Towards an ecoregion scale evaluation of eDNA metabarcoding primers: a case-study for the freshwater fish biodiversity of the Murray-Darling Basin (Australia)

Jonas Bylemans¹,²*, Dianne M. Gleeson¹,², Christopher M. Hardy³,², Elise Furlan¹,²

¹ Institute for Applied Ecology, University of Canberra, Canberra, ACT, Australia
² Invasive Animals Cooperative Research Centre, University of Canberra, Canberra, ACT 2617, Australia
³ CSIRO Land and Water, GPO Box 1700, Canberra, ACT, Australia

* Corresponding author: Jonas.Bylemans@canberra.edu.au
Contents

Amplification and sequencing of the 12S ribosomal RNA gene for all freshwater fish species of the Murray-Darling Basin (MDB). .................................................................................................................. 4
List of vertebrate families with occurrence records in the MDB. .............................................. 10
Sequence length distribution of all sequence reads assigned to their respective samples. ...... 14
Best fitting linear regression model for the eDNA metabarcoding data obtained from the artificial community sample. .................................................................................................................. 15
Summary of the metabarcoding data obtained from environmental DNA samples collected from two sites in the MDB. ................................................................................................................................. 16
References ........................................................................................................................................ 18

Tables

Table S1. Complete list of all freshwater fish species the Murray-Darling Basin (MDB) and the details of all the samples used for the PCR amplification and Sanger sequencing of the entire mitochondrial 12S ribosomal RNA gene (NCBI accession codes: KY798443-KY798504). .................................................................................................................................................. 6
Table S2. List of all major vertebrate families with occurrence records in the Darling River Drainage (Atlas of Living Australia). ........................................................................................................... 10
Table S3. Summary of the metabarcoding data obtained from environmental DNA samples collected for two sites within the Murray-Darling Basin (i.e. 8 and 12 samples collected for the Blakney Creek and Murrumbidgee River sites respectively) and analysed with the MiFish-U, Teleo and AcMDB07 primer pairs. Results are given as the number of samples testing positive for the different species and the average proportion of sequence reads ± the standard deviation given in between brackets. ................................................................. 16

Figures

Figure S1. The number of internally amplified barcodes for each primer pair plotted against the length of the internal barcode sequences. The data are derived from all sequence records that were successfully assigned to their respective samples and the vertical dashed lines represent the sequence length threshold used to remove short sequence records for each primer pair. ................................................................. 14

Figure S2. The best fitting model describing the relationship between the proportional read abundances and the PrimerMiner penalty scores for the artificial community. ....................... 15
Amplification and sequencing of the 12S ribosomal RNA gene for all freshwater fish species of the Murray-Darling Basin (MDB).

A genetic database for all freshwater Actinopterygii species with established populations in the MDB was obtained using a PCR amplification of the complete mitochondrial 12S ribosomal RNA gene followed by Sanger sequencing. For all species, either extracted DNA or tissue samples were obtained from previous studies (Hardy et al., 2011; MacDonald, Young, Lintermans, & Sarre, 2014) (Table S1). When only tissues samples were available, genomic DNA was extracted using the DNeasy Blood and Tissue Kit following the manufacturer instructions (Qiagen, Hilden, Germany).

For most samples, successful amplification of the entire 12S gene was achieved using primer combinations 12SR and 12SL or Marinefish-12SrRNA-F and Marinefish-12SrRNA-R (Jin, Zhao, & Wang, 2013; Wang, Tsai, Tu, & Lee, 2000). PCR reactions contained 12.50 µL MyTaq™ HS Red Mix (Bioline Australia Pty Ltd, NSW, Australia), 0.25-1.00 µL of each primer (10µM), 1.00-4.00 µL genomic DNA and DEPC-treated water to a final volume of 25 µL. Cycling conditions consisted of an initial activation of 2 min at 95°C; 35 3-step cycles of 1 min at 94°C, 1 min at 50°C and 1 min 30 sec at 72°C; and a final extension of 10 min at 72°C. For three species (i.e. Galaxias ornatus, Maccullochella peeli and Pseudaphritis urvillii) modifications to the PCR protocol were needed. For G. ornatus and M. peeli the 12SV5 primers described by Riaz et al. (Riaz et al., 2011) were used as internal PCR primers in combination with Marinefish-12S-F and 12SR. Additionally, a touchdown cycling stage (i.e. 10 3-step cycles of 1 min at 94°C, 1 min at 60°C and 1 min 30 sec at 72°C with annealing temperatures decreasing with 1°C per cycle) was added after the initial activation step to increase specificity and yield. Successful amplification of the 12S gene of P. urvillii required
newly developed primers Not-12S-F (5’-TATTTAAAACGTAACTGAAAATG-3’) and Not-12S-R (5’-TCATGATGCAAAAGGTACGAG-3’) as previously used primers contained significant base-pair mismatches with sequence records of other species within the suborder Notothenioidei.

The presence of a single PCR product was confirmed through gel electrophoresis using a 2% agarose gel containing SYBR® Safe DNA gel stain and a run time of 60 min at 90 volts. Amplicons were purified using the MinElute PCR Purification Kit (Qiagen, Hilden, Germany) and Sanger sequenced using an AB 3730xl DNA Analyzer at the ACRF Biomolecular Resource Facility (The John Curtin School of Medical Research, Australian National University). PCR primers were used for sequencing and an internal sequencing primer (MT1478H) was used to improve sequencing quality of the 5’ region of the 12S gene for most samples (excluding *G. ornatus* and *M. peelii*) (Fuller, Baverstock, & King, 1998). Sequences were imported into Geneious v8.1.8 and assembled into contigs using the “DeNovo Assembly” option (Kearse et al., 2012). Assemblies were manually checked for quality and a consensus sequence was obtained containing a partial sequence of the Phenylalanyl-tRNA gene, the whole 12S ribosomal RNA gene and Valine-tRNA gene, and a partial sequence of the 16S ribosomal RNA gene (NCBI accession codes: KY798443-KY798504).
| Species Name                        | Origin  | Isolate | Source                                                                 |
|------------------------------------|---------|---------|------------------------------------------------------------------------|
| *Afurcagobius tamarensis*          | Native  | CES-088 | Donovans Landing (SA); Hardy et al. (2011)                            |
| *Ambassis agassizii*               | Native  | CES-224 | Brewster Outlet Channel (NSW); Hardy et al. (2011)                    |
| *Anguilla australis*               | Native  | CES-080 | Onkaparinga River (SA); Hardy et al. (2011)                           |
| *Anguilla reinhardtii*             | Native  | CES-064 | Seafood Trade (VIC); Hardy et al. (2011)                              |
| *Atherinosoma microstoma*          | Native  | CES-084 | Mundoo Channel (SA); Hardy et al. (2011)                              |
| *Bidyanus bidyanus*                | Native  | CES-043 | Narrandera Fisheries Centre (NSW); Hardy et al. (2011)                |
| *Carassius auratus*                | Invasive| CES-025 | Narrandera Fisheries Centre (NSW); Hardy et al. (2011)                |
| *Carassius carassius*              | Invasive| TR-1709 | Campaspe River (VIC); Raadik T.A.                                    |
| *Craterocephalus amniculus*        | Native  | CES-675 | Gwydir River (NSW); Hardy et al. (2011)                               |
| *Craterocephalus fluviatilis*      | Native  | CES-007 | Cardross Lakes (VIC); Hardy et al. (2011)                             |
| *Craterocephalus stercusmuscarum fulvus* | Native | CSF9934.1 | Murray River (NSW); Unmack P.J.                                    |
| *Cyprinus carpio*                  | Invasive| CES-005 | Lower Torrens River (SA); Hardy et al. (2011)                        |
| *Gadopsis bispinosus*              | Native  | CES-009 | Cotter River (ACT); Hardy et al. (2011)                              |
| *Gadopsis marmoratus*              | Native  | CES-016 | LaTrobe River (VIC); Hardy et al. (2011)                              |
| Species                  | Status | Catalogue Number | Location                  | Reference                          |
|--------------------------|--------|------------------|---------------------------|------------------------------------|
| **Galaxias arcanus**     | Native | CES-021          | King River (VIC); Hardy et al. (2011) |
| **Galaxias brevipinnis** | Native | CES-010          | Victoria Creek (SA); Hardy et al. (2011) |
| **Galaxias fuscus**      | Native | CES-022          | Plain Creek (VIC); Hardy et al. (2011) |
| **Galaxias maculatus**   | Native | CES-087          | Lower Myponga River (SA); Hardy et al. (2011) |
| **Galaxias olidus**      | Native | CES-019          | Lachlan River (NSW); Hardy et al. (2011) |
| **Galaxias oleriros**    | Native | CES-023          | King River (VIC); Hardy et al. (2011) |
| **Galaxias ornatus**     | Native | TR-4399          | Hirts Creek (VIC); Raadik T.A. |
| **Galaxias rostratus**   | Native | CES-024          | Goulburn River (VIC); Hardy et al. (2011) |
| **Galaxias tantangara**  | Native | TR-4382          | Tantangara Creek (NSW); Raadik T.A. |
| **Galaxias truttaceus**  | Native | CES-079          | McIvor River (VIC); Hardy et al. (2011) |
| **Gambusia holbrooki**   | Native | CES-026          | Narrandera Fisheries Centre (NSW); Hardy et al. (2011) |
| **Geotria australis**    | Native | CES-082          | Goolwa Barrage (SA); Hardy et al. (2011) |
| **Hypseleotris kluinzeri** | Native | CES-003          | Murray River (VIC); Hardy et al. (2011) |
| **Hypseleotris sp.1 “midgley’s”** | Native | CES-030          | Calperum (SA); Hardy et al. (2011) |
| **Hypseleotris sp.2 “Lake”** | Native | HLak3            | Black Swamp (VIC); Unmack P.J. |
| **Hypseleotris sp.3 “murray-darling”** | Native | CES-034          | Dunns Swamp (NSW); Hardy et al. (2011) |
| **Leiopotherapon unicolor** | Native | CES-264          | Caliguel Lagoon (QLD); Hardy et al. (2011) |
| Scientific Name                        | Status     | Code    | Location Details                                      | Reference               |
|---------------------------------------|------------|---------|-------------------------------------------------------|-------------------------|
| *Maccullochella macquariensis*        | Native     | UC0524  | Bendora Reservoir (ACT); MacDonald et al. (2014)      |                         |
| *Maccullochella peelii*               | Native     | MP-#28  | Murrumbidgee River (ACT); Couch A.J.                  |                         |
| *Macquaria ambiguam ambiguag*         | Native     | GPMB.1  | Murray River (SA); Unmack P.J.                       |                         |
| *Macquaria australasica*              | Native     | CES-208 | Cotter River (ACT); Hardy et al. (2011)               |                         |
| *Melanotaenia fluviatilis*            | Native     | CES-028 | Murray/Darling confluence (NSW); Hardy et al. (2011) |                         |
| *Melanotaenia splendida tatei*        | Native     | CES-029 | Paroo River (QLD); Hardy et al. (2011)                |                         |
| *Misgurnus anguillicaudatus*          | Invasive   | CES-074 | Murrumbidgee River (ACT); Hardy et al. (2011)         |                         |
| *Mogurnda adspersa*                   | Native     | CES-006 | Murray Bridge (SA); Hardy et al. (2011)               |                         |
| *Mordacia mordax*                     | Native     | CES-083 | Goolwa Barrage (SA); Hardy et al. (2011)              |                         |
| *Nannoperca australis*                | Native     | CES-012 | Finniss River (SA); Hardy et al. (2011)               |                         |
| *Nannoperca obscura*                  | Native     | CES-011 | Finniss River (SA); Hardy et al. (2011)               |                         |
| *Nematalosa erebi*                    | Native     | CES-002 | Lake Alexandrina (SA); Hardy et al. (2011)            |                         |
| *Neosilurus hyrtlii*                  | Native     | CES-001 | Warrego River (QLD); Hardy et al. (2011)              |                         |
| *Oncorhynchus mykiss*                 | Invasive   | CES-077 | Eucumbene Trout Farm (NSW); Hardy et al. (2011)       |                         |
| *Oxyeleotris lineolata*               | Native     | CES-232 | Aquarium Trade (ACT); Hardy et al. (2011)             |                         |
| *Perca fluviatilis*                   | Invasive   | CES-004 | Murray River (SA); Hardy et al. (2011)                |                         |
| *Percalates colonorum*                | Native     | CES-040 | Snowy River (VIC); Hardy et al. (2011)                |                         |
| Species                      | Status  | CES   | Location                                      | Source                |
|------------------------------|---------|-------|-----------------------------------------------|-----------------------|
| *Percalates novemaculeata*   | Native  | CES-231 | Aquarium Trade (ACT); Hardy et al. (2011)     |                       |
| *Philypnodon gandiceps*      | Native  | CES-014 | Martins Bend Wetland (SA); Hardy et al. (2011)|                       |
| *Philypnodon macrostomus*    | Native  | CES-013 | Martins Bend Wetland (SA); Hardy et al. (2011)|                       |
| *Porochilus rendahli*        | Native  | CES-170 | Beardmore Dam (QLD); Hardy et al. (2011)      |                       |
| *Pseudaphritis urvillii*     | Native  | CES-085 | Mundoo Channel (SA); Hardy et al. (2011)      |                       |
| *Pseudogobius olorum*        | Native  | CES-081 | Finniss River (SA); Hardy et al. (2011)       |                       |
| *Retropinna semoni*          | Native  | CES-035 | Martins Bend Wetland (SA); Hardy et al. (2011)|                       |
| *Rutilus rutilus*            | Invasive| CES-075 | Moorabool River (VIC); Hardy et al. (2011)    |                       |
| *Salmo salar*                | Invasive| CES-070 | Seafood Trade (ACT); Hardy et al. (2011)      |                       |
| *Salmo trutta*               | Invasive| CES-069 | Gellibrand River (VIC); Hardy et al. (2011)   |                       |
| *Salvelinus fontinalis*      | Invasive| CES-071 | Eucumbene Trout Farm (NSW); Hardy et al. (2011)|                       |
| *Tandanus tandanus*          | Native  | CES-050 | Namoi River (NSW); Hardy et al. (2011)        |                       |
| *Tasmanogobius lasti*        | Native  | CES-086 | Lake Bonney (SA); Hardy et al. (2011)         |                       |
| *Tinca tinca*                | Invasive| CES-076 | Campaspe River (VIC); Hardy et al. (2011)     |                       |
List of vertebrate families with occurrence records in the MDB.

Table S2. List of all major vertebrate families with occurrence records in the Darling River Drainage (Atlas of Living Australia).

| Class            | Order         | Family                          |
|------------------|---------------|---------------------------------|
| Actinopterygii   | Anguilliformes| Anguillidae                      |
|                  | Clupeiformes  | Clupeidae                       |
|                  | Galaxiiformes | Galaxiidae                      |
|                  | Osmeriformes  | Retropinnidae                   |
|                  | Siluriformes  | Plotosida                       |
|                  | Atheriniforms | Atherinidae; Melanotaeniida     |
|                  | Perciformes   | Ambassidae; Bovichtidae; Percidae|
|                  | Centrarchiformes | Percichthyidae; Terapontida   |
|                  | Gobiiformes   | Eleotridae; Gobiidae           |
|                  | Salmoniformes | Salmonidae                      |
|                  | Cypriniformes | Cyprinidae; Cobitidae          |
|                  | Cyprinodontiformes | Poeciliidae                   |
| Chondrichthyes   | Carcharhiniformes | Triakidae; Sphyrnidae; Carcharhinidae; Scyliorhinidae |
| Class          | Order                  | Families                                           |
|---------------|------------------------|---------------------------------------------------|
| Lamniformes   | Lamnidae; Mitsukurinidae; Odontaspidae               |
| Pristiophoriformes | Pristiophoridae           |
| Myliobatiformes | Myliobatidae; Dasyatidae        |
| Chimaeriformes | Callorhinchidae            |
| Hexanchiformes | Hexanchidae              |
| Orectolobiformes | Orectolobidae              |
| Heterodontiformes | Heterodontidae          |
| Amphibia      | Anura                  | Myobatrachidae; Hylidae; Bufonidae; Microhylidae |
| Reptilia      | Squamata                | Scincidae; Agamidae; Diplodactylidae; Elapidae; Gekkonidae; Pygopodidae; Varanidae; Typhlopidae; Carphodactylidae; Boidae; Colubridae; Acrochordidae |
| Testudines    |                        | Chelidae; Cheloniidae; Dermochelyidae             |
| Crocodylia    |                        | Crocodylidae                                      |
| Aves          | Passeriformes           | Meliphagidae; Artamidae; Acanthizidae; Pachycephalidae; Rhipiduridae; Monarchidae; Corvidae; Pardalotidae; Maluridae; Petroicidae; Sturnidae; Hirundinidae; Campephagidae; Climacteridae; Passeridae; Corcoracidae; Estrildidae; Timaliidae; Turdidae; Pomatostomidae; Megaluridae; Nectariniidae; Motacillidae; Fringillidae; Oriolidae; Oreoididae; Neosittidae; Acrocephalidae; Ptilonorhynchidae; Psophodidae; Alaudidae; Cisticolidae; Menuridae; Dasyornithidae; Dicruridae; Orthonychidae; Pittidae; Paradisaeidae; Pycnonotidae; Atrichornithidae; Ploceidae; Sylviidae |
|              | Psittaciformes          | Psittacidae; Cacatuidae                           |
| Clade          | Subclades                                                                 |
|---------------|---------------------------------------------------------------------------|
| Anseriformes  | Anatidae; Anseranatida                                                   |
| Falconiformes | Accipitridae; Falconida                                                   |
| Charadriiformes | Charadriidae; Laridae; Scolopacida; Recurvirostrida; Burhinidae; Haematopodida; Pedionomidae; Glareolida; Rostratulidae; Jacanidae; Stercorariidae |
| Columbiformes | Columbidae                                                                |
| Ciconiiformes | Threskiornithidae; Ardeidae; Ciconiidae                                  |
| Pelecaniformes | Phalacrocoracidae; Pelecanidae; Anhingidae; Phaethontidae; Sulidae; Fregatidae |
| Coraciiformes | Alcedinidae; Meropidae; Coraciida                                        |
| Gruiformes    | Rallidae; Gruidae; Otidida                                                |
| Cuculiformes  | Cuculidae; Centropodida                                                  |
| Podicipediformes | Podicipedida                                                               |
| Strigiformes  | Strigidae; Tytonida                                                       |
| Struthioniformes | Casuariidae; Struthionida                                               |
| Galliformes   | Phasianidae; Megapodiida; Numidida                                        |
| Apodiformes   | Aegothelidae; Apodida                                                    |
| Caprimulgiformes | Podargidae; Caprimulgida                                                |
| Turniciformes | Turnicidae                                                                |
| Kingdom          | Subkingdom | Class             | Order                      |
|------------------|------------|-------------------|----------------------------|
| **Procellariiformes** |            | Procellariidae; Diomedeidae; Oceanitidae |
| **Sphenisciformes**    |            | Spheniscidae       |
| **Accipitriformes**    |            | Accipitridae       |
| **Mammalia**          | Diprotodontia | Macropodidae; Phalangeridae; Vombatidae; Pseudocheiridae; Phascolarctidae; Pterauridae; Potoroidae; Burramyidae; Acrobatidae; Hypsiprymnodontidae |
| **Chiroptera**        |            | Vespertilionidae; Molossidae; Miniopteridae; Pteropodidae; Emballonuridae; Rhinolophidae; Rhinonycteridae; Megadermatidae; Hipposideridae |
| **Carnivora**         |            | Canidae; Felidae; Otariidae; Mustalidae; Phocidae |
| **Rodentia**          |            | Muridae            |
| **Dasyuromorphia**    |            | Dasyuridae; Myrmecobiidae |
| **Lagomorpha**        |            | Leporidae          |
| **Artiodactyla**      |            | Bovidae; Cervidae; Suidae; Camelidae |
| **Monotremata**       |            | Tachyglossidae; Ornithorhynchidae |
| **Peramelemorphia**   |            | Peramelidae; Thylacomyidae; Chaeropodidae |
| **Perrisodactyla**    |            | Equidae            |
| **Cetacea**           |            | Delphinidae; Balaenidae; Physeteridae; Ziphiidae; Phocoenidae; Balaenopteridae; Neobalaenidae; Kogiidae |
| **Sirenia**           |            | Dugongidae         |
Sequence length distribution of all sequence reads assigned to their respective samples.

Figure S1. The number of internally amplified barcodes for each primer pair plotted against the length of the internal barcode sequences. The data are derived from all sequence records that were successfully assigned to their respective samples and the vertical dashed lines represent the sequence length threshold used to remove short sequence records for each primer pair.
Best fitting linear regression model for the eDNA metabarcoding data obtained from the artificial community sample.

Figure S2. The best fitting model describing the relationship between the proportional read abundances and the PrimerMiner penalty scores for the artificial community.
**Summary of the metabarcoding data obtained from environmental DNA samples collected from two sites in the MDB.**

**Table S3.** Summary of the metabarcoding data obtained from environmental DNA samples collected for two sites within the Murray-Darling Basin (i.e. 8 and 12 samples collected for the Blakney Creek and Murrumbidgee River sites respectively) and analysed with the MiFish-U, Teleo and AcMDB07 primer pairs. Results are given as the number of samples testing positive for the different species and the average proportion of sequence reads ± the standard deviation given in between brackets.

| Species name         | Blakney Creek | Murrumbidgee River |
|----------------------|---------------|--------------------|
|                      | MiFish-U      | Teleo              | AcMDB07 | MiFish-U      | Teleo              | AcMDB07              |
| C. auratus           | 0             | 0                  | 0       | 1 (0.004)     | 0                  | 0                   |
| C. carpio            | 8 (0.448 ± 0.074) | 8 (0.064 ± 0.019) | 8 (0.317 ± 0.118) | 12 (0.847 ± 0.134) | 12 (0.369 ± 0.132) | 12 (0.757 ± 0.175) |
| G. bispinosus        | 0             | 8 (0.057 ± 0.036) | 2 (0.044 ± 0.009) | 0       | 0                  | 0                   |
| G. holbrooki         | 0             | 0                  | 0       | 0       | 2 (0.022 ± 0.012) | 0                   |
| Galaxias sp.         | 8 (0.223 ± 0.065) | 8 (0.461 ± 0.098) | 8 (0.331 ± 0.095) | 11 (0.036 ± 0.017) | 12 (0.119 ± 0.070) | 8 (0.057 ± 0.030) |
| H. klunzingeri       | 0             | 1 (0.004)          | 0       | 8 (0.083 ± 0.195) | 11 (0.152 ± 0.243) | 6 (0.168 ± 0.277) |
| H. sp.'Midgley's'    | 3 (0.020 ± 0.007) | 1 (0.008)          | 0       | 0       | 0                  | 0                   |
| M. ambigua           | 0             | 0                  | 0       | 6 (0.018 ± 0.008) | 7 (0.020 ± 0.021) | 2 (0.037 ± 0.026) |
| M. anguillicaudatus  | 0             | 0                  | 0       | 8 (0.028 ± 0.016) | 12 (0.044 ± 0.044) | 5 (0.024 ± 0.003) |
| Species               | Count 0 | Count 1 | Count 2 | Count 3 | Mean ± SD 0 | Mean ± SD 1 | Mean ± SD 2 | Mean ± SD 3 |
|----------------------|---------|---------|---------|---------|-------------|-------------|-------------|-------------|
| M. australasica      | 0       | 0       | 0       | 0       | 3 (0.040 ± 0.011) | 2 (0.025 ± 0.006) |
| M. macquariensis     | 0       | 0       | 0       | 1 (0.015) | 2 (0.031 ± 0.039) | 1 (0.028) |
| M. peelii peelii     | 0       | 0       | 0       | 11 (0.036 ± 0.022) | 12 (0.115 ± 0.066) | 9 (0.043 ± 0.013) |
| N. australis         | 8 (0.183 ± 0.048) | 8 (0.231 ± 0.054) | 8 (0.183 ± 0.085) | 0       | 0       | 0       |
| O. mykiss            | 0       | 0       | 0       | 0       | 2 (0.055 ± 0.021) | 0       |
| P. fluviatilis       | 8 (0.123 ± 0.068) | 8 (0.111 ± 0.058) | 8 (0.101 ± 0.029) | 2 (0.013 ± 0.000) | 3 (0.014 ± 0.004) | 1 (0.028) |
| P. grandiceps        | 3 (0.017 ± 0.010) | 3 (0.013 ± 0.004) | 2 (0.044 ± 0.015) | 0       | 0       | 0       |
| R. semoni            | 4 (0.018 ± 0.007) | 8 (0.069 ± 0.043) | 6 (0.061 ± 0.034) | 2 (0.010 ± 0.009) | 12 (0.161 ± 0.105) | 10 (0.074 ± 0.048) |
| S. trutta            | 0       | 0       | 0       | 0       | 3 (0.040 ± 0.017) | 1 (0.025) |
References

Fuller, S., Baverstock, P., & King, D. (1998). Biogeographic origins of goannas (Varanidae): a molecular perspective. *Molecular Phylogenetics and Evolution, 9*(2), 294–307. https://doi.org/10.1006/mpev.1997.0476

Hardy, C. M., Adams, M., Jerry, D. R., Court, L. N., Morgan, M. J., & Hartley, D. M. (2011). DNA barcoding to support conservation: species identification, genetic structure and biogeography of fishes in the Murray—Darling River Basin, Australia. *Marine and Freshwater Research, 62*(8), 887–901.

Jin, X. X., Zhao, S. L., & Wang, R. X. (2013). Universal primers to amplify the complete mitochondrial 12S rRNA gene in marine fish species. *Genetics and Molecular Research, 12*(4), 4575–4578.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., … Drummond, A. (2012). Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics, 28*(12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

MacDonald, A. J., Young, M. J., Lintermans, M., & Sarre, S. D. (2014). Primers for detection of Macquarie perch from environmental and trace DNA samples. *Conservation Genetics Resources, 6*(3), 551–553.

Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., & Coissac, E. (2011). ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Research, 39*(21), e145.

Wang, H. Y., Tsai, M. P., Tu, M. C., & Lee, S. C. (2000). Universal primers for amplification of the complete mitochondrial 12S rRNA gene in vertebrates. *Zoological Studies, 39*(1), 61–66.