DNA barcoding to support conservation: Species identification, genetic structure and biogeography of fishes in the Murray-Darling River Basin, Australia

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Abstract

Freshwater fish stocks worldwide are under increasing threat of overfishing, disease, pollution and competition from introduced species. In the Murray-Darling River Basin (MDB), the largest river system of Australia, over half the native species are listed as rare or endangered. Active management is required to counteract reduction in population sizes, prevent local extinctions and to maintain genetic diversity that needs to be defined. We describe the first comprehensive set of DNA barcodes able to discriminate between all 58 native and introduced species of freshwater fish recorded in the MDB. These barcodes also distinguish populations from those in adjacent basins with estimated separation times as short as 0.1 Mya. We demonstrate the feasibility of using DNA fingerprinting of ribosomal RNA (12S and 18S rRNA) and mitochondrial DNA control region (mtDNA CR) sequences to identify species from eggs, larvae, tissues and predator gut contents as well as differentiate populations, morphologically cryptic species and hybrids. The DNA barcode resource will enhance capacity in many areas of fish conservation biology that can benefit from improved knowledge of genetic provenance. These include captive breeding and restocking programs, life history studies and ecological research into the interactions between populations of native and exotic species.

Introduction

Molecular genetic information has the potential to address two great voids in conservation and management. First, genetic markers can provide fast and accurate species identification for all life stages, including those for which taxonomic keys are not available and for *ex vivo* tissue samples. Second, they offer valuable insights into population structure and evolutionary history and contribute to the definition and monitoring of Evolutionary Significant Units (ESUs) and Management Units (MUs) in conservation management strategies (Waples 1991; Moritz 1994; Swartz *et al.* 2008). Reflecting these strengths, DNA barcodes are now widely used to identify species (Moritz and Cicero 2004). The Fish Barcode of Life campaign (FISH-BOL) has been established to create a standardised reference DNA library based on the mitochondrial cytochrome oxidase subunit 3 gene (COI barcode) for all fishes (Ward *et al.* 2009). The stated goals of barcoding fishes include facilitating species identification, revealing range expansions, detecting previously overlooked species and enabling identifications where traditional methods cannot be applied. (Ward *et al.* 2009). Few Australian freshwater fishes have so far been sequenced under this initiative and small subunit ribosomal RNA (mitochondrial 12S and nuclear 18S rRNA) and mitochondrial DNA (mtDNA) control region (CR) sequences are amongst the most widely preferred barcoding regions to discriminate and infer genetic relationships in selected Australian fishes (Jerry *et al.* 2001; Wang *et al.* 2001; Huey *et al.* 2006; Jansen *et al.* 2006; Faulks *et al.* 2010a; Faulks *et al.* 2010b; Page and Hughes 2010).

Native and exotic fish management is high on the agenda of Australian government and research agencies and accurate recording and monitoring of fish species is vital to ensure that management programs are having a positive effect (Higham *et al.* 2005; Barrett and Mallen-Cooper 2006; Moore *et al.* 2010). Australia's Murray-Darling River Basin (MDB) is the sixth largest catchment in the world (Walker 1985) yet over half the native freshwater fish species it contains are threatened with extinction. Over the past 200 years, the fish of the MDB have suffered as a result of habitat loss, changes in flow regimes, barriers to passage and over-allocation of water for human use. Further exacerbating their plight, the Murray-Darling is currently rated one of the world's top 10 river systems under serious threat from introduced fish species (Wong *et al.* 2007) and native species are now considered to have declined to abundances of just 10% of pre-European levels (Moore *et al.* 2010). The freshwater fish fauna of the Murray-Darling is comprised of 58 species, including one translocated native and 12 exotic species (Lintermans 2007; Davies *et al.* 2010). A number of other, essentially marine species are also routinely encountered in the estuarine reaches of the Murray River, lower lakes and the Coorong, an extensive and occasionally hyper-saline system separated by barrages from the mouth of the river (Higham *et al.* 2002). Museum records exist for an additional two Australian native species (*Macquaria novemaculeata* and *Oxyeleotris lineolata*) that were translocated but failed to establish self-sustaining populations (Hammer and Walker 2004). The need for detailed genetic data is now being addressed through extensive population studies on key species in the MDB, but information is still lacking on many fish in the system, including both abundant species and several of the 26 natives listed as rare or endangered (Lintermans 2007; Davies *et al.* 2010; Moore *et al.* 2010).

The aim of our study was to produce and demonstrate the applicability for conservation research of the first comprehensive nuclear and mitochondrial DNA sequence inventory for all the freshwater fish species known to inhabit the MDB, including comparisons between conspecific populations that are allopatric and occur outside the MDB. We hypothesised that the use of functionally matched nuclear and mitochondrial genes with different evolutionary rates as DNA barcodes would facilitate species identification as well as overcome potential problems identified with the use of single barcodes in fish (Page and Hughes 2010). We therefore selected 18S rRNA (relatively slow evolutionary rate), 12S rRNA (medium) and mtDNA CR (fast) sequences for their individual and combined utility for species identification, estimating phylogenetic and biogeographic relationships, determining predation by introduced species and for identifying hybrids.

Methods

Species selection

Seventy one species recorded from the MDB (Hammer and Walker 2004; Lintermans 2007; Davies *et al.* 2010) were examined in this study to cover the full extent of the freshwater fish fauna, including several species commonly encountered in estuarine reaches of the lower Murray River, lower lakes and Coorong (Table 1, Fig 1). Specimens of 11 additional species closely-related to Murray-Darling species were also included for barcoding comparisons and phylogenetic analyses (Table 1).

DNA and RNA extraction and PCR amplification

Tissue or DNA samples were obtained from pre-existing collections and the aquaculture and seafood trade (Table 1). Wherever possible at least two specimens from each species were obtained. Where specimens were obtained alive, fish were first anesthetised using 5% w/v tricaine methanesulfonate (MS-222, Argent Chemical Laboratories, WA, USA) in aged water until breathing ceased and then frozen at -20^{o}C. Tissues were processed using the DNeasy Tissue Kit (Qiagen) according to the manufacturer's instructions. Total RNA extraction was performed using the RNeasy mini kit (Qiagen) with the inclusion of RNase-Free DNase (Qiagen). Complementary DNA (cDNA) from up to 1 μg total RNA was prepared using random hexamer primers and the Superscript III First Strand Synthesis System (Invitrogen).

Approximately 1.8kb of the 18S rRNA gene between helix stems 5 and 49' (Wuyts *et al.* 2004) was amplified between universal primers 18se (5'-

CTGGTTGATCCTGCCAGT-3’) and 18sp (5’-TAATGATCCTTCCGCAGGTTCACCT-3’) (Winchell *et al.* 2002). Approximately 390 bp of the 12S rRNA gene between helix stems 27’ and 32’ (Wuyts *et al.* 2004) was amplified between vertebrate universal PCR primers MT 1091L (5’-CAAACTGGGATTAGATACCCCACTAT-3’) and MT 1478H (5’-TGACTGCAGAGGGTGACGGGCGGTGTGT-3’) (Fuller *et al.* 1998). PCR amplification and sequencing of a highly variable 0.3-1.0 kb domain in the control region of the mitochondrial genome was conducted using primers Proline gene F (5’-CCACTAGCTCCCAAAGCTA-3’), Central conserved region R (5’-CCTGAAGTAGGAACCAGATG-3’) (Ovenden *et al.* 2002), MT 1091R (5’-GGGTATCTAATCCCAGTTTG -3’) and *Anguilla*-specific Proline gene F mod (5’-TCCTCAACTCCCAAAGCTG-3’).

DNA was amplified in 50 µL containing 0.4 µM of each primer, 200 µM dNTP, 2.5 mM $MgCl_2$, 10-50ng DNA, Q solution, 1X supplied buffer and 1 unit *Taq* (Qiagen *Taq* PCR Core kit) under the following cycle conditions: 2 min at 94ºC; then 35 cycles of 1 min at 94ºC, 1 min at 55ºC and 1 min 30 sec at 72ºC, followed by a final post-extension for 10 min at 72ºC. PCR products were purified using QIAquick PCR Purification Kit (Qiagen) and sequenced directly on both strands using a Coulter CEQ 8800 capillary sequencer (Beckman). Additional 18S rRNA gene internal primers used for sequencing were: 18S_470R (5’-TTGGATGTGGTAGCCGTTTC-3’), 18S_internalF (5’-GCCCTATCAACTTTCGATGG-3’), 18S_580F (5’-AGCCGCGGTAATTCCAGCTC-3’), 18S_internalR (5’-CGTTATCGGAATTAACCAGAC-3’), All18SF (5’-TGGTGCATGGCCGTTCTTAGT-3’), All18SR (5’-CATCTAAGGGCATCACAGACC-3’).

All sequences were lodged in GenBank under Accession numbers: FJ710812-FJ710909, HQ615525-HQ615586 (18S rRNA); FJ710910-FJ711007, HQ615461-HQ615524 (12S rRNA); and HQ615423-HQ615460, HQ682191-HQ682193 (mtDNA CR).

Phylogenetic analyses

Pairwise sequence comparisons to determine nucleotide similarities were conducted using BLASTn (Altschul *et al.* 1990). Sequences were initially aligned using MAFFT version 6.815 (Katoh and Toh 2008) and manually adjusted using Bioedit version 7.0.5.3 (Hall 1999). Predicted rRNA secondary structure models were used to designate regions of ambiguous alignment and to partition putatively homologous nucleotide positions into paired and unpaired character sets (Kjer 1995; Gillespie 2004). Phylogenetic analyses for the partitioned 18S and 12S rRNA alignments were performed at CIPRES Science Gateway v3.0 (Miller *et al.* 2009). Maximum Likelihood (ML) analyses were performed with RAxMLv7.2.7 (Stamatakis 2006; Stamatakis *et al.* 2008) using 100 searches from distinct randomized Maximum Parsimony (MP) starting trees. Each partition was assigned a separate General Time Reversible (GTR) model of nucleotide substitution (Tavaré 1986). Among-site rate variation was modelled using the Γ model of rate variation (Yang 1996) with four discrete rate categories (GTR+ Γ model). Alternative models of rate variation implemented in RAxML had no effect on the results (data not shown). Clade support was assessed using 1000 replicates of ML bootstrapping (MLBP).

Bayesian posterior probabilities (BPP) were estimated using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003). Each partition was assigned a GTR+ Γ model of evolution with flat Dirichlet priors for base frequencies and substitution rate matrices and uniform priors for among-site rate parameters. As preliminary 18S rRNA analyses using the default value (10) for the rate parameter of the exponential prior on branch lengths exhibited greatly inflated branch length estimates compared to ML results, a more reasonable prior value for this dataset (606.31) was derived (Brown *et al.* 2010). Two parallel Markov Chain Monte Carlo (MCMC) runs of four chains each were performed (one cold and three heated, temperature parameter = 0.1) with a length of 10,000,000 generations, a sampling frequency of one per 1000 generations and a burn-in corresponding to the first 2,500,000 generations. Adequate mixing was confirmed by examining the proportion of successful chain swaps and convergence was confirmed by examining the posterior distributions of parameters. Clades were considered strongly supported for values of MLBP >70% and BPP >0.95.

Detection of predation using DNA barcodes

Stomach contents from two specimens of *Perca fluviatilis,* containing unrecognisable remains of prey fish, were obtained as ethanol preserved samples from a monitoring program conducted by the Department of Industry & Investment NSW in the upper Lachlan River catchment. This area contains some of the few remaining populations in the MDB of the endangered native *Nannoperca australis* and *Macquaria australasica*. DNA was extracted from the remains as described above and sequenced to test the ability of DNA barcodes to identify the prey species that were consumed.

Results

18S rRNA barcodes

Sequences were obtained for a 1.8 kb nucleotide region of the 18S rRNA gene from 158 individuals covering 71 species of fish from the MDB (Hammer and Walker 2004; Lintermans 2007; Davies *et al.* 2010), as well as 11 additional related species/subspecies (Table 1). Direct sequencing of 18S rRNA amplicons was not possible in four species (*Tinca tinca*, *Aldrichetta forsteri*, *Porochilus rendahli* and *Maquaria ambigua* 'Eyre') due to the existence of intra-individual 18S rRNA variants similar to those reported in sturgeons (Krieger *et al.* 2006). Amplification and direct sequencing of cDNA was therefore employed for these species and this confirmed that only a single rRNA gene variant was expressed in muscle tissue in each case.

All genera and most (59 of the 71) Murray-Darling species were uniquely identified on the basis of the relatively conserved 18S rRNA barcodes, with up to 25 nucleotides differences separating genera over 1.8 kb (Table 2). The exceptions were 14 species, from 4 genera (*Hypseleotris, Galaxias, Melanotaenia* and *Maccullochella*) which could only be uniquely identified from other species within the MDB on the basis of 12S rRNA sequences. Specifically, taxa sharing identical 18S rRNA sequences were four species in the *Hypseleotris* complex: *H. klunzingeri, H.* sp.1 'midgley's'*, H.* sp.3 'murray-darling' and some *H.* sp.2 'lake's' hybrids; two species each in *Melanotaenia* (*M. fluviatilis* and *M. splendida tatei*), *Maccullochella* (*M. peelii peelii* and *M. macquariensis*) and *Galaxias (G. maculatus* and *G. rostratus)*; and lastly, the various members of the *Galaxias olidus* complex (*G. olidus*, *G. fuscus, G.* 'riffle' and *G.* 'oliros'). Only 3 MDB species (*Gadopsis marmoratus*, *Hypseleotris* sp.2 ‘lake’s’ and *Retropinna semoni* exhibited 18S rRNA variation (0.1-0.2%) among populations.

12S rRNA barcodes

All 71 MDB species were uniquely identified on the basis of the shorter and less conserved 390 bp nucleotide region of the 12S rRNA gene. There were between 1 (*Galaxias olidus* species complex) and 47 (*Macquaria* species) nucleotide differences within a genus (Table 2). In addition, for native species that also occur in adjacent drainages, the 12S rRNA barcodes were able to distinguish MDB specimens from their conspecific populations in neighbouring basins in 12 of 24 possible comparisons. In these cases up to 20 nucleotide differences were present (Table 2). The lack of differentiation for some populations of *Ambassis agassizii*, *Anguilla australis*, *Galaxias maculatus*, *Hypseleotris* sp.1 ‘midgley’s’, *Hypseleotris* sp.2 ‘lake’s’, *Leiopotherapon unicolor*, *Macquaria ambigua*, *Macquaria australasica*, *Nannoperca australis*, *Neosilurus hyrtlii*, *Porochilus rendahli* and *Tandanus tandanus* was subsequently addressed by generating additional CR barcodes. *Mogurnda adspersa* was also included as the 12S rRNA sequence of a GenBank entry (AF265367) with uncertain provenance (Wang *et al.* 2001) was identical to one MDB individual.

Mitochondrial DNA control region barcodes

Additional sequences spanning between 0.3-1.0 kb nucleotides of a highly variable domain in the mtDNA CR were obtained for 13 species for which 12S rDNA gene barcodes could not be used to distinguish populations in the MDB from those of the same species in other river basins. In addition to these, we obtained mtDNA CR barcodes for two comparisons involving MDB fish and specimens representing subspecies or closely-related congeners found in adjacent drainages, namely *Bidyanus bidyanus* versus *B. welchi* and *Maccullochella peelii peelii* versus *M. peelii mariensis*. Intra-species sequence differences of up to 26 nucleotides (8.1% divergence) across catchments for the mtDNA CR were present in all but one species (Table 2). Only *Ambassis agassizii* from the Lachlan River (southern MDB, NSW) and Burnett River (east coast catchments, QLD) could not be identified to basin by mtDNA CR, 12S or 18S rRNA barcodes.

Phylogenetic relationships

The potential for 18S and 12S rRNA barcode sequences to generate phylogenetically useful information was examined after exclusion of nucleotide regions that could not be aligned unambiguously across all taxa. The mtDNA CR barcode sequences were not analysed in this way as they were too divergent to align across different species. Species-specific sequence barcode differences and some phylogenetic information present at sub-taxa level were therefore not included and the final alignments comprised 1663 bp and 288 bp respectively. Maximum Likelihood and Bayesian analyses of each alignment did not strongly conflict, although many nodes were unsupported in both analyses. The Maximum Likelihood trees and Bayesian support values for 18S (Fig. 2) and the more variable 12S (Fig. 3) rRNA sequences revealed phylogenetic relationships consistent with established taxonomy (shown grouped by Order for 18S and by Family for 12S trees). The 18S rRNA tree lends some support for separation of Osmeriformes into more than one Order. There was only one unusual discrepancy between the inferred phylogenetic relationships using the 18S and 12S rRNA sequences. *Nannoperca australis* was more closely related to *N. obscura* than *N. variegata* on the basis of 12S, but not 18S rRNA relationships. The only two described species with identical 18S and 12S rRNA sequences were *Bidyanus bidyanus* and *B. welchi* (currently restricted to the Lake Eyre basin), although the more highly variable mtDNA CR sequences differed by 4.5%.

Identification of fish using DNA barcodes

The ability of the DNA barcode reference dataset to enable identification of specimens that cannot confidently be assigned to species by morphology was tested using larval specimens and partially digested fish remains (all less than 1 cm in length) present in the stomachs of two *Perca fluviatilis*. By comparison to the reference dataset, a larval *Hypseleotris* species “carp gudgeon” from the Shoalhaven River (east coast catchments) was identified as *H. klunzingeri* with only a single 12S rRNA barcode nucleotide difference (HQ615480) from the MDB barcode (FJ710943). Another fish from Paddy’s River, a tributary of another east coastal river, the Hawkesbury-Nepean, was identified as *Hypseleotris* sp.2 ‘lake’s’. Its 18S (HQ615549) and 12S (HQ615485) barcodes were identical to MDB specimens and the mtDNA CR barcodes (HQ682191-HQ682193) differed by only 1-2 nucleotides. Similarly, freeze-dried gut tissues of fish larvae from the Murray River at Mildura (Hardy *et al.* 2010) were confirmed by 12S rRNA barcode comparisons as identical to *Retropinna semoni* (FJ710982), *Hypseleotris* sp.1 'midgley's' (FJ710948) and *Melanotaenia fluviatilis* (FJ710958). Finally, native fish (*Hypseleotris* sp.1 'midgley's' HQ615489, HQ615490) as well as the introduced *Gambusia holbrooki* (HQ615474, HQ615475) were detected in the stomach samples of *P. fluviatilis*, each with identical 12S rRNA barcodes to MDB specimens.

Discussion

Value of DNA barcodes

A register of small subunit rRNA and mtDNA CR sequence barcodes contributes greatly to our knowledge of genetic provenance and the likely higher order evolutionary significant units and management units for MDB fish species (Unmack 2001; Moore *et al.* 2010). We demonstrate that the use of functionally matched nuclear and mitochondrial genes as DNA barcodes with different molecular evolutionary rates enables species identification as well as overcomes many of the problems associated with the use of single barcodes such as COI. These include the ability to discriminate hybrids, recent radiations, regional differentiation in barcode sequences, availability of universal primers for all species and the existence of nuclear copies of the barcode region (Ward *et al.* 2009).

Comparisons of sequences to the reference DNA barcodes enabled us to detect previously undescribed natural and/or translocated fish populations outside the MDB.

For example, sequences of juvenile carp gudgeons (*Hypseleotris* species) from the upper Hawkesbury-Nepean and the Shoalhaven catchments in coastal NSW were identified as *H. klunzingeri* and *Hypseleotris* sp.2 ‘lake’s’ from initial DNA barcode comparisons. One individual in particular, later confirmed as *H.* sp.2 ‘lake’s’ by closer morphological examination and scale counts, was genetically very similar (indistinguishable 18S, 12S rRNA and only a single mtDNA CR nucleotide barcode difference) to a specimen collected near Cohuna in Victoria. These occurrences represent disjunct east coast range extensions for both species and, considering their phylogenetic affinities and lack of historical records, implicate human assisted translocation from MDB stocks.

In another application, natural or induced fertile hybrids have been reported between a range of MDB fish species (Douglas *et al.* 1995; Bertozzi *et al.* 2000; Gleeson *et al.* 2000; Waters *et al.* 2001; McDowall 2006), including estuarine and marine members of *Macquaria* and *Acanthopagrus* (Rowland 1984; Jerry *et al.* 1999). However, hybrid identification has relied on appropriately collected tissues, expertise and often knowledge of allozyme frequencies based on multiple samples. Combined nuclear and mt DNA barcode analysis can now be used to facilitate the identification of species and hybrids using material otherwise unsuitable for allozyme characterization such as very small fry, ethanol preserved and degraded tissue. The barcodes can also provide evidence for hybrids arising from the introduction of species into new catchments. For example, the presence of a *H.* sp.2 ‘lake’s’ 18S rRNA allele was detected in a specimen of *H. galii* from the Georges River in coastal NSW, a catchment adjacent to where *H.* sp.2 ‘lake’s’ now appears present. However, further sampling will be required to confirm the presence or impacts of gene flow between these species.

Accurate identification of fish using DNA barcodes has proven essential in a previous study examining food webs in the MDB, albeit for a limited number of small fish species (Hardy *et al.* 2010). The ability to identify any species consumed by larger fish by sequencing stomach contents represents a further practical demonstration of the value of comprehensive reference DNA barcodes for monitoring the impacts of predation.

Endemism in MDB fish

In addition to providing a resource for conservation monitoring, the multi-gene DNA barcode inventory has revealed valuable information on the levels of endemism, genetic structure and evolutionary relationships of native MDB fish populations relative to those outside the basin. Specifically, 23 of 33 native species restricted to freshwater have distinct populations outside the MDB. Inter-basin sequence differences were evident for 15 of these using 12S rRNA barcodes and a further seven using the more variable mtDNA CR barcodes. A more detailed description by family of MDB fish barcode results is available online (see Accessory Publication).

Estimates for separation times between MDB and coastal populations have previously been made using mtDNA CR sequences or allozymes. These dates range between 0.6-1.6 Mya for subspecies within *Craterocephalus stercusmuscarum* (McGlashan and Hughes 2001), *Maccullochella peeli* (Rowland 1993; Nock *et al.* 2010), *Macquaria australasica* (Faulks *et al.* 2010a), *Macquaria ambigua* (Faulks *et al.* 2010b) and *Mogurnda adspersa* (Faulks *et al.* 2008). Comparable divergence times (0.1-1.5 Mya) have also been proposed between MDB and Lake Eyre Basin populations of *Nematalosa erebi* and *Retropinna semoni* (Hughes and Hillyer 2006). For those species listed above, nucleotide differences were in the order of 0-1.5% (0-7 nucleotides) between the 12S rRNA and up to 7.5% (26 nucleotides) for the mtDNA CR barcodes (Table 2). Most of the other species lack previous population divergence time estimates, but showed comparable 12S rRNA sequence differences (0-1.3%) between allopatric coastal and MDB populations. This supports the conclusion that most movements of fish across or around the Great Dividing Range occurred around the same time (during the Pleistocene, 0.1-1.8 Mya), facilitated by a range of possible factors (Unmack 2001). The species likely to have most recently moved between the MDB and coast is *Ambassis agassizii*, as there was an absence of sequence variation between populations. This raises the possibility of human assisted translocation, although this appears unlikely as widespread records exist for *A. agassizii* in the MDB from up to a century ago and abundance has since declined to the stage that inland populations are currently listed as endangered or extinct in some states (McNeil *et al.* 2008).

Cryptic speciation

Relatively large sequence differences and phylogenetic splits, indicative of older population separation times between the MDB and coastal catchments, were detected in three species, supporting genetic evidence from previous studies for undescribed species complexes linked to biogeographical regions. These complexes involve *Gadopsis marmoratus* (Jerry *et al.* 2001; Miller *et al.* 2004), *Retropinna semoni* (Hughes and Hillyer 2006; Hammer *et al.* 2007) and *Tandanus tandanus* (Jerry 2005; Jerry 2008; Rourke *et al.* 2010). The greatest intra-species sequence differences between the MDB and adjacent coastal catchments were observed in *Gadopsis marmoratus* (18S: 0.2%; 12S: 3.9%) and *Retropinna semoni* (18S: 0.2%; 12S: 5.1%). These differences are sufficiently large to strongly support species level separation and are comparable to those that occur between closely related species (18S: 0-1.3%; 12S: 0.3-12.1 %) (Table 2, Fig 2, 3) or even between genera such as *Salmo* and *Salvelinus-Oncorhynchus* (18S: 0.5%; 12S: 2.8-4.6%; 18.6 Mya) and *Salvelinus* and *Oncorhynchus* (18S: 0.6%; 12S: 3.1%; 12.3 Mya) (Osinov and Lebedev 2000; Wilson and Turner 2009).

A *Galaxias olidus* species complex (Raadik 2001) both within and outside the MDB is also supported by a high degree of polymorphism in the 12S rRNA sequences between populations, although the diversity is likely to have arisen through relatively recent radiation and isolation (Raadik *et al.* 1996). Specimens from each sub-catchment within the Murray-Darling were genetically distinct according to the 12S barcodes (0.5-1.3%), although the number of nucleotide substitutions are relatively few, even for populations that are recognised as morphologically distinct such as *G.* sp. ‘riffle’ and *G.* sp. ‘oliros’ (Raadik 2001). In this respect, the 12S rRNA sequence of *Galaxias fuscus,* the only other described species in the complex, differs to some *G. olidus* populations by as little as 0.8 %. *G. olidus* therefore appears particularly susceptible to genetic isolation due to its preference for upland habitats and, whilst abundant in some habitats, gene flow is likely to become further constrained by increasing habitat fragmentation and predation by introduced fish species (Lintermans 2000). At deeper phylogenetic levels, the 18S (Fig 2) and 12S (Fig 3) rRNA phylogenies conflict over the relationships between Osmeriformes and Salmoniformes, but the 18S tree supports polyphyly of Osmeriformes and the creation of a separate Order Galaxiiformes as proposed on the basis of complete mitochondrial genome sequences (Li *et al.* 2010).

Evolutionary relationships within *Nannoperca* appear the most complex due to incongruence between the two rRNA gene phylogenies. Specifically, *N. australis* is the sister taxon to *N. obscura* by 12S rRNA, but is more closely related to *N. variegata* using 18S rRNA. This finding is consistent with mtDNA capture between the sympatric species *N. australis* and *N. obscura*. Furthermore, sequence differences within *N. australis* (18S: 0.2%; 12S: 0.8%; mtDNA CR: 4.0%), support the existence of two allopatric species or subspecies, of which only one is present in the MDB (Kuiter 2008). Other widely distributed species such as *Craterocephalus stercusmuscarum, Philypnodon* species and *Nematalosa erebi* exhibit sufficient divergence between 12S rRNA sequences (0.8-1.3%) as well as other genetic and morphological markers across basins (McGlashan and Hughes 2001; Raadik 2001; Thacker *et al.* 2008) to warrant further examination for the presence of cryptic species.

Conclusions

Comprehensive sets of unique sequence barcodes such as those described in this study for the MDB fish fauna are proving to be of increasing value to researchers in many areas of ecology particularly for monitoring species distributions, breeding, recruitment, translocations and introductions (Ward *et al.* 2009; Page and Hughes 2010). These datasets also create opportunities to develop high throughput and accurate genetic identification techniques such as DNA microarrays (Hardy *et al.* 2010) and multiplex real-time PCR (Harper *et al.* 2005) to complement morphological examination in studies involving complex food webs, multiple predator-prey interactions and life stages or degraded material. In more practical terms, DNA barcodes can be used to determine population structures that need to be maintained or manipulated and provide improved certainty for conservation stocking and maintenance of broodstock. For example, routine DNA barcoding will assist fisheries and conservation managers to assess genetic structure and avoid issues such as reduction in effective population sizes from inbreeding depression or loss of genetic diversity due to isolation or stocking with progeny from too few individuals (Cook *et al.* 2007), as well as outbreeding depression (formation of sterile hybrids or gene introgression) from inappropriate stocking with genetically divergent strains (McDonald *et al.* 2008). Furthermore, fisheries management plans that propose to regulate genetic resources, set research priorities, define hatchery and stocking genetic protocols, provide rules for translocations and demographic and genetic rescues (Moore *et al.* 2010) can only be realistically implemented once suitable genetic markers are made available.

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References

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., and Lipman, D.J. (1990). Basic local alignment search tool. *Journal of Molecular Biology* **215**, 403-410.

Barrett, J., and Mallen-Cooper, M. (2006). The Murray River's 'Sea to Hume Dam' fish passage program: Progress to date and lessons learned. *Ecological Management and Restoration* **7**, 173-183.

Bertozzi, T., Adams, M., and Walker, K.F. (2000). Species boundaries in carp gudgeons (Eleotrididae: *Hypseleotris*) from the River Murray, South Australia: evidence for multiple species and extensive hybridization. *Marine and Freshwater Research* **51**, 805-815.

Brown, J.M., Hedtke, S.M., Lemmon, A.R., and Lemmon, E.M. (2010). When trees grow too long: Investigating the causes of highly inaccurate Bayesian branch-length estimates. *Systematic Biology* **59**, 145-161.

Cook, B.D., Bunn, S.E., and Hughes, J.M. (2007). Molecular genetic and stable isotope signatures reveal complementary patterns of population connectivity in the regionally vulnerable southern pygmy perch (*Nannoperca australis*). *Biological Conservation* **138**, 60-72.

Davies, P.E., Harris, J.H., Hillman, T.J., and Walker, K.F. (2010). The Sustainable Rivers Audit: Assessing river ecosystem health in the Murray–Darling Basin, Australia. *Marine and Freshwater Research* **61**, 764-777.

Douglas, J.W., Gooley, G.J., Ingram, B.A., Murray, N.D., and Brown, L.D. (1995). Natural hybridization between murray cod, *Maccullochella peelii peelii* (Mitchell), and trout cod, *Maccullochella macquariensis* (Cuvier) (Percichthyidae), in the Murray River, Australia. *Marine and Freshwater Research* **46**, 729-734.

Faulks, L.K., Gilligan, D.M., and Beheregaray, L.B. (2008). Phylogeography of a threatened freshwater fish (*Mogurnda adspersa*) in eastern Australia: conservation implications. *Marine and Freshwater Research* **59**, 89-96.

Faulks, L.K., Gilligan, D.M., and Beheregaray, L.B. (2010a). Evolution and maintenance of divergent lineages in an endangered freshwater fish, *Macquaria australasica*. *Conservation Genetics* **11**, 921-934.

Faulks, L.K., Gilligan, D.M., and Beheregaray, L.B. (2010b). Clarifying an ambiguous evolutionary history: range-wide phylogeography of an Australian freshwater fish, the golden perch (*Macquaria ambigua*). *Journal of Biogeography* **37**, 1329-1340.

Fuller, S., Baverstock, P., and King, D. (1998). Biogeographic origins of goannas (Varanidae): A molecular perspective. *Molecular Phylogenetics and Evolution* **9**, 294-307.

Gillespie, J.J. (2004). Characterizing regions of ambiguous alignment caused by the expansion and contraction of hairpin-stem loops in ribosomal RNA molecules. *Molecular Phylogenetics and Evolution* **33**, 936-943.

Gleeson, D.J., McCallum, H.I., and Owens, I.P.F. (2000). Differences in initial and acquired resistance to *Ichthyohthirius multifiliis* between populations of rainbowfish. *Journal of Fish Biology* **57**, 466-475.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95-98.

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.P., Adams, M., Unmack, P.J., and Walker, K.F. (2007). A rethink on *Retropinna*: conservation implications of new taxa and significant genetic sub-structure in Australian smelts (Pisces: Retropinnidae). *Marine and Freshwater Research* **58**, 327-341.

Hardy, C.M., Krull, E.S., Hartley, D.M., and Oliver, R.L. (2010). Carbon source accounting for fish using combined DNA and stable isotope analyses in a regulated lowland river weir pool. *Molecular Ecology* **19**, 197-212.

Harper, G.L., King, R.A., Dodd, C.S., Harwood, J.D., Glen, D.M.*, et al.* (2005). Rapid screening of invertebrate predators for multiple prey DNA targets. *Molecular Ecology* **14**, 819-827.

Higham, J., Hammer, M., and Geddes, M. (2002). Fish and invertebrates. In 'The Murray Mouth. Exploring the implications of closure or restricted flow. ' pp. 53-64. (Murray-Darling Basin Commission and Department of Land and Biodiversity Conservation: Canberra.)

Higham, J., Ye, Q., and Ferguson, G. (2005). Murray-Darling Basin drought monitoring. Monitoring small-bodied fish in the lower Murray during and after drought conditions in 2003-2004. RD04/0154. South Australian Research Development Institute Aquatic Sciences, Adelaide.

Huey, J.A., Hughes, J.M., and Baker, A.M. (2006). Patterns of gene flow in two species of eel-tailed catfish, *Neosilurus hyrtlii* and *Porochilus argenteus* (Siluriformes: Plotosidae), in western Queensland's dryland rivers. *Biological Journal of the Linnean Society* **87**, 457-467.

Hughes, J.M., and Hillyer, M.J. (2006). Mitochondrial DNA and allozymes reveal high dispersal abilities and historical movement across drainage boundaries in two species of freshwater fishes from inland rivers in Queensland, Australia. *Journal of Fish Biology* **68**, 270-291.

Jansen, G., Devaere, S., Weekers, P.H.H., and Adriaens, D. (2006). Phylogenetic relationships and divergence time estimate of African anguilliform catfish (Siluriformes: Clariidae) inferred from ribosomal gene and spacer sequences. *Molecular Phylogenetics and Evolution* **38**, 65-78.

Jerry, D.R., Raadik, T.A., Cairns, S.C., and Baverstock, P.R. (1999). Evidence for natural interspecific hybridization between the Australian bass (*Macquaria novemaculeata*) and Estuary perch (*M. colonorum*). *Marine and Freshwater Research* **50**, 661-666.

Jerry, D.R., Elphinstone, M.S., and Baverstock, P.R. (2001). Phylogenetic relationships of Australian members of the family Percichthyidae inferred from mitochondrial 12S rRNA sequence data. *Molecular Phylogenetics and Evolution* **18**, 335-347.

Jerry, D.R. (2005). Electrophoretic evidence for the presence of *Tandanus tandanus* (Pisces: Plotosidae) immediately north and south of the Hunter River, New South Wales. *Proceedings of the Linnean Society of New South Wales* **126**, 121-124.

Jerry, D.R. (2008). Phylogeography of the freshwater catfish *Tandanus tandanus* (Plotosidae): a model species to understand evolution of the eastern Australian freshwater fish fauna. *Marine and Freshwater Research* **59**, 351-360.

Katoh, T., and Toh, H. (2008). Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* **9**, 286-298.

Kjer, K.M. (1995). Use of ribosomal RNA secondary structure in phylogenetic studies to identify homologous positions: An example of alignment and data presentation from the frogs. *Molecular Phylogenetics and Evolution* **4**, 314-330.

Krieger, J., Hett, A.K., Fuerst, P.A., Birstein, V.J., and Ludwig, A. (2006). Unusual intraindividual variation of the nuclear 18S rRNA gene is widespread within the Acipenseridae. *Journal of Heredity* **97**, 218-225.

Kuiter, R.H. (2008). The southern pygmy perch. *Fishes of Sahul* **22**, 414-417.

Li, J., Xia, R., McDowall, R.M., Lopez, J.A., Lei, G., and Fu, C. (2010). Phylogenetic position of the enigmatic *Lepidogalaias salamandroides* with comment on the orders of lower euteleosean fishes. *Molecular and Phylogenetics and Evolution* **57**, 932-936.

Lintermans, M. (2000). Recolonization by the mountain galaxias *Galaxias olidus* of a montane stream after the eradication of rainbow trout *Oncorhynchus mykiss*. *Marine and Freshwater Research* **51**, 799-804.

Lintermans, M. (2007). 'Fishes of the Murray-Darling Basin: An Introductory Guide.' (Murray-Darling Basin Commission: Canberra).

McDonald, D.B., Parchman, T.L., Bower, M.R., Hubert, W.A., and Rahel, F.J. (2008). An introduced and a native vertebrate hybridize to form a genetic bridge to a second native species. *Proceedings of the National Academy of Sciences of the United States of America* **105**, 10837-10842.

McDowall, R.M. (2006). Crying wolf, crying foul, or crying shame: alien salmonids and a biodiversity crisis in the southern cool-temperate galaxioid fishes? *Reviews in Fish Biology and Fisheries* **16**, 233-422.

McGlashan, D.J., and Hughes, J.M. (2001). Genetic evidence for historical continuity between populations of the Australian freshwater fish *Craterocephalus stercusmuscarum* (Atherinidae) east and west of the Great Dividing Range. *Journal of Fish Biology* **59**, 55-67.

McNeil, D.G., Wilson, P.J., Hartwell, D., and Pellizzari, M. (2008). Olive perchlet (*Ambassis agassizii*) in the Lachlan River: Population status and sustainability in the Lake Brewster region: A report submitted to the Lachlan Catchment Management Authority. South Australian Research and Development Institute (Aquatic Sciences), No. F2008/000846-1, Adelaide.

Miller, A.D., Waggy, G., Ryan, S.G., and Austin, C.M. (2004). Mitochondrial 12S rRNA sequences support the existence of a third species of freshwater blackfish (Percicthyidae: *Gadopsis*) from south-eastern Australia. *Memoirs of Museum Victoria* **61**, 121-127.

Miller, M.A., Holder, M.T., Vos, R., Midford, P.E., Liebowitz, T., *et al.* (2009). 'The CIPRES Portals (CIPRES 2009-08-04).' Available at http://www.phylo.org/sub_sections/portal [Verified 4 March 2011].

Moore, A., Ingram, B.A., Friend, S., King Ho, H., Robinson, N., *et al.* (2010). 'Management of genetic resources for fish and crustaceans in the Murray-Darling Basin.' (Bureau of Rural Sciences: Canberra).

Moritz, C. (1994). Defining evolutionarily-significant-units for conservation. *Trends in Ecology and Evolution* **9**, 373-375.

Moritz, C., and Cicero, C. (2004). DNA barcoding: Promise and pitfalls. *PLoS Biology* **2**, 1529-1531.

Nock, C.J., Elphinstone, M.S., Rowland, S.J., and Baverstock, P.R. (2010). Phylogenetics and revised taxonomy of the Australian freshwater cod genus, *Maccullochella* (Percichthyidae). *Marine and Freshwater Research* **61**, 980-991.

Osinov, A.G., and Lebedev, V.S. (2000). Genetic divergence and phylogeny of the Salmoninae based on allozyme data. *Journal of Fish Biology* **57**, 354-381.

Ovenden, J.R., Lloyd, J., Newman, S.J., Keenan, C.P., and Slater, L.S. (2002). Spatial genetic subdivision between northern Australian and southeast Asian populations of *Pristipomoides multidens*: a tropical marine reef fish species. *Fisheries Research* **59**, 57-69.

Page, T.J., and Hughes, J.M. (2010). Comparing the performance of multiple mitochondrial genes in the analysis of Australian freshwater fishes. *Journal of Fish Biology* **77**, 2093–2122.

Raadik, T. (2001). When is a mountain galaxias not a mountain galaxias? *Fishes of Sahul* **15**, 785-789.

Raadik, T.A., Saddlier, S.R., and Koehn, J.D. (1996). Threatened fishes of the world: *Galaxias fuscus* Mack, 1936 (Galaxiidae). *Environmental Biology of Fishes* **47**, 108.

Ronquist, F., and Huelsenbeck, J.P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-1574.

Rourke, M., Teske, P., Attard, C., Gilligan, D., and Beheregaray, L. (2010). Isolation and characterisation of microsatellite loci in the Australian freshwater catfish (*Tandanus tandanus*). *Conservation Genetics Resources* **2**, 245-248.

Rowland, S.J. (1984). Hybridization between the estuarine fishes yellowfin bream, *Acanthopagrus australis* (Gunther), and black bream, *Acanthopagrus butcheri* (Munro) (Pisces, Sparidae). *Australian Journal of Marine and Freshwater Research* **35**, 427-440.

Rowland, S.J. (1993). *Maccullochella ikei*, an endangered species of freshwater cod (Pisces: Percichthyidae) from the Clarence River system, NSW and *M. peelii* *mariensis*, a new subspecies from the Mary River system, Qld. *Records of the Australian Museum* **45**, 121-145.

Stamatakis, A. (2006). RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688-2690.

Stamatakis, A., Hoover, P., and Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology* **57**, 758-771.

Swartz, E.R., Mwale, M., and Hanner, R. (2008). A role for barcoding in the study of African fish diversity and conservation. *South African Journal of Science* **104**, 293-298.

Tavaré, S. (1986). Some probabilistic and statistical problems in the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* **17**, 57–86.

Thacker, C.E., Unmack, P.J., Matsui, L., Duong, P., and Huang, E. (2008). Phylogeography of *Philypnodon* species (Teleostei: Eleotridae) across south-eastern Australia: testing patterns of connectivity across drainage divides and among coastal rivers. *Biological Journal of the Linnean Society* **95**, 175-192.

Unmack, P.J. (2001). Biogeography of Australian freshwater fishes. *Journal of Biogeography* **28**, 1053-1089.

Walker, K.F. (1985). A review of the ecological effects of river regulation in Australia. *Hydrobiologia* **125**, 111-129.

Wang, H.Y., Tsai, M.P., Dean, J., and Lee, S.C. (2001). Molecular phylogeny of gobioid fishes (Perciformes: Gobioidei) based on mitochondrial 12S rRNA sequences. *Molecular Phylogenetics and Evolution* **20**, 390-408.

Waples, R.S. (1991). Genetic interactions between hatchery and wild salmonids - Lessons from the Pacific-Northwest. *Canadian Journal of Fisheries and Aquatic Sciences* **48**, 124-133.

Ward, R.D., Hanner, R., and Hebert, P.D.N. (2009). The campaign to DNA barcode all fishes, FISH-BOL. *Journal of Fish Biology* **74**, 329-356.

Waters, J.M., Esa, Y.B., and Wallis, G.P. (2001). Genetic and morphological evidence for reproductive isolation between sympatric populations of *Galaxias* (Teleostei : Galaxiidae) in South Island, New Zealand. *Biological Journal of the Linnean Society* **73**, 287-298.

Wilson, W.D., and Turner, T.F. (2009). Phylogenetic analysis of the Pacific cutthroat trout (*Oncorhynchus clarki* ssp.: Salmonidae) based on partial mtDNA ND4 sequences: A closer look at the highly fragmented inland species. *Molecular Phylogenetics and Evolution* **52**, 406-415.

Winchell, C.J., Sullivan, J., Cameron, C.B., Swalla, B.J., and Mallatt, J. (2002). Evaluating hypotheses of deuterostome phylogeny and chordate evolution with new LSU and SSU ribosomal DNA data. *Molecular Biology and Evolution* **19**, 762-776.

Wong, C.M., Williams, C.E., Pittock, J., Collier, U., and Schelle, P. (2007). 'World’s top 10 rivers at risk.' (WWF International. Gland, Switzerland).

Wuyts, J., Perriere, G., and Van de Peer, Y. (2004). The European ribosomal RNA database. *Nucleic Acids Research* **32**, D101-D103.

Yang, Z. (1996). Among-site rate variation and its impact on phylogenetic analyses. *Trends in Ecology and Evolution* **11**, 367–372.

Figure Legends

Fig 1. Map of south-eastern Australia showing fish collection localities. Bioregions: Murray-Darling Basin (M); Coastal Queensland and NSW drainages (E); Lake Eyre and Bulloo Basins (C); Coastal Victoria and SE South Australia (S).

Fig 2. Bayesian majority-rule consensus tree arranged by Order for Murray-Darling Basin fish based on segments of 18S rRNA sequence that could be unambiguously aligned across all taxa. Species-specific sequence differences and phylogenetic information present in regions of ambiguous alignment are not represented. Branch support is indicated above branches. MLBP: Maximum Likelihood bootstrap proportion; BPP: Bayesian posterior probability. Branch labels contain acronyms for species scientific names followed by the number of individuals sampled and bioregion source: M, Murray-Darling Basin; E, Eastern; C, Central; S, Southern; W, Western; O, Overseas; U, Uncertain.

Fig 3. Bayesian majority-rule consensus tree arranged by Family for Murray-Darling Basin fish based on segments of 12S rRNA sequence that could be unambiguously aligned across all taxa. Species-specific sequence differences and phylogenetic information present in regions of ambiguous alignment are not represented. Branch support is indicated above branches. MLBP: Maximum Likelihood bootstrap proportion; BPP: Bayesian posterior probability. Branch labels contain acronyms for species scientific names followed by the number of individuals sampled and bioregion source: M, Murray-Darling Basin; E, Eastern; C, Central; S, Southern; W, Western; O, Overseas; U, Uncertain.

Table 1: Fish specimens used for DNA barcoding and phylogenetic comparisons

Scientific Name	Species Code	Common Name	Life cycle	Source [c]	Bioregion [d]	Accession Number		
						18S rRNA	12S rRNA	mtDNA CR
Native to MDB (45)								
Afurcagobius tamarensis	Afu tam	Tamar River Goby	Freshwater-Marine	Glenelg River SA	S	FJ710896	FJ710994	
				North Maroochy River QLD	E	HQ615525	HQ615461	
Ambassis agassizii [a]	Amb aga	Olive Perchlet	Freshwater	Lachlan River NSW	M	HQ615526	HQ615462	HQ615424
				Lachlan River NSW	M	HQ615527	HQ615463	
				Burnett River QLD	E	FJ710812	FJ710910	HQ615423
Anguilla australis	Ang aus	Short-finned Eel	Catadromous	Onkaparinga River SA	S	FJ710814	FJ710912	HQ615426
				Seafood Trade Eden NSW	E	FJ710813	FJ710911	HQ615425
Anguilla reinhardtii	Ang rei	Long-finned Eel	Catadromous	Seafood Trade Euroa VIC	E	FJ710815	FJ710913	
Atherinosoma microstoma	Ath mic	Small-mouthed Hardyhead	Anadromous	Mundoo Channel SA	M	FJ710900	FJ710998	
Bidyanus bidyanus [a]		Silver Perch	Freshwater	Hatchery-Narrandera NSW	M	FJ710817	FJ710915	
				Aquarium Trade ACT	M	FJ710818	FJ710916	HQ615427
Craterocephalus amniculus [a]	Cra amn	Darling River Hardyhead	Freshwater	Garrawilla Creek NSW	M	FJ710821	FJ710919	
Craterocephalus fluviatilis [a]	Cra flu	Murray Hardyhead	Freshwater	Cardross Lakes VIC	M	FJ710822	FJ710920	
Craterocephalus stercusmuscarum [a]	Cra ste	Fly-speckled Hardyhead	Freshwater	Lake Alexandrina SA	M	FJ710823	FJ710921	
				Mildura Weir VIC	M	FJ710824	FJ710922	
				Isis River QLD	E	HQ615532	HQ615468	
				Aquarium Trade ACT	E	FJ710825	FJ710923	
Gadopsis bispinosus [a]	Gad bis	Two-spined Blackfish	Freshwater	Cotter River ACT	M	FJ710828	FJ710926	
Gadopsis marmoratus [a]	Gad mam	River Blackfish	Freshwater	Marne River SA	M	FJ710829	FJ710927	
				Gwydir River NSW	M	FJ710831	FJ710929	
				LaTrobe River VIC	S	FJ710830	FJ710928	
Galaxias brevipinnis	Gal bre	Climbing Galaxias	Amphidromous	Victoria Creek SA	M	FJ710832	FJ710930	
Galaxias fuscus [a]	Gal fus	Barred Galaxias	Freshwater	Plain Creek VIC	M	FJ710833	FJ710931	
Galaxias maculatus	Gal mac	Common Galaxias	Catadromous	Myponga River SA	S	FJ710834	FJ710932	HQ615429
				Woronora River NSW	E	HQ615533	HQ615469	HQ615430
Galaxias olidus [a]	Gal oli	Mountain Galaxias	Freshwater	Severn River NSW	M	FJ710835	FJ710933	
				Lachlan River NSW	M	FJ710836	FJ710934	
				Nangkita Creek SA	M	FJ710837	FJ710935	
				Brindle Creek NSW	E	HQ615535	HQ615471	
				Shoalhaven River NSW	E	HQ615534	HQ615470	
Galaxias sp. ‘oliros’ [a]	Gal olr	Obscure Galaxias	Freshwater	King River VIC	M	FJ710838	FJ710936	
				Jews Harp Creek VIC	M	HQ615536	HQ615472	

Galaxias sp. ‘riffle’ [a]	Gal rif	Riffle Galaxias	Freshwater	King River VIC	M	FJ710839	FJ710937	
				Mitta Mitta River VIC	M	HQ615537	HQ615473	
Galaxias rostratus [a]	Gal ros	Flat-headed Galaxias	Freshwater	Goulburn River VIC	M	FJ710840	FJ710938	
Geotria australis	Geo aus	Pouched Lamprey	Anadromous	Coorong SA	M	FJ710844	FJ710942	
Hypseleotris klunzingeri [a]	Hyp klu	Western Carp Gudgeon	Freshwater	Murray River VIC	M	FJ710845	FJ710943	
				Dunn’s Swamp NSW	M	FJ710846	FJ710944	
				Lake Ginninderra ACT	M	FJ710847	FJ710945	
				Monkeybong Ck QLD	E	HQ615544	HQ615481	
				Shoalhaven River NSW	E	HQ615543	HQ615480	
				Barcoo River QLD	C	HQ615545	HQ615482	
Hypseleotris sp.1 ‘midgley’s’ [a]	Hyp mid	Midgley’s Carp Gudgeon	Freshwater	Murray River SA	M	FJ710848	FJ710946	HQ615431
				Warrego River NSW	M	FJ710849	FJ710947	
				Mildura Weir VIC	M	FJ710850	FJ710948	
				Kolan River QLD	E	HQ615552	HQ615488	HQ615433
				Barcoo River QLD	C	HQ615551	HQ615487	HQ615432
Hypseleotris sp.2 ‘lake’s’ [a]	Hyp lak	Lake’s Carp Gudgeon	Freshwater	Severn River QLD [e]	M	HQ615546 HQ615547	HQ615483	HQ682191
				Black Swamp VIC	M	HQ615548	HQ615484	HQ682192
				Paddy’s River NSW	E	HQ615549	HQ615485	HQ682193
				Barcoo River QLD	C	HQ615550	HQ615486	
Hypseleotris sp.3 ‘murray-darling’ [a]	Hyp mur	Murray-Darling Carp Gudgeon	Freshwater	Dawson Creek SA	M	FJ710851	FJ710949	
				Dunn’s Swamp NSW	M	FJ710852	FJ710950	
Leiopotherapon unicolor [a]	Lei uni	Spangled Perch	Freshwater	Caliguel Lagoon QLD	M	HQ615554	HQ615492	HQ615436
				Tenterfield Creek NSW	M	HQ615555	HQ615493	
				Coongie Lakes SA	C	FJ710853	FJ710951	HQ615434
				Aquarium Trade ACT	E	HQ615553	HQ615491	HQ615435
Maccullochella macquariensis [a]	Macc mac	Trout Cod	Freshwater	Hatchery-Narrandera NSW	M	FJ710854	FJ710952	
Maccullochella peelii peelii [a]	Macc ppe	Murray Cod	Freshwater	Hatchery-Narrandera NSW	M	FJ710855	FJ710953	HQ615439
Macquaria ambigua [a]	Mac amb	Murray-Darling Golden Perch	Freshwater	Hatchery-Narrandera NSW	M	FJ710856	FJ710954	
				Mildura Weir VIC	M	FJ710857	FJ710955	HQ615440
		Fitzroy-Dawson Golden Perch	Freshwater	Hatchery - Beenleigh QLD	E	HQ615559	HQ615497	HQ615441
		Lake Eyre Golden Perch	Freshwater	Barcoo River QLD	C	HQ615560	HQ615498	HQ615442
Macquaria australasica [a]	Mac aus	Macquarie Perch	Freshwater	Hatchery-Narrandera NSW	M	FJ710858	FJ710956	
				Cotter River ACT	M	HQ615561	HQ615499	HQ615443
				Little River (Nattai) NSW	E	HQ615562	HQ615500	HQ615444
				Little River (Nattai) NSW	E	HQ615563	HQ615501	
				Cordeaux Dam NSW	E	HQ615564	HQ615502	HQ615445

Macquaria colonorum	Mac col	Estuary Perch	Catadromous	Snowy River VIC	S	FJ710903	FJ711001	
				Arthur River TAS	S	FJ710904	FJ711002	
Melanotaenia fluviatilis[a]	Mel flu	Murray-Darling Rainbowfish	Freshwater	Lower Darling River NSW	M	FJ710859	FJ710957	
				Mildura Weir VIC	M	FJ710860	FJ710958	
				Aquarium Trade ACT	M	FJ710861	FJ710959	
Melanotaenia splendida tatei[a]	Mel spl	Desert Rainbowfish	Freshwater	Paroo River QLD	M	FJ710862	FJ710960	
				Coongie Lakes SA	C	HQ615567	HQ615505	
Mogurnda adspersa[a]	Mog ads	Southern Purple-spotted Gudgeon	Freshwater	Murray Bridge SA	M	FJ710864	FJ710962	HQ615446
				Pallal Creek NSW	M	FJ710865	FJ710963	HQ615447
				Sheep Station Creek QLD	E	HQ615569	HQ615507	HQ615448
Mordacia mordax	Mor mor	Short-headed Lamprey	Anadromous	Coorong SA	M	FJ710866	FJ710964	
Nannoperca australis[a]	Nan aus	Southern Pygmy Perch	Freshwater	Finniss River SA	M	FJ710867	FJ710965	HQ615449
				Tookayerta Creek SA	M	FJ710868	FJ710966	
				Macquarie River TAS	S	HQ615570	HQ615508	HQ615450
				Snowy River VIC	S	HQ615571	HQ615509	HQ615451
Nannoperca obscura[a]	Nan obs	Yarra Pygmy Perch	Freshwater	Finniss River SA	M	FJ710869	FJ710967	
				Waurn Ponds Ck VIC	S	HQ615572	HQ615510	
Nematalosa erebi[a]	Nem ere	Bony Bream	Freshwater	Lake Alexandrina SA	M	FJ710870	FJ710968	
				Mildura Weir VIC	M	FJ710871	FJ710969	
				Ross River QLD	E	HQ615574	HQ615512	
				Diamantina River QLD	C	HQ615575	HQ615513	
Neosilurus hyrtlii[a]	Neo hyr	Hyrtl’s Tandan	Freshwater	Warrego River QLD	M	FJ710872	FJ710970	HQ615452
				Return Creek QLD	E	HQ615576	HQ615514	HQ615453
				Cooper Creek QLD	C	HQ615577	HQ615515	HQ615454
Philypnodon grandiceps[a]	Phi gra	Flat-headed Gudgeon	Freshwater	Murray River SA	M	FJ710877	FJ710975	
				Woronora River NSW	E	HQ615579	HQ615517	
Philypnodon macrostomus[a]	Phi mac	Dwarf Flat-headed Gudgeon	Freshwater	Murray River SA	M	FJ710878	FJ710976	
				Tuross River NSW	E	HQ615580	HQ615518	
Porochilus rendahli[a]	Por ren	Rendahl’s Tandan	Freshwater	Balonne River QLD	M	HQ615581	HQ615519	HQ615457
				Stradbroke Island QLD	E	FJ710879	FJ710977	HQ615455
				Hunters Creek QLD	E	FJ710880	FJ710978	HQ615456
Pseudogobius olorum	Pse olo	Swan River Goby	Freshwater-Marine	Finniss River SA	M	FJ710907	FJ711005	
Pseudaphritis urvillii	Pse urv	Congoli	Catadromous	Mundoo Channel SA	M	FJ710881	FJ710979	
Retropinna semoni[a]	Ret sem	Australian Smelt	Freshwater	Murray River SA	M	FJ710882	FJ710980	
				Black Swamp VIC	M	FJ710883	FJ710981	
				Mildura Weir VIC	M	FJ710884	FJ710982	
				Paddy’s River NSW	E	HQ615582	HQ615520	

				Coongie Lakes SA	C	HQ615583	HQ615521	
Tandanus tandanus[a]	Tan tan	Tandan	Freshwater	Namoi River NSW	M	FJ710890	FJ710988	HQ615458
				Thane Creek QLD	M	FJ710891	FJ710989	HQ615459
				Stradbroke Island QLD	E	FJ710893	FJ710991	HQ615460
				Aquarium Trade ACT	U	FJ710892	FJ710990	
Tasmanogobius lasti	Tas las	Lagoon Goby	Freshwater-Marine	Lake Bonney SA	S	FJ710909	FJ711007	
Natives translocated to MDB (3)								
Galaxias truttaceus	Gal tru	Spotted Galaxias	Amphidromous	McIvor River VIC	M	FJ710841	FJ710939	
Macquaria novemaculeata[b]	Mac nov	Australian Bass	Catadromous	Aquarium Trade ACT	E	HQ615565	HQ615503	
Oxyeleotris lineolata[b]	Oxy lin	Sleepy Cod	Freshwater	Aquarium Trade ACT	E	HQ615578	HQ615516	
Exotics established in MDB (12)								
Carassius auratus[a]	Car aur	Goldfish	Freshwater	Murrumbidgee River NSW	M	FJ710819	FJ710917	
				Aquarium Trade ACT	U	FJ710820	FJ710918	
Carassius carassius[a]	Car car	Crucian Carp	Freshwater	Seafood Trade China	O	HQ615531	HQ615467	
Cyprinus carpio[a]	Cyp car	Carp	Freshwater	Torrens River SA	S	FJ710826	FJ710924	
				Lake Ginninderra ACT	M	FJ710827	FJ710925	
Gambusia holbrooki[a]	Gam hol	Eastern Gambusia	Freshwater	Murrumbidgee River NSW	M	FJ710842	FJ710940	
				Lake Ginninderra ACT	M	FJ710843	FJ710941	
Misgurnus anguillicaudatus[a]	Mis ang	Oriental Weatherloach	Freshwater	Murrumbidgee River ACT	M	FJ710863	FJ710961	
Oncorhynchus mykiss[a]	Onc myk	Rainbow Trout	Freshwater	Hatchery-Eucumbene NSW	E	FJ710874	FJ710972	
				Seafood Trade TAS	S	FJ710873	FJ710971	
Perca fluviatilis[a]	Per flu	Redfin Perch	Freshwater	Murray River SA	M	FJ710875	FJ710973	
				Lake Ginninderra ACT	M	FJ710876	FJ710974	
Rutilus rutilus[a]	Rut rut	Roach	Freshwater	Moorabool River VIC	S	FJ710885	FJ710983	
Salmo salar[a]	Sal sal	Atlantic Salmon	Freshwater	Seafood Trade TAS	S	FJ710886	FJ710984	
Salmo trutta[a]	Sal tru	Brown Trout	Freshwater	Gellibrand River VIC	S	FJ710887	FJ710985	
				Thredbo River NSW	E	FJ710888	FJ710986	
Salvelinus fontinalis[a]	Sal fon	Brook Charr	Freshwater	Hatchery-Eucumbene NSW	E	FJ710889	FJ710987	
Tinca tinca[a]	Tin tin	Tench	Freshwater	Campaspe River VIC	M	FJ710894	FJ710992	
Estuarine natives in MDB (11)								
Acanthopagrus butcheri	Aca but	Black Bream	Estuarine	Seafood Trade VIC	S	FJ710895	FJ710993	
Acentrogobius bifrenatus	Ace bif	Bridled Goby	Estuarine-Marine	Sydney Harbour NSW	E	FJ710899	FJ710997	
Aldrichetta forsteri	Ald for	Yelloweye Mullet	Catadromous	Seafood Trade NSW	E	FJ710897	FJ710995	
				Seafood Trade VIC	S	FJ710898	FJ710996	

Ammotretis rostratus	Amm ros	Long-nosed Flounder	Estuarine-Marine	Murray Mouth SA	M	HQ615528	HQ615464	
Argyrosomus hololepidotus	Arg hol	Mulloway	Estuarine-Marine	Seafood Trade WA	W	FJ710816	FJ710914	
Arripis truttaceous	Arr tru	Southern Australian Salmon	Estuarine-Marine	Murray Mouth SA	M	HQ615529	HQ615465	
Hyporhamphus regularis	Hyp reg	River Garfish	Estuarine-Marine	Seafood Trade NSW	E	FJ710901	FJ710999	
Rhombosolea tapirina	Rho tap	Greenback Flounder	Estuarine-Marine	Seafood Trade SA	M	FJ710908	FJ711006	
Hyperlophus vittatus	Hyp vit	Sandy Sprat	Estuarine-Marine	Seafood Trade NSW	E	HQ615538	HQ615476	
Liza argentea	Liz arg	Flat-tailed Mullet	Catadromous	Seafood Trade QLD	E	FJ710902	FJ711000	
				Seafood Trade NSW	E	HQ615556	HQ615494	
Mugil cephalus	Mug cep	Sea Mullet	Catadromous	Seafood Trade NSW	E	FJ710905	FJ711003	
				Seafood Trade WA	W	FJ710906	FJ711004	
Natives not in MDB (11)								
Bidyanus welchi	Bid wel	Welch's Grunter	Freshwater	Coongie Lakes SA	C	HQ615530	HQ615466	HQ615428
Hypseleotris compressa	Hyp com	Empire Gudgeon	Freshwater	Aquarium Trade ACT	E	HQ615539	HQ615477	
Hypseleotris galii	Hyp gal	Firetail Gudgeon	Freshwater	Aquarium Trade QLD	E	HQ615540	HQ615478	
				Georges River NSW [e]	E	HQ615541 HQ615542	HQ615479	
Melanotaenia splendida splendida	Mel spl	Eastern Rainbowfish	Freshwater	Branch Creek QLD	E	HQ615568	HQ615506	
Melanotaenia duboulayi	Mel dub	Crimson-spotted Rainbowfish	Freshwater	Bunya Creek QLD	E	HQ615566	HQ615504	
Nannoperca variegata	Nan var	Ewen's Pygmy Perch	Freshwater	Glenelg River VIC	S	HQ615573	HQ615511	
Maccullochella ikei	Macc ike	Eastern Freshwater Cod	Freshwater	Clarence River NSW	E	HQ615557	HQ615495	HQ615437
Maccullochella peelii mariensis	Macc pma	Mary River Cod	Freshwater	Mary River QLD	E	HQ615558	HQ615496	HQ615438
Retropinna tasmanica	Ret tas	Tasmanian Smelt	Anadromous	Mersey River TAS	S	HQ615584	HQ615522	
Tandanus sp.1	Tan sp1	Bellinger Tandan	Freshwater	Bellinger River NSW	E	HQ615585	HQ615523	
Tandanus sp.2	Tan sp2	Northern Tandan	Freshwater	Mulgrave River QLD	E	HQ615586	HQ615524	
***Perca fluviatilis* gut contents**								
Gambusia holbrooki Gut 1		Eastern Gambusia	Freshwater	Blakney Creek NSW	M		HQ615474	
Gambusia holbrooki Gut 2		Eastern Gambusia	Freshwater	Blakney Creek NSW	M		HQ615475	
Hypseleotris sp.1 'midgley's' Gut 1		Midgley's Carp Gudgeon	Freshwater	Blakney Creek NSW	M		HQ615489	
Hypseleotris sp.1 'midgley's' Gut 2		Midgley's Carp Gudgeon	Freshwater	Blakney Creek NSW	M		HQ615490	

[a] Murray-Darling Basin (MDB) species (n=45, 33 native) entirely restricted to freshwater.

[b] Records exist, but not established in the MDB.

[c] ACT, Australian Capital Territory; NSW, New South Wales; QLD, Queensland; SA, South Australia; VIC, Victoria; WA, Western Australia.

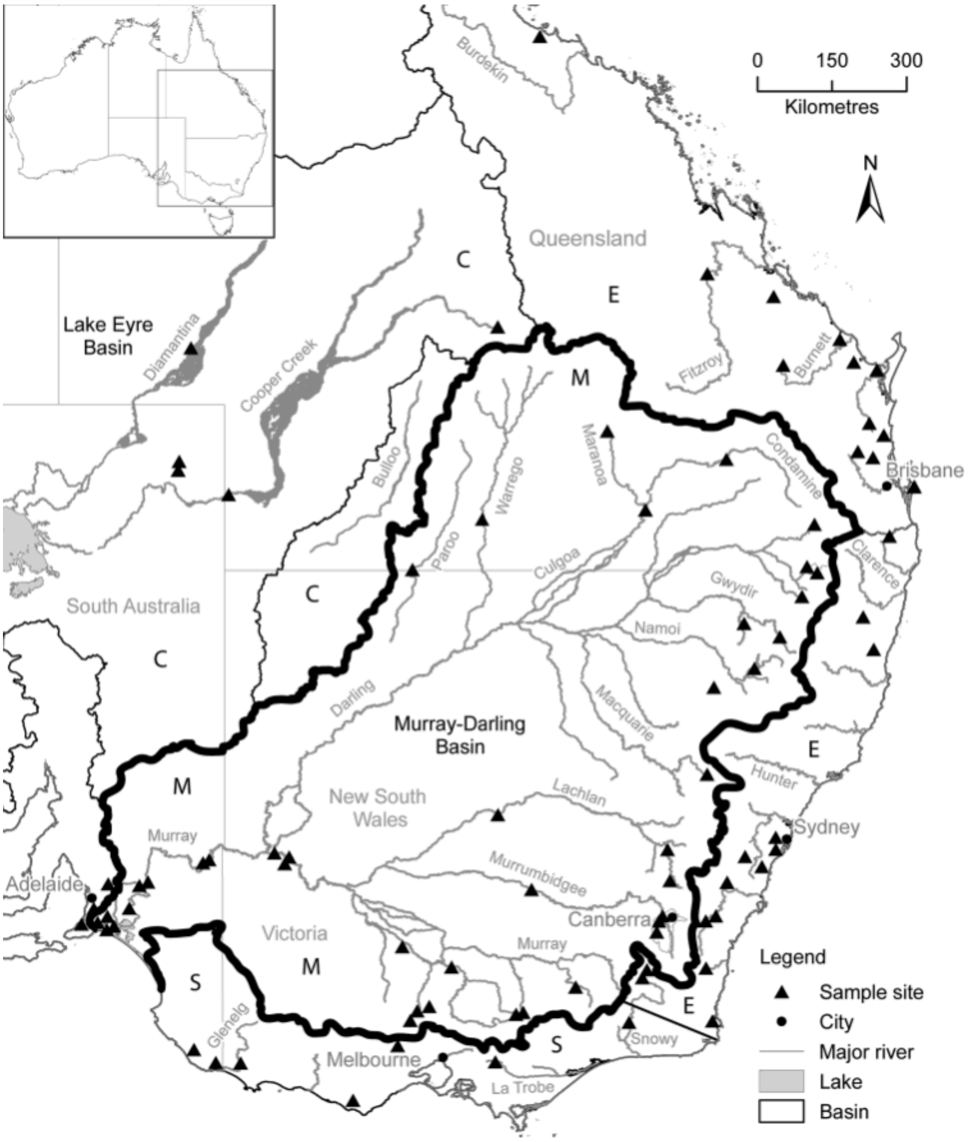
[d] Bioregions: M, MDB; E, Eastern; C, Central; S, Southern; W, Western; O, Overseas; U, Uncertain.

[e] *Hypseleotris sp.* individuals possessing two different 18S rRNA sequences.

Table 2: Sequence differences between fish from the Murray-Darling Basin and the same or related species in other river basins

		Number of nucleotide differences		
	Taxonomic Level	18S rRNA (~1.8 kb)	12S rRNA (~0.4 kb)	mtDNA CR (~0.4 kb)
	Intra-Species			
1	*Ambassis agassizi*	0	0	0
2	*Anguilla australis*	0	0	18
3	*Craterocephalus stercusmuscarum*	0	4-5	
4	*Gadopsis marmoratus*	4	15	
5	*Galaxias olidus*	0	6-7	
6	*Galaxias maculatus*	0	0	1
7	*Hypseleotris klunzingeri*	0	1-4	
8	*Hypseleotris* sp.1 'midgley's'	0	0-4	2
9	*Hypseleotris* sp.2 'lake's'	0-2[a]	0-15[a]	1-2
10	*Leiopotherapon unicolor*	0	0-1	2-17
11	*Macquaria ambigua*	0	0-1	7-22
12	*Macquaria australasica*	0	0-1	9
13	*Melanotaenia splendida*	0	1	
14	*Mogurnda adspersa*	0	1-2	6
15	*Maccullochella peelii*	1	1	26
16	*Nannoperca australis*	0-4	0-3	3-15
17	*Nannoperca obscura*	0	2	
18	*Nematalosa erebi*	0	3-7	
19	*Neosilurus hyrtlii*	0	0-1	2
20	*Philypnodon grandiceps*	0	4	
21	*Philypnodon macrostomus*	1	3	
22	*Porochilus rendahli*	0	0-4	2-9
23	*Retropinna semoni*	1-4	2-20	
24	*Tandanus tandanus*	0	0	6
	Intra-Genus			
A	*Bidyanus* (2 sp.)	0	0	27
B	*Craterocephalus* (3 sp.)	3-11	3 -16	
C	*Gadopsis complex* (2-3 sp.)	3-5	7-15	
D	*Galaxias* (6-8 sp.)	0-14	1-49	
E	*Galaxias olidus complex* (2-4 sp.)	0	1-9	
F	*Hypseleotris* (6 sp.)	0-2[a]	0[a] -16	
G	*Melanotaenia* (3 sp.)	0	3-5	
H	*Maccullochella* (3 sp.)	0-1	0-26	>6
I	*Macquaria* (4-6 sp.)	0-3	0-47	>6
J	*Melanotaenia* (3 sp.)	0	3-5	
K	*Nannoperca complex* (3-4 sp.)	4-25	2-11	
L	*Philypnodon* (2 sp.)	6-7	23-27	
M	*Retropinna complex* (2-3 sp.)	1-5	4 -22	
N	*Tandanus complex* (2-3 sp.)	0-2	10-24	

[a] Value due to hybrid *Hypseleotris* sp.2 'lake's' individuals. A range of values reflects sub-catchment sequence differences observed in some species.


0
150
300
Kilometres
N
Burdekin
Queensland
C
E
Lake Eyre
Basin
Diamantina
Cooper Creek
Fitzroy
Burnett
M
Maranoa
Condamine
Brisbane
Bulloo
Warrego
Paroo
Culgoa
Clarence
Gwydir
Namoi
South Australia
C
C
Darling
Macquarie
Murray-Darling
Basin
E
Hunter
M
New South
Wales
Lachlan
Sydney
Murray
Murrumbidgee
Adelaide
Canberra
Victoria
M
Murray
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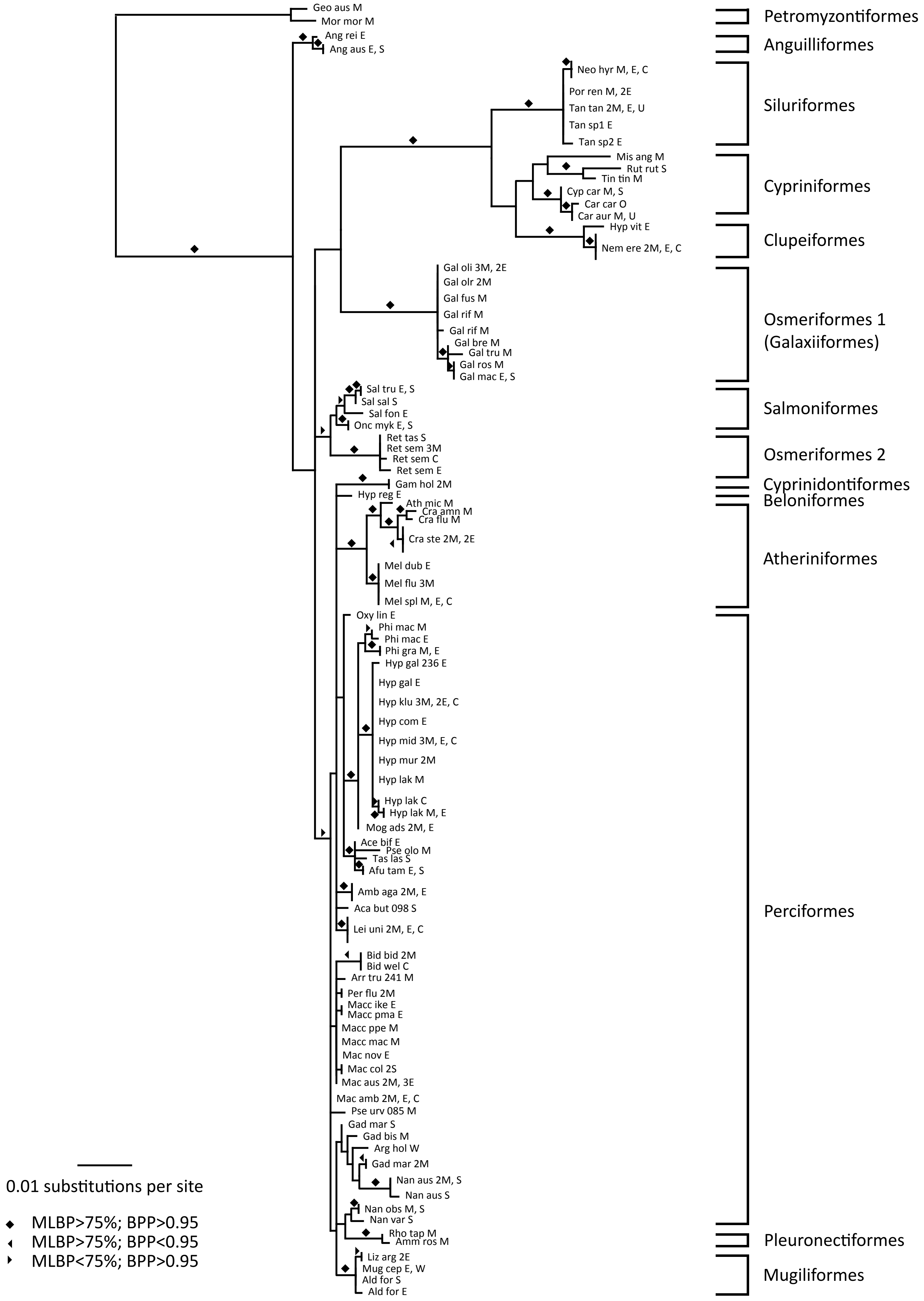
▲ Sample site
● City
Major river
Lake
Basin
S
Glenelg
E
S
Snowy
Melbourne
La Trobe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Ang rei E
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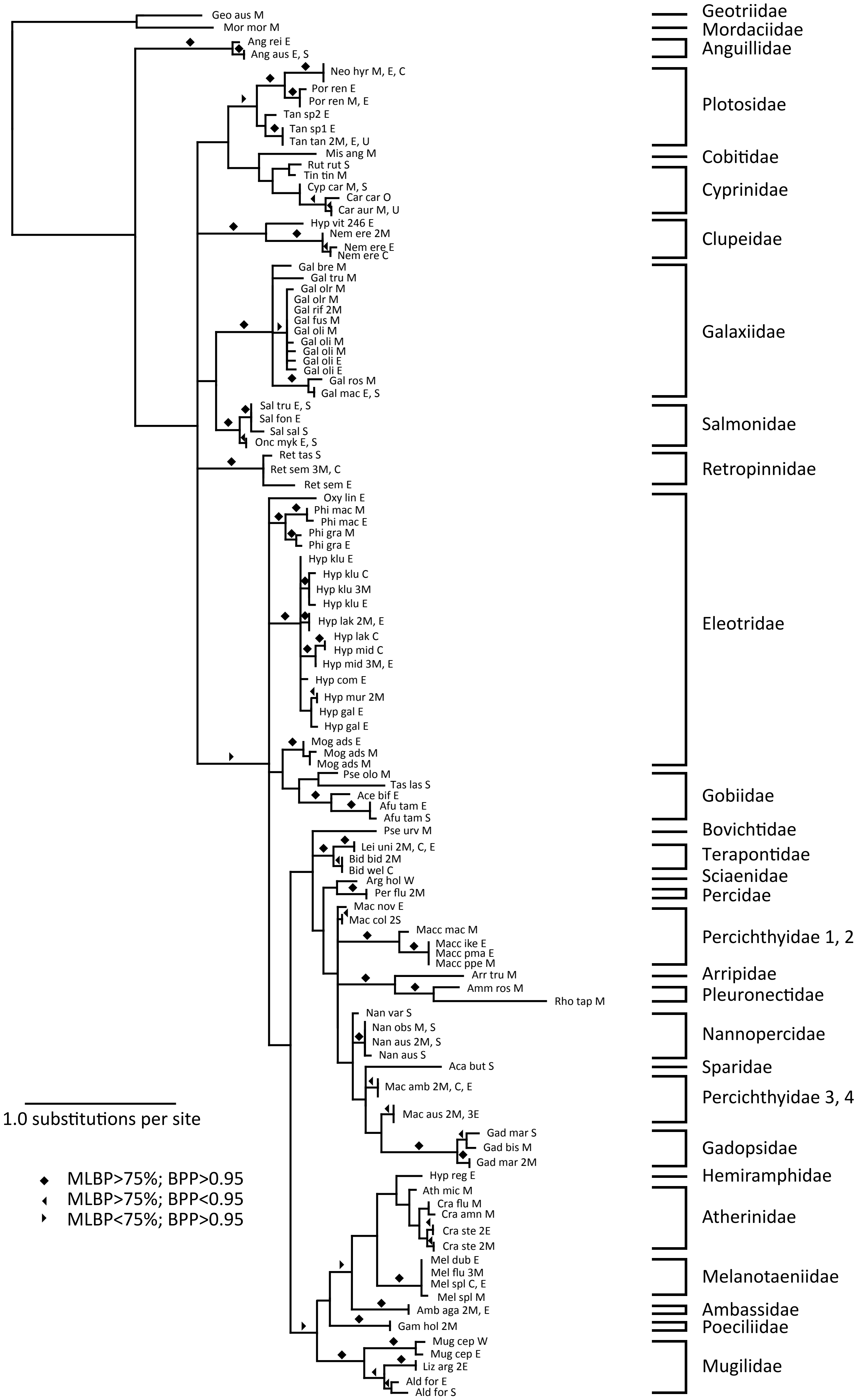
Neo hyr M, E, C
Por ren M, 2E
Tan tan 2M, E, U
Tan sp1 E
Tan sp2 E
Mis ang M
Rut rut S
Tin tin M
Cyp car M, S
Car car O
Car aur M, U
Hyp vit E
Nem ere 2M, E, C
Gal oli 3M, 2E
Gal olr 2M
Gal fus M
Gal rif M
Gal rif M
Gal bre M
Gal tru M
Gal ros M
Gal mac E, S
Sal tru E, S
Sal sal S
Sal fon E
Onc myk E, S
Ret tas S
Ret sem 3M
Ret sem C
Ret sem E
Gam hol 2M
Hyp reg E
Ath mic M
Cra amn M
Cra flu M
Cra ste 2M, 2E
Mel dub E
Mel flu 3M
Mel spl M, E, C
Oxy lin E
Phi mac M
Phi mac E
Phi gra M, E
Hyp gal 236 E
Hyp gal E
Hyp klu 3M, 2E, C
Hyp com E
Hyp mid 3M, E, C
Hyp mur 2M
Hyp lak M
Hyp lak C
Hyp lak M, E
Mog ads 2M, E
Ace bif E
Pse olo M
Tas las S
Afu tam E, S
Amb aga 2M, E
Aca but 098 S
Lei uni 2M, E, C
Bid bid 2M
Bid wel C
Arr tru 241 M
Per flu 2M
Macc ike E
Macc pma E
Macc ppe M
Macc mac M
Mac nov E
Mac col 2S
Mac aus 2M, 3E
Mac amb 2M, E, C
Pse urv 085 M
Gad mar S
Gad bis M
Arg hol W
Gad mar 2M
Nan aus 2M, S
Nan aus S
Nan obs M, S
Nan var S
Rho tap M
Amm ros M
Liz arg 2E
Mug cep E, W
Ald for S
Ald for E
Petromyzontiformes
Anguilliformes
Siluriformes
Cypriniformes
Clupeiformes
Osmeriformes 1
(Galaxiiformes)
Salmoniformes
Osmeriformes 2
Cyprinidontiformes
Beloniformes
Atheriniformes
Perciformes
Pleuronectiformes
Mugiliformes
0.01 substitutions per site
◆ MLBP>75%; BPP>0.95
◂ MLBP>75%; BPP<0.95
▸ MLBP<75%; BPP>0.95


Geo aus M
Mor mor M
Ang rei E
Ang aus E, S
Neo hyr M, E, C
Por ren E
Por ren M, E
Tan sp2 E
Tan sp1 E
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Mis ang M
Rut rut S
Tin tin M
Cyp car M, S
Car car O
Car aur M, U
Hyp vit 246 E
Nem ere 2M
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Gal bre M
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Gal oli E
Gal ros M
Gal mac E, S
Sal tru E, S
Sal fon E
Sal sal S
Onc myk E, S
Ret tas S
Ret sem 3M, C
Ret sem E
Oxy lin E
Phi mac M
Phi mac E
Phi gra M
Phi gra E
Hyp klu E
Hyp klu C
Hyp klu 3M
Hyp klu E
Hyp lak 2M, E
Hyp lak C
Hyp mid C
Hyp mid 3M, E
Hyp com E
Hyp mur 2M
Hyp gal E
Hyp gal E
Mog ads E
Mog ads M
Mog ads M
Pse olo M
Tas las S
Ace bif E
Afu tam E
Afu tam S
Pse urv M
Lei uni 2M, C, E
Bid bid 2M
Bid wel C
Arg hol W
Per flu 2M
Mac nov E
Mac col 2S
Macc mac M
Macc ike E
Macc pma E
Macc ppe M
Arr tru M
Amm ros M
Rho tap M
Nan var S
Nan obs M, S
Nan aus 2M, S
Nan aus S
Aca but S
Mac amb 2M, C, E
Mac aus 2M, 3E
Gad mar S
Gad bis M
Gad mar 2M
Hyp reg E
Ath mic M
Cra flu M
Cra amn M
Cra ste 2E
Cra ste 2M
Mel dub E
Mel flu 3M
Mel spl C, E
Mel spl M
Amb aga 2M, E
Gam hol 2M
Mug cep W
Mug cep E
Liz arg 2E
Ald for E
Ald for S
Geotriidae
Mordaciidae
Anguillidae
Plotosidae
Cobitidae
Cyprinidae
Clupeidae
Galaxiidae
Salmonidae
Retropinnidae
Eleotridae
Gobiidae
Bovichtidae
Terapontidae
Sciaenidae
Percidae
Percichthyidae 1, 2
Arripidae
Pleuronectidae
Nannopercidae
Sparidae
Percichthyidae 3, 4
Gadopsidae
Hemiramphidae
Atherinidae
Melanotaeniidae
Ambassidae
Poeciliidae
Mugilidae
1.0 substitutions per site
◆ MLBP>75%; BPP>0.95
◂ MLBP>75%; BPP<0.95
▸ MLBP<75%; BPP>0.95