Table 3) and has undergone significant range contraction in MD (Sim *et al.* 2000), exacerbated since 1997 by the loss to irrigation diversions of more than half of the spring-fed habitats in the Marne River, one of few remaining refuges (Hammer 2002b). Similarly, range contraction and on-going local extirpations have been recorded for *N. australis* (Hammer[1]). Estuary perch *Macquaria colonorum* was once more widespread in the lower Murray prior to the construction of barrages near to the Murray Mouth (Sim *et al.* 2000). For SEC, *N. cleaveri* and the Australian grayling *Prototroctes maraena* have not been reported since 1974 and 1982 respectively and

other SEC species including *G. pusilla* have likely suffered large range reductions coinciding with massive loss of wetland habitat (Hammer 2002a).

Other species are confined to small areas, including five endemic species in Dalhousie Springs (LE) (Wager & Unmack 2000), *M. clivicola* (recorded only from Balcanoona Creek in the Flinders Ranges (LE): e.g. SAMA F3042), Murray hardyhead *Craterocephalus fluviatilis* (very few sites in the lower Murray (MD): Lloyd & Walker 1986; Wedderburn & Hammer 2003); *N. obscura* (three habitat fragments in SEC and a small section of MD: Hammer 2002a; Wedderburn & Hammer 2003) and the variegated pygmy perch *Nannoperca variegata* (a 4-km^2 spring-fed area in SEC: Hammer *et al.* 2000).

Discussion

This catalogue is a contribution toward an inventory of state and regional biodiversity. Well-maintained historic collections and voucher specimens are critical to record information, validate doubtful records and sustain progress in taxonomy, ecology and conservation. Ideally, this information should be updated frequently, as work progresses.

Although surface waters in South Australia are limited (NLWRA 2001), the state harbours about one fifth of the continental freshwater fish fauna. As the state borders intersect, rather than enclose, some drainage divisions, and as most divisions allow access to the sea, the number of endemic species is comparatively low. Some 'new' records here arise from minor re-alignments of physiographic boundaries between drainage divisions or states (e.g. South Australian Gulf Drainage Division: *N. australis*; South East Coast Drainage Division: *G. truttaceus*), but others represent significant range extensions (e.g. Murray Darling Drainage Division: *N. obscura*; South East Coast Drainage Division: *N. cleaveri*; Lake Eyre Drainage Division: *C. s. ?stercusmuscarum*).

Biodiversity assessments and monitoring should favour obligate freshwater fishes isolated within particular drainage divisions or regions, because they are most likely to have diverged (cf. Crowley & Ivanstoff 1990a,b; Musyl & Keenan 1992; Larson 1995; Allen & Jenkins 1996; Allen & Feinberg 1998; Hammer[1]). These studies may gain impetus from assessments of ecosystem 'health', as fishes are potential indicators (e.g. Harris 1995). Clarifications are needed in regard to the taxonomy of undescribed taxa, species complexes and the biogeographic status of some species, especially where there are few historical data. Fine-scale molecular markers may help to distinguish natural and translocated populations (e.g. Waters *et al.* 2002).

Alien freshwater fishes are ubiquitous in South Australia. They are most apparent in areas directly affected by human industry, particularly in the Murray Darling and South Australian Gulf drainage divisions. All such species are potential vectors for pathogens and parasites (e.g. Langdon & Humphrey 1987). Predators like brown trout *Salmo trutta*, rainbow trout *Oncorhynchus mykiss* and European perch *Perca fluviatilis* are implicated in the decline of small native fishes (e.g. Crowl *et al.* 1992; Morgan *et al.* 2002). *Gambusia holbrooki* is an aggressive, highly fecund competitor that undoubtedly has affected native species (e.g. Lloyd[3]). The feeding behaviour and high abundance of common carp *Cyprinus carpio* have contributed to destruction of wetlands associated with the River Murray (e.g. Sim *et al.* 2000), and thereby affected native fishes. There is also some risk of genetic contamination of native stocks by translocated native species (Arthington 1991).

The preservation of native biota is a management priority in South Australia (e.g. Kahrimanis *et al.* 2001; EPA 2003), and avenues for the introduction of non-native fishes such as the government-sanctioned releases of salmonids, sales of fingerling angling species to the public, "conservation" stocking, releases of unwanted aquarium fishes and inter-basin transfers from the River Murray all need review within broadly-based programs of flow and habitat protection, particularly where small isolated populations of native fish occur.

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RANGE EXTENSIONS FOR FOUR ESTUARINE GOBIES (PISCES: GOBIIDAE) IN SOUTHERN AUSTRALIA: HISTORICALLY OVERLOOKED NATIVE TAXA OR RECENT ARRIVALS?

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Summary

Targeted sampling for gobiid fishes in the Port River estuarine system adjacent to Adelaide, South Australia, identified four previously unrecorded species. Significant range extensions along the east-west coastline of southern Australia are reported for the Australian endemic flatback mangrove goby *Mugilogobius platynotus* (Günther, 1861), largemouth goby *Redigobius macrostoma* (Günther, 1861) and Krefft's frill goby *Bathygobius kreffti* (Steindachner, 1866) plus the alien Trident goby *Tridentiger trigonocephalus* (Gill, 1859). Moreover, *M. platynotus, R. macrostoma* and *T. trigonocephalus* are new records to the fish fauna of the state of South Australia. While it is clear that *T. trigonocephalus* has invaded another southern Australian port, there is difficulty in determining the status of the three Australian endemics as being either native to the area or recent introductions (e.g. through ship mediated translocation) due to a previous paucity of sampling and the cryptic nature of goby behaviour that may have prevented historic detection. The long-term existence of suitable habitat on the one hand suggests that these populations are naturally occurring in the Port River. However, a drastically altered estuarine environment, the high incidence of other translocated marine organisms in the system and goby biological traits suiting transportation in ship ballasts or hull fouling conversely casts doubts over their origin. Contrasting management scenarios of conservation versus potential eradication for these newly discovered species highlights a dilemma for biodiversity conservation in an altered environment.

KEY WORDS: Aquatic biodiversity, environmental change, Gobiidae, marine bioinvasion

Introduction

Small cryptic fishes such as gobies (Family Gobiidae, >1,500 species occurring almost globally: Hoese 1998) are not infrequently encountered in ballast water and as exotics established in world ports (Wonham *et al.* 2000). Introductions of these fishes represent an increasing ecological problem in areas such as southern Australia where three oriental species, the yellowfin goby *Acanthogobius flavimanus*, striped sand goby *Acentrogobius pflaumii* and Trident goby *Tridentiger trigonocephalus* are established in ports within or nearby major cities (Pollard & Hutchings 1990; Hoese & Larson 1994; Lockett & Gomon 2001). There is also obvious potential for the transportation of local species over shorter distances (e.g. Middleton 1982; Willis *et al.* 1999; Francis *et al.* 2003), posing genetic risks such as introgression and swamping of distinct units (Avise 2004) in addition to ecological threats (e.g. Corkum *et al.* 2004).

Although several new gobiid arrivals have been documented for temperate southern Australia, the natural baseline of native species distributions remains poorly documented. The fauna comprises small species (generally <100mm) which typically exhibit cryptic behaviour, often occur in habitats rarely sampled for fishes (e.g. unappealing muddy areas, structurally complex habitat), and have no direct commercial value. Collectively these factors tend to inhibit the gathering of detailed information regarding the spatial occurrence of many gobies (i.e. range, distribution, abundance and habitat), particularly for species in the state of South Australia (Scott *et al.* 1974; Kuiter 1993; Hoese & Larson 1994). Nevertheless, such information is vital to our understanding of the overall biological diversity, ecology and biogeography of marine and estuarine systems (Irish & Norse 1996), especially as gobies are often reported as significant components of fish communities in shallow near-shore areas exposed to anthropogenic impacts (e.g. Bell *et al.* 1984; Hoese 1991; Gill & Potter 1993; Clynick & Chapman 2002).

The Port River estuary is a prominent system in southern Australia both in terms of its size and habitat, and due to its development as a shipping port servicing the major city of Adelaide (population over one million people). A host of exotic species have been translocated to the estuary, most likely due to shipping, including Sabellid fan worm *Sabella spallanzanii*, European shore crab *Carcinus maenas*, New Zealand screw shell *Maoricolpus roseus*, the bryozoan *Bugula flabellata* and the red alga *Polysiphonia brodiaei* (Furlani 1996). Previous studies have examined the fish fauna of the estuary (Connolly 1994; Jones *et al.* 1996; Connolly *et al.* 1997; Jackson & Jones 1999); however, these concentrated on species with commercial value and sampled only a few of the different macro and micro-habitats in the system. This study was designed to broaden the scope of sampling in the Port River estuary to help elucidate the true species richness of the local gobiid community.

This study details significant range extensions for four gobies, and provides an initial assessment of their spatial occurrence, ecology and conservation status.

Methods

Study region

The Port River/Barker Inlet system is a c.100 km^2 temperate zone estuary in St Vincent Gulf adjacent to the Adelaide Plains, South Australia (34°48´S, 138°32´E), and central to the Flindersian Biogeographical Province (Bennett & Pope 1953). As is typical with many estuaries of the world, significant physical and chemical alterations have occurred (Kraehenbuehl 1996; Edyvane 1999; Wade 2002), with particular hydrological changes including the diversion of the major natural freshwater input (River Torrens) and warm-water discharge from the Torrens Island power station (Thomas *et al.* 1986). Remaining habitat is highly modified, especially in the upper reaches of the system which comprises shipping docks, rock levees, cement embankments (e.g. West Lakes) and small patches of mudflats supporting grey mangrove *Avicennia marina*.

Sampling

Targeted non-destructive sampling using dip-nets (400 mm^2 frame, 3mm stretch mesh) investigated areas of high structural integrity, such as artificial vertical surfaces (e.g. cement walls), crevices and rock banks. Sampling was from shore and by wading at sites accessible by road, with a site covering a 30m stretch of bank. Two supplementary techniques were also employed: at night specimens could be observed under torchlight and coerced into one of two dip nets used in unison, and at low tide the turning of rocks in some cases revealed specimens for hand capture or with small aquarium nets. Sampling was designed to cover a range of daily and seasonal conditions such as tide height and diurnal phase (day or night) and to record specific characteristics of captured species habitat and ecology. Some laboratory observations using aquaria were also undertaken. Equipment was sterilised (dilute bleach solution and sun-drying) between use in different parts of the Port River system. Representative vouchers were euthanased, then fixed in 10% formalin, and subsequently transferred to 70% ethanol and lodged at the South Australian Museum, Adelaide (SAMA). Identification followed the keys of Hoese & Larson (1994) and incorporates subsequent updates in nomenclature (Larson 2001; Larson & Murdy 2001). Fish lengths are given in Total Length (TL) for live specimens to be consistent with Hoese & Larson (1994) and in Standard Length (SL) taken from preserved material.

Location	Site/habitat	Date/conditions	*Acentrogobius bifrenatus* (Kner, 1865)	*Afurcagobius tamarensis* (Johnston, 1883)	*Bathygobius kreffiii* (Steindachner, 1866)	*Callogobius mucosus* (Günther, 1872)	*Favonigobius lateralis* (Macleay, 1881)	*Gobiopterus semivestita* (Munro, 1949)	*Mugilogobius platynotus* (Günther, 1861)	*Pseudogobius olorum* (Sauvage, 1880)	*Redigobius macrostoma* (Günther, 1861)	*Tridentiger trigonocephalus* (Gill, 1859) *
North Arm	1 RP, mangroves	20/09/03 N, M					3	20	2	25		
		30/10/03 N, H						35	3	4		
		06/11/03 D, L							13	30		
	2 RP, mangroves	05/08/03 N, H					35	15				
		08/08/03 N, L	3	5			15	30		5		
	3 Rocks	06/11/03 D, L										
Angas Inlet	4 RP	05/08/03 N, H					10	60	5	25		
		08/08/03 N, L					10	15	1	10		
		06/11/03 D, L							5	3		
	5 VW, rocks	06/11/03 D, L					10	5				
Port River	6 Rock pools	06/11/03 D, L					25					
Old Port Reach	7 RP	06/11/03 D, L	15				1		3	28		
	8 Mangroves	21/10/01 N, M	3				7	100		5		
		30/07/03 N, H								3		
West Lakes	9 RP and VW	21/10/01 N	10	2						50		
	10 VW, rocks at base	01/03/01 D	15							8	12	1
		21/10/01 N								10	4	
		10/01/04 D	100	3						13	10	1
	11 RP and VW	30/07/03 N								5	1	
	12 VW	10/02/03 D	3							3	12	
		30/07/03 N								10	3	
	13 VW, seagrass bed	28/02/04 D	8	1						35	40	
	14 RP	10/02/03 N	1	4						12	2	
	15 VW	10/02/03 N								1	5	
	16 VW	21/10/01 N	5			1				5	2	
		29/07/03 N	2	2						10	4	
		05/08/03 N								7	4	
		01/02/04 D	10								32	1
	17 VW	29/07/03 N									14	
		03/08/03 D	1							5	4	
		05/08/03 N									13	
		24/12/03 N	2	1							25	
		01/02/04 D	5		2						28	

Table 1 *Relative abundance of goby species sampled at 17 sites in the upper Port River estuary between March 2001 and February 2004.* [Conditions: night (N) or day (D), tide height low (L), med. (M) or high (H) - note water levels are relatively constant in West Lakes. Habitat: rock pile (RP), vertical wall (VW)] * Introduced to Australia

Results

Sampling for gobies was undertaken at 17 sites in the upper sections of the Port River between March 2001 and February 2004 (Fig. 1). Ten goby species were located in or near the targeted habitat (Table 1). There was considerable variation in the detection of goby species interrelating between habitat, environmental conditions (e.g. tidal height) and time of day, and broader temporal replication at sites often revealed contrasting catches (Table 1).

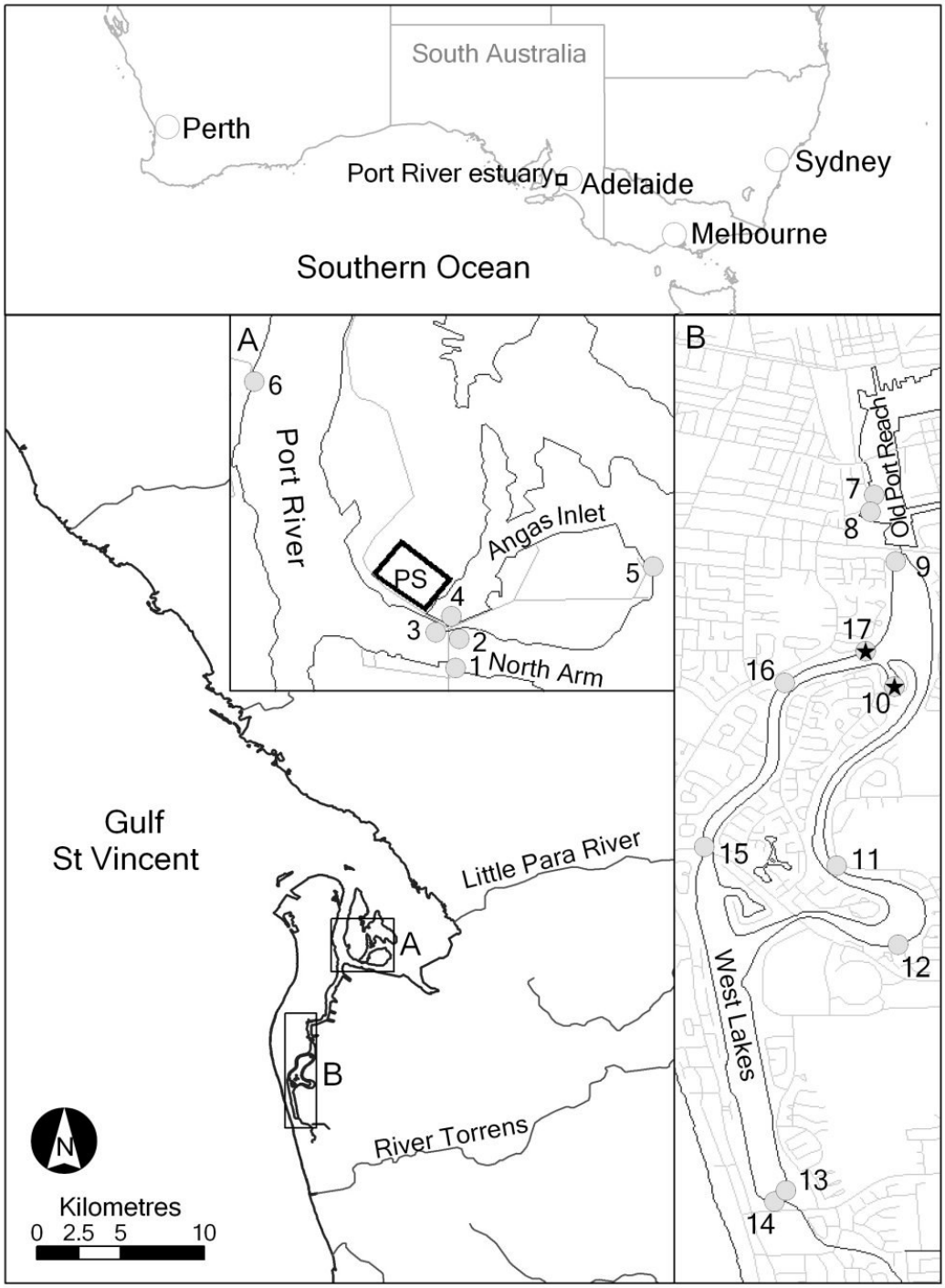

Figure 1 Map showing the location of goby sampling sites in the upper Port River estuary within urban Adelaide, South Australia. PS = Torrens Island Power Station; stars indicate *Tridentiger trigonocephalus* capture locations.

Range extensions

Four species previously unknown from the Port River were collected. These included three additions to the known fish fauna of the state of South Australia: (a) flatback mangrove goby *Mugilogobius platynotus* collected at three sites in the upper Port River (Table 1; SAMA F10130, 10132, 10133), representing a range extension of some 1000km westward along Australia's southern coastline (Larson 2001); (b) largemouth goby *Redigobius macrostoma* collected from nine of ten sites in West Lakes (Table 1; SAMA F10137, 10138, 10312), representing a westerly range extension from the Glenelg River of approximately 550 km (Kuiter 1993; Hoese & Larson 1994), and (c) *T. trigonocephalus* collected from two sites in West Lakes (Table 1; SAMA F10134, F10141), a species otherwise native to the north-west Pacific and now known from the immediate vicinity of all capital cities (major shipping ports) on the coastline of mainland southern Australia (Hoese 1973; Chubb *et al.* 1979; Gill & Potter 1993; Lockett & Gomon 2001). The fourth new Port River record, Krefft's frill goby *Bathygobius krefftii*, collected from one site in West Lakes (Table 1; SAMA F10142) extends the range of this species by 440 km to the east (by sea) to include St Vincent Gulf as a second distinct western population within a broader disjunct distribution – the species is also known from upper Spencer Gulf, South Australia (recent presence confirmed by the author at Whyalla Marina: SAMA F10453) and the east coast of Australia (Kuiter 1993; Hoese & Larson 1994).

Habitat

Field data suggest that the occupied habitat of the four newly recorded gobies is quite specific. Between and within site observations indicate that the distribution of *M. platynotus* is patchy and related to select microhabitat of sheltered intertidal rock piles over silty mud (as opposed to coarse sand) within or near mangrove stands. Here they were sympatric with western bluespot goby *Pseudogobius olorum* at low tide located in moist depressions under rocks, and with additional species when habitats were immersed, mainly the pelagic smallmouth hardyhead *Atherinosoma microstoma* and glass goby *Gobiopterus semivestita*, and the benthic southern longfin goby *Favonigobius lateralis* and bridled goby *Acentrogobius bifrenatus*.

Redigobius macrostoma was located almost exclusively at vertical algal and mussel covered surfaces (particularly at night), occasionally being caught at weedy and rocky areas nearby (more so during the day). Removal and vigorous shaking of groups of mussels from vertical surfaces often released fish from within cavities or dead shells. At the southern end of West Lakes (Site 13b) specimens (mostly juveniles) were netted from a *Zostera* seagrass bed. *Redigobius macrostoma* and oyster blenny *Omobranchus anolius* were generally the exclusive inhabitants of vertical surface microhabitats with other sympatric species such as *P. olorum*, *A. bifrenatus*, and Tamar River goby *Afurcagobius tamarensis* captured from nearer to the benthos. *Bathygobius krefftii* and *T. trigonocephalus* occupied structurally complex habitat such as rocks and clumps of dead mussels.

Population status

The relative abundance of *M. platynotus* was typically low with up to 13 individuals located in a 30m stretch of bank (usually five or less). The total length of 32 fish sampled ranged from a 21 mm juvenile to a 74 mm TL adult male (16-59 mm SL) and a number were larger than the reported 60 mm TL maximum size for the species (Hoese & Larson 1994). The fore-mentioned adult male displayed nuptial colours and was located under an exposed rock at Site 4 beside a 59 mm TL (48 mm SL) female with distended abdomen on 6/xi/03, suggesting that breeding was imminent (SAMA F10131).

Redigobius macrostoma was more common within its restricted range (total of 215 captured) with up to 40 fish (often >10) captured at a site and higher catches in greater water depths (i.e. increased vertical surface). Population size-structure was evident with fish ranging between 22-50 mm TL (17-41 mm SL) and this indication of recruitment was matched with observations of local reproduction. Ripe fish were captured in early August 2003 through to February 2004 (some

transferred to an aquarium spawned in a rock cave, within four days at approximately 22°C whereby the male guarded the eggs). In December 2003 two natural spawning sites were discovered (Site 16). Adult male fish displaying distinct enlarged mouths (~40-50 mm TL) were found inside dead mussel shells guarding patches of eggs. One patch preserved and examined (SAMA F10136) covered an area of ~13 cm^2 (in the order of 3000-3500 eggs) in two roughly symmetrical patches on either shell half. The semi-transparent eggs were cylindrical, adhesive at one end and just over 1mm in length. Observations on the behaviour of *R. macrostoma* larvae were made following the transferral of an egg patch to the laboratory (maintained at room temperature; 18-22°C, and with artificial aeration): 2-3 mm larvae hatched after six days and swam with difficulty throughout the water column, often resting against surfaces.

Both *T. trigonocephalus* and *B. krefftii* were apparently rare in the habitats sampled (i.e. 3 and 2 captured respectively). Records for *T. trigonocephalus* spanning the three year sampling program suggest it is persistent in its small area of occupancy with adult and sub-adult specimens caught: 37 mm TL (29 mm SL), 52 mm TL (43 mm SL) and 80 mm TL (voucher not retained for SL). The two *B. krefftii* were adults (56 and 50 mm TL; 46 and 39 mm SL) with the smaller specimen a ripe female.

Discussion

The discovery of three species new to South Australia at a location adjacent to the state capital city shows that the ichthyofauna of near-shore environments in the region is poorly understood. It is clear that targeted, temporally replicated and intensive sampling of different microhabitats is necessary for confidence in regional species lists, especially for diminutive and cryptic species such as gobies.

The gobiid community of the Port River estuary is species-rich by southern Australian standards (cf. Potter & Hyndes 1999). Evidence of both the reproduction and recruitment of *M. platynotus* and *R. macrostoma* indicates that these species are well established in suitable habitats of the upper Port River. Conversely *B. krefftii* and *T. trigonocephalus* do not appear to be widespread or in high abundance. It is clear that *T. trigonocephalus* is an introduced species which almost certainly arrived via international and/or domestic ships. However, determining whether *M. platynotus, R. macrostoma* and *B. krefftii* were present prior to the arrival of Europeans is less certain and more complex, with a resolution on their native or introduced status currently unknown due to evidence consistent with both scenarios (explored below).

The restricted distribution, cryptic behaviour and micro-habitat noted for the three species may have prevented their previous detection as collections from littoral areas of high structural integrity do not appear in the literature (Connolly 1994; Jones *et al.* 1996; Connolly *et al.* 1997; Jackson & Jones 1999) or in institutions that maintain historical voucher specimens such as SAMA (note however, that a single *M. platynotus* was captured during a concurrent research program that targeted varied microhabitats in the Port River system: Bloomfield & Gillanders 2005). Hence the available survey coverage is inadequate for determining historic presence or absence.

The current study supports observations that two of the species are habitat specialists, with *M. platynotus* occurring in areas with mangroves and *R. macrostoma* preferring vertical structure and rocky areas in estuaries (Kuiter 1993; Hoese & Larson 1994; Larson 2001). These habitats are limited in southern Australia. Mangrove habitat east of the Port River is absent coastally until southern New South Wales, with the exception of one small patch in Western Port, Victoria (Butler *et al.* 1977; Busby & Bridgewater 1986), and the few estuaries are widely separated by exposed, high-energy coastlines, particularly west of the Glenelg River. For *B. krefftii* its broader distribution matches relictual subtropical distribution patterns for other marine fauna such as the tiger pipefish *Filicampus tigris* (Kuiter & Debelius 2000), blue swimmer crab *Portunus pelagicus* (Bryars & Adams 1999) and numerous molluscs (K. Gowlett-Holmes, CSIRO Marine Research, pers. comm. 2004), as well as a highly divergent northern lineage of the sea-star *Coscinasterias muricate*

(Waters & Roy 2003). Hence, outlying natural goby populations in the Port River could be explained by the long-term existence of suitable habitat.

A natural presence in the region may also be explained by occasional or episodic marine dispersal of larval or adult gobies to the Port River/St Vincent Gulf. This is documented along the east coast of Australia and northern New Zealand where ephemeral populations of tropical and subtropical fish species have been reported to range southward to temperate areas (Kuiter 1993; Francis *et al.* 1999).

An alternate explanation for recent detection could be the result of human mediated translocation from shipping (i.e. *M. platynotus* and *R. macrostoma* as recent arrivals from the east and *B. krefftii* from the east and/or Spencer Gulf). A precedent exists for such introductions given the array of exotic biota in the Port River system (Furlani 1996), which notably is also now known to include an introduced goby, *T. trigonocephalus*. The species in question also have biological traits suited to transportation via ship ballasts or hull fouling. Wonham *et al*. (2000) matched the crevicolous nature of gobies with entry through ballast-intake holes on ships, and judging from the occupied habitat of *R. macrostoma* and *M. platynotus* in South Australia, both actively seek refuge and spawning sites in confined spaces. Observations on the small size and behaviour of *R. macrostoma* larvae are consistent with them being pelagic (Hoese 1998) and thus entrapment with ballast intake could easily occur (Carlton & Geller 1993). Moreover, *R. macrostoma* is known to occur in close proximity to ships (i.e. pylons in harbours: Kuiter 1993) and appears to have an affinity for hull fouling organisms such as mussels.

The physically altered Port River environs appear suitable for colonisation of newly arrived gobies. For example warm water discharge from the Torrens Island Power Station may provide conditions to sustain subtropical species though winter (a warm water plume can extend from Angas Inlet, through the North Arm and on to the Port River: Thomas *et al.* 1986). Similarly, artificial structure such as rock piles and debris common to the area provides structural habitat for colonisation. Nonetheless, *R. macrostoma* and *B. krefftii* populations in West Lakes may be relicts from former seagrass/mangrove habitat prior to development (Kraehenbuehl 1996), and imported man-made rock piles may provide alternate habitat for *M. platynotus* offsetting habitat loss (e.g. mangrove clearance, channel deepening, swamp reclamation). Other altered and artificially maintained habitat nearby in the lower River Murray is known to provide refuge for rare or threatened native fishes (Wedderburn & Hammer 2003).

The dilemma over the status of gobies in the Port River highlights a problem concerning species origin that is going to be increasingly difficult to answer in areas where faunas are poorly catalogued, loss of habitat continues and where increasing number of species introductions occur. Further assessment of the status of Port River gobies would be assisted by examination of genetic and morphological variation (Hickley *et al.* 2004) and potentially by sampling other regional estuaries not frequented by ships. The examination of preserved material after extirpation will however, do little to protect unique lineages or even discrete species adapted to local conditions, and hence the three Australian endemic gobies should best be treated as species native to the Port River until evidence to the contrary is provided.

In principle, management decisions will differ significantly depending upon whether these gobies are indigenous or recently-translocated, since the former would involve measures for species conservation whereas the latter would address eradication. This notion has practical significance given populations in the Port River appear to be restricted, leaving them vulnerable to extirpation (e.g. fish kills, further habitat loss, treatment methods to control introduced organisms). Vigilance is required with respect to the population dynamics and ecological impact of *T. trigonocephalus* and other potential piscine invaders, especially *A. flavimanus* and *A. pflaumi* that are already established elsewhere in southern Australia.

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