Received: 5 June 2020 | Revised: 5 July 2021 | Accepted: 6 July 2021
DOI: 10.1111/mec.16082

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

The roles of aridification and sea level changes in the diversification and persistence of freshwater fish lineages

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Funding information
Australian Research Council, Grant/Award Number: FT130101068

Abstract

While the influence of Pleistocene climatic changes on divergence and speciation has been well-documented across the globe, complex spatial interactions between hydrology and eustatics over longer timeframes may also determine species evolutionary trajectories. Within the Australian continent, glacial cycles were not associated with changes in ice cover and instead largely resulted in fluctuations from moist to arid conditions across the landscape. We investigated the role of hydrological and coastal topographic changes brought about by Plio-Pleistocene climatic changes on the biogeographic history of a small Australian freshwater fish, the southern pygmy perch *Nannoperca australis*. Using 7958 ddRAD-seq (double digest restriction-site associated DNA) loci and 45,104 filtered SNPs, we combined phylogenetic, coalescent and species distribution analyses to assess the various roles of aridification, sea level and tectonics and associated biogeographic changes across southeast Australia. Sea-level changes since the Pliocene and reduction or disappearance of large waterbodies throughout the Pleistocene were determining factors in strong divergence across the clade, including the initial formation and maintenance of a cryptic species, *N.* 'flindersi'. Isolated climatic refugia and fragmentation due to lack of connected waterways maintained the identity and divergence of inter- and intraspecific lineages. Our historical findings suggest that predicted increases in aridification and sea level due to anthropogenic climate change might result in markedly different demographic impacts, both spatially and across different landscape types.

KEYWORDS
climate change, ddRAD, ecological genomics, phylogeography, southeast Australia, teleost

1 | INTRODUCTION

Dramatic changes in climate, hydrology and topography have long been recognised to have lasting impacts on the diversity, distribution and divergence of species and populations (Pelletier et al., 2015). Understanding the relationship between the historical environment and the genealogy of species remains critical for interpreting how contemporary climate change may impact on species currently and in the near future. Most notably, increasing aridification and rising sea-levels (1–2 m increase by the end of the century; Overpeck & Weiss, 2009) predicted by climate change projections for many regions across the globe call into question the adaptive capacity and resilience of organisms, especially those with poor dispersal potential and narrow ranges (Davis et al., 2013; Falkenmark, 2013; Grummer et al., 2019). However, applying broadscale inferences about environmental changes to understand biodiversity resilience in the future is further complicated by spatial variation in environmental factors that might impact on how within-species responses vary across their ranges (Razgour et al., 2019). For example, the influence of historical eustatic changes would have been greater for coastal or marine ecosystems, while the effects of aridification would have been stronger inland (Beheregaray et al., 2002; Pinceel et al., 2013). Thus, understanding the relative role of different environmental changes between regions is important to more accurately predicting species' responses.

Complex impacts of climatic change are particularly exacerbated in freshwater ecosystems, as increasing temperature and aridity alters the stability and structure of hydrological systems (Blöschl et al., 2019; Middelkoop et al., 2001; Nijssen et al., 2001; Pinceel et al., 2013). With limited dispersal capability and reliance on available freshwater for survival, aquatic-dependent species often demonstrate strong evolutionary associations with hydrological changes. Even minor alterations to hydrologic structure can have profound impacts on the evolution of freshwater taxa (Inoue et al., 2014; Thomaz et al., 2017; Wallis et al., 2017). For example, tectonic activity can reshape waterways, leading to river capture across new areas and shifting distributional patterns of water-dependent species (Murphy & Austin, 2004; Waters et al., 2001). Thus, freshwater biodiversity functions as an important indicator of the impact of historical environmental changes.

Inferences of phylogeographic responses to past environmental change relies upon a combination of genetic, spatial and modelling approaches. Determining the relative role and chronology of past climatic events is difficult when resolution is low due to few genetic markers or limited model capability (Carstens et al., 2012; Cutter, 2013; Nakhleh, 2013). To this end, the collection of thousands of genetic markers enables better capture of the diverse array of demographic processes influenced by Earth history (Carstens et al., 2012; Edwards et al., 2016). In tandem, recent advancements in coalescent modelling, informed by detailed information of geological and ecological history, have improved the ability to provide more nuanced inferences (Cutter, 2013; Excoffier et al., 2013). This combination of greater data and sophisticated modelling techniques provides the analytical framework to address questions about the spatial variance of species responses to climate change.

A suitable biogeographic setting to test hypotheses of spatial and temporal variation of Earth history on evolution is one including both inland and coastal regions. In this regard, the temperate southeast of Australia is well-suited given it has been influenced by aridification across the continent, as well as changes in exposed land area attributed to eustatic changes (McLaren & Wallace, 2010). Major aridification of the region originated in the mid Miocene ~15 million years ago (Ma), driving the formation of Australia's arid zone which dominates much of the contemporary mainland ~1–4 Ma (Byrne et al., 2008). However, coastal areas of southeast Australia remained somewhat buffered against historical aridification and sustained cool temperate rainforest habitats throughout the Plio-Pleistocene (Gallagher et al., 2003). This region is characterised by complex geography and geology, affected by a history of uplift, subsidence and volcanism (McLaren et al., 2011). The region is subdivided by the Great Dividing Range, which runs parallel to the coastline from northern Australia to the southern coast. This range acts as a barrier that separates the inland Murray-Darling Basin (MDB) from coastal areas and is a key biogeographic feature of the region (Figure 1b; Chapple et al., 2011; Unmack, 2001).

The MDB spans an area of 1.06 M km^2 (~14% of Australia's landmass) and consists of a single dendritic network with an outlet along the southern coast of the mainland (Pittock & Finlayson, 2011). Environment characteristics vary dramatically across the region, with high-rainfall alpine regions along the Great Dividing Range contrasting with arid floodplain regions further inland. Within the MDB, major hydrological changes in the past were mostly associated with aridification and tectonics, such as the formation and decline of the paleo megalake Bungunnia during the Plio-Pleistocene (from 3 Ma until ~700 Ka), which spanned 90,000 km^2 across the lower section of the MDB at its largest size – larger than any contemporary freshwater lake in the world (McLaren et al., 2011, 2012). Lake Bungunnia has been suggested to have acted as a barrier for some terrestrial species (Ansari et al., 2019; Cooper et al., 2000; Joseph et al., 2008; Kawakami et al., 2009; Neal et al., 2019), and inversely may have acted as a conduit for aquatic species (Waters et al., 2019). Similarly, the shrinking and salinisation of Lake Corangamite south of the Great Dividing Range (Figure 1b) during the Holocene (1.6 Ma–0.01 Ka) may also have presented a barrier to dispersal for freshwater species (Schultz et al., 2008; White, 2000). These historical factors are probably exacerbated by recent anthropogenic modification to waterways, particularly within the MDB, where extensive agricultural development, water removal and weir construction since European colonisation (~200 ya) has dramatically impacted species abundance and connectivity across the basin (Cole et al., 2016; Koehn, 2013).

Contrastingly, environmental changes across the coastal habitats south of the MDB were more associated with eustatic changes. Particularly, the formation and submergence of the Bassian Isthmus, which connected the island of Tasmania to the mainland during glacial maxima (Blom & Alsop, 1988; Porter-Smith et al., 2012), is a well-documented driver of biogeographic patterns for a variety of terrestrial, marine and freshwater taxa (Schultz et al., 2008; Waters, 2008). This combination of relevant Earth history factors across southeast Australia, and their potentially interactive nature, provides a scenario to investigate the relative role of different past environmental changes on phylogeographic patterns.

An ideal system for studying biogeographic changes in southeast Australia is the southern pygmy perch, *Nannoperca australis* (Percichthyidae). This small-bodied (<80 mm), habitat-specialist fish prefers slow flowing and vegetated ephemeral streams (Hammer et al., 2013; Wedderburn et al., 2012). It is distributed throughout the temperate southeast Australia region, occupying the MDB, coastal Victoria and northern Tasmanian rivers. Previous phylogenetic work indicated that southern pygmy perch from eastern Victoria, Flinders Island and north-eastern Tasmania belong to a genetically distinct cryptic species referred to as *Nannoperca* 'flindersi' (Buckley et al., 2018; Unmack et al., 2013). Estimates of divergence time using the formation of the Nullarbor Plain as a calibration point suggest this split occurred ~6 Ma (Buckley et al., 2018), but the biogeographic

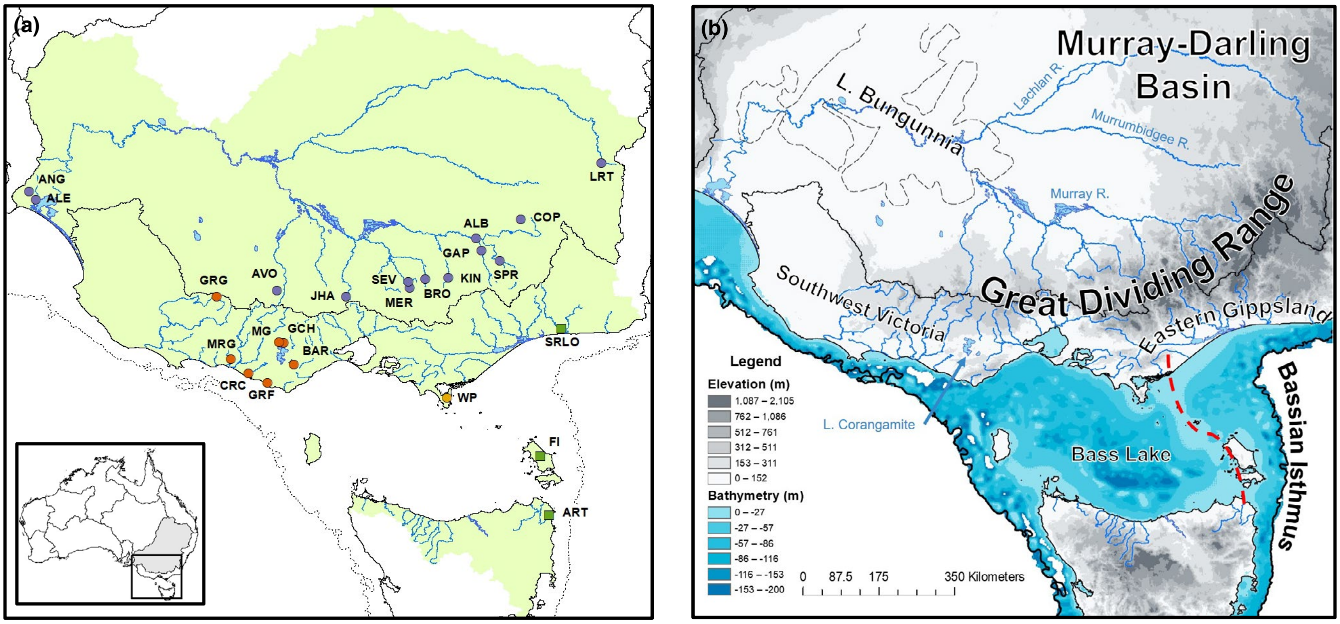


FIGURE 1 (a) Distribution and sampling map for southern pygmy perch. Inset depicts study extent and major drainage basins of Australia, with the Murray-Darling Basin shaded in grey. The shaded area denotes the putative distribution of the species, spanning multiple major basins (black lines). Locality abbreviations are detailed in Table 1. Colours denote major clades explored within coalescent models (refer to Section 3) whilst shapes denote "species" (circles = *N. australis*; squares = *N.* 'flindersi'). The extent of the continental shelf (−135 m), which was exposed during glacial periods, is indicated by the dotted line. (b) Topographic map of southeast Australia, highlighting topographic heterogeneity and major biogeographic regions across the area. Grey shading indicates elevation (m above current sea level) and blue shading indicates bathymetry (m below current sea level). Solid black lines indicate major basin boundaries and contemporary coastline whilst the red dashed line indicates the drainage divide across the Bassian Isthmus. The maximum extent of Lake Bungunnia (at 1.2 Ma) is also indicated with a dashed line

forces driving this speciation remain unknown. Being an ancient lineage, *N. australis* would have responded to a variety of environmental changes across inland and coastal habitats since the Miocene. For instance, landscape genomics (Brauer et al., 2016) and ecological transcriptomic studies (Brauer et al., 2017) have shown that the strong contemporary east-west hydroclimatic gradient of the MDB has led to the evolution of several adaptively divergent populations in *N. australis*. Furthermore, *N. australis* is threatened, particularly within the MDB, due to extreme pressure from anthropogenic changes to water flow, introduced predators and contemporary climate change (Balcombe et al., 2011; Brauer et al., 2016). The low dispersal capability, small population sizes and high genetic structure of the species makes their survivability of great concern (Brauer et al., 2016, 2017; Cole et al., 2016). In fact, the species has been the target of multiple genetic-based captive-breeding and reintroduction programs in the MDB (Attard et al., 2016; Beheregaray et al., 2021). Here, we used genome-wide data to assess the relative roles of hydrological and coastal topographic changes as drivers of evolutionary diversification and lineage persistence. We hypothesised that demographic changes and lineage diversification of populations from inland basins would be more closely linked to aridification, whereas changes linked to eustatic variation would be comparatively common for populations from coastal or island habitats. Given the significant environmental changes that both aridification and eustatic changes have caused in the region, we hypothesised that both factors would have stronger impacts on phylogeographic patterns than other factors such as tectonic changes or river capture. We tested the impact of these factors using a hierarchical framework that incorporates complex, hypothesis-driven coalescent modelling, model-free demographic analyses and spatial (species distribution) modelling.

2 | MATERIALS & METHODS

2.1 | Sample collection and genomic library preparation

A total of 109 samples across 21 known populations of *N. australis* and three populations of *N.* 'flindersi' (n = 4–5 individuals per population) were used (Table 1). This sampling spans the full geographic range of the species and includes at least one population from each management unit identified in previous population genetic and genomic studies (Figure 1a; Cole et al., 2016; Unmack et al., 2013). The sister species *N. obscura* (Buckley et al., 2018) was included as the outgroup for phylogenetic analyses (n = 5). Specimens were collected using a combination of electrofishing, dip-, fyke- or seine-netting. Specimens (either caudal fin or entire specimen) were stored dry at −80°C at the South Australian Museum, or in 99% ethanol at Flinders University.

TABLE 1 Locality data for samples used in this study

Species	Population	Abbreviation	Field code	*n*
N. australis	Angas R., Strathalbyn	NauANG	F-FISH84	5
	Lake Alexandrina	NauALE	SPPBrA*	4
	Middle Ck, Warrenmang, Avoca	NauAVO	F-FISH75: PU99-33SPP	5
	Jew Harp Ck, Sidonia	NauJHA	F-FISH78: PU00-01SPP	3
	Tributary to Seven Creeks	NauSEV	PU13-65SPP	4
	Merton Ck, Goulburn Rvr.	NauMER	F-FISHY6: PU09-01SPP	5
	Broken R., Lima South	NauBRO	F-FISHY6: PU09-02SPP	5
	King R., Cheshunt, Ovens Rvr.	NauKIN	F-FISHY6: PU09-06SPP	4
	Spring Ck, Mitta Mitta	NauSPR	F-FISHY6: PU09-13SPP	5
	Gap Ck, Kergunyah, Kiewa	NauGAP	F-FISHY6: PU09-12SPP F-FISH77: PU99-81SPP	5
	Murray R. lagoon, Albury	NauALB	F-FISH53: IW94-47	4
	Coppabella Ck, Coppabella	NauCOP	F-FISH75: PU99-82SPP	5
	Blakney Ck, Lachlan Rvr.	NauLRT	F-FISH98: LPP-*	5
	Glenelg R., Glenisla	NauGRG	F-FISH78: PU0014-SPP	5
	Merri R., Grassmere	NauMRG	F-FISH78: PU00-22SPP	5
	Curdies R., Curdie	NauCRC	F-FISH78: PU00-24SPP	4
	Gellibrand R. floodplain	NauGRF	F-FISH97: PU02-92SPP	5
	Barongarook Ck, Colac	NauBAR	SPP08-13	4
	Mundy Gully	NauMG	F-FISHY8: PU08-11SPP	4
	Gnarkeet Ck, Hamilton	NauGCH	F-FISHY2: PU00-27SPP	4
	Wilsons Promontory	NauWP	F-FISH97: PU02-70SPP	5
N. 'flindersi'	Snowy R. lagoon, Orbost	NflSRLO	F-FISH77: PU99-85SPP	5
	Flinders Island	NflFI	F-FISH84: FI-*	4
	Anson R. tributary	NflANS	F-FISH82: HT–2*	5
N. obscura	Lake Alexandrina	*Outgroup*	YPBR*	5
Total		24		109 *(114)*

Note: Abbreviations described in the table were those used for further analyses, while *n* refers to the number of individuals sequenced per population. *Nannoperca obscura* samples were only included as an outgroup in the phylogenetic analysis.

DNA was extracted from muscle tissue or fin clips using a modified salting-out method (Sunnucks & Hales, 1996) or a Qiagen DNeasy kit (Qiagen Inc.). Genomic DNA quality was assessed using a spectrophotometer (NanoDrop, Thermo Scientific), 2% agarose gels, and a fluorometer (Qubit, Life Technologies). All ddRAD genomic libraries were prepared in-house following (Peterson et al., 2012), with modifications as described in Brauer et al., (2016). Of the 109 samples, 73 were previously paired-end sequenced as part of a landscape genomics study (Brauer et al., 2016) using an Illumina HiSeq 2000 at Genome Quebec. The additional 36 samples were single-end sequenced on an Illumina HiSeq 2500 at the South Australia Health and Medical Research Institute.

2.2 | Bioinformatics

The resultant reads (forward reads only for paired-end samples) were filtered and demultiplexed using the process_radtags module of Stacks 1.29 (Catchen et al., 2013), allowing ≤2 mismatches in barcodes. Barcodes were removed and reads trimmed to 80 bp to remove low-quality bases from the ends. Cut reads were then aligned using PyRAD 3.0.6 (Eaton, 2014), and further filtered by removing reads that had >5 bp with a Phred score of <20. Loci were retained at a minimum sequencing depth of 5 and occurring in at least ~90% of samples (103). The final alignment contained 7958 ddRAD loci and 45,104 SNPs. Although tree-based analyses predominantly used a concatenation of this alignment, coalescent-based analyses that formed the basis of more thorough investigations of demography and divergence were based upon “putatively unlinked” SNPs (i.e., a single randomly sampled SNP per ddRAD locus).

2.3 | Phylogenetic analysis

To determine evolutionary relationships as a basis for phylogeographic modelling, a maximum likelihood (ML) phylogeny was estimated using RAxML 8.2.11 (Stamatakis, 2014) and the 7958 concatenated ddRAD loci data set. This was done using rapid hill-climbing and 1000 resampling estimated log-likelihood (Pante et al., 2015) bootstraps under a GTR+Γ substitution model. Additionally, we estimated a ML tree in RAxML by partitioning the alignment by ddRAD locus, as well as inferring gene trees separately per ddRAD locus, using the same parameters. Concordance between individual gene trees from the latter approach and the concatenated tree were summarized using site concordance factors (Minh et al., 2020) in IQ-TREE2 (Minh, Schmidt, et al., 2020). These approaches were done to better account for the potential role of genome-wide rate heterogeneity and incomplete lineage sorting (Liu et al., 2015). The resultant phylogenetic trees were visualised using MEGA 7 (Kumar et al., 2016) and rooted with *N. obscura* as the outgroup.

To determine if dendritic river hierarchy alone could explain phylogenetic patterns across the Murray-Darling Basin lineage, and to clarify coalescent models (see Section 3), linear correlations between genetic and riverine distance were estimated using StreamTree (Kalinowski et al., 2008). StreamTree models genetic divergence across a dendritic river system and assigns a cost to each riverine segment, comparing this modelled distance with the empirical data. While StreamTree is often used with pairwise F_{ST} values (e.g., Brauer et al., 2018) to assess contemporary spatial patterns, we used uncorrected genetic distances (*p*-distance) as this more likely contains signal of historic patterns of divergence (Nei, 2001). Pairwise *p*-distances between individuals were estimated using PAUP* 4 (Swofford, 2002) and averaged per population for all 13 MDB populations.

2.4 | Divergence time estimation

We estimated divergence times using r8s 1.81 (Sanderson, 2003). Given the lack of suitable fossils for pygmy perches, we calibrated the node between *N. australis* and *N.* 'flindersi' at 5.9–6.1 Ma based on a previous estimate that includes all pygmy perch species (Buckley et al., 2018). We applied a broader range around the calibrated node to accommodate potential variation in dating not captured by the methods in Buckley et al., (2018). Divergence times for each node were estimated using a penalized-likelihood model under a truncated Newton algorithm (Nash, 2000), which uses a parametric branch substitution rate model with a nonparametric roughness penalty (Sanderson, 2003). Cross-validation was used to determine the best value of the smoothing parameter for the roughness penalty between $\log_{10} 0$ and $\log_{10} 100$. The optimum smoothing parameter of $\log_{10} 41$, with a chi-square error of −12,836.285, was used to estimate divergence times between populations and higher order clades across the lineage.

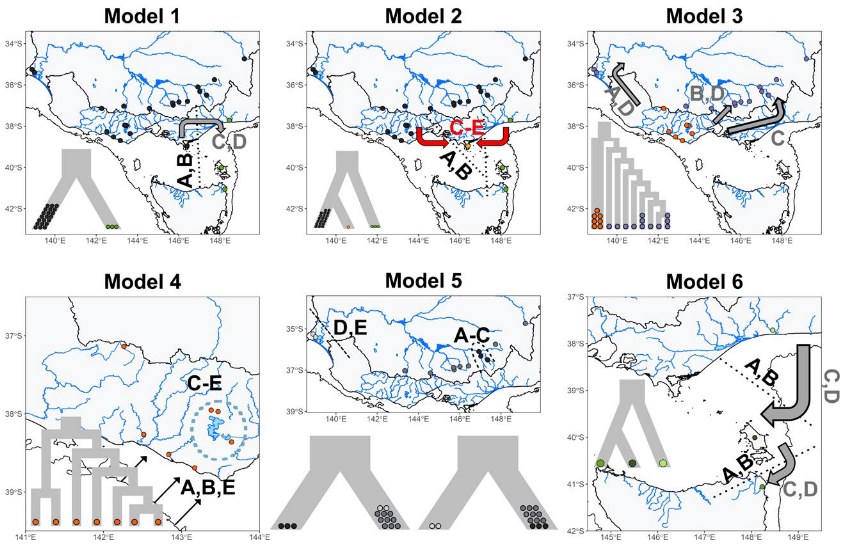
2.5 | Ancestral range estimation

We used a phylogenetic tree-based method to estimate ancestral ranges across the maximum likelihood tree with the R package BioGeoBEARS (Matzke, 2013). The maximum likelihood tree was collapsed down to individual populations using the R package ape (Paradis et al., 2004). Given the paraphyletic nature of the Albury population (NauALB) within the phylogenetic tree (see Section 3), this population was pruned from the tree. The tree was then converted to ultrametric format using the divergence time estimates from r8s.

Tips were assigned to one of six main geographic regions based on current hydrogeology (McLaren et al., 2011) and biogeographic regions of interest. Individual *N.* 'flindersi' populations were assigned to unique geographic states given their current isolation and to allow for the explicit testing of vicariance versus dispersal scenarios across the Bassian Isthmus. Ranges spanning multiple states were filtered to only those composed of neighbouring ranges (total number of possible ranges = 21). Furthermore, given extensive marine inundation of the lower MDB during the Pliocene (7–5 Ma) indicated by sand strandlines throughout the region (McLaren et al., 2011) which would have precluded the presence of southern pygmy perch, time-stratification was used to exclude “MDB” as a geographic state prior to 5 Ma or 3 Ma. These times reflect a conservative estimate of marine inundation (the most recent time at which marine sediments have been accurately identified within paleolake Bungunnia; McLaren et al., 2011) and a more relaxed estimate that is possibly the most recent time marine water could have been present within the basin. Ancestral ranges were estimated under all six available models (DEC, DIVA-LIKE and BAYAREA-LIKE, with and without founder-event speciation, +J). All models were run under both time-stratification scenarios and compared using the Akaike Information Criterion (AIC) within each set.

2.6 | Biogeographic hypothesis testing using coalescent modelling

We expanded upon our tree-based approaches using a suite of population-level SNP-based analyses to more thoroughly investigate historical patterns of demography and divergence. These analyses provided a more robust analytical framework for testing specific hypotheses of the impact of Earth history on the evolution of southern pygmy perches. Specific hypotheses based on biogeographic events were tested using a coalescent framework within FastSimCoal 2.6 (Figure 2; Excoffier et al., 2013). These hypotheses expand on the broad interpretations based on phylogenetic analyses. These models were focused around particular divergence events across the lineage, with hypotheses built around the separation of major clades within the phylogeny (Figure 3). For each major divergence event, we estimated coalescent models considering various competing hypotheses that may have driven the divergence. These models primarily varied in the order of population coalescence (akin to topology), the timing of divergence events and other demographic parameters such as the presence of bottlenecks and bidirectional gene flow, based on the expected impact of biogeographic patterns previously identified within the literature (Table S1) and information from both prior and current phylogenetic and coalescent analyses (Buckley et al., 2018).



🟦 Eustatics 🟥 Aridification 🟪 Both 🟨 Other

Model		Biogeographic factor		
1	A	Vicariance due to early marine inundation	🟦	-
	B		🟦	+
	C	Dispersal after early marine inundation	🟨	-
	D		🟨	+
2	A	Vicariance due to tectonics, marine inundation or volcanism	🟦	-
	B		🟦	+
	C	NauWP hybrid lineage of 50% *N. australis* and 50% *N.* 'flindersi'	🟨	-
	D	2C but with variable contributions of lineages	🟨	-
	E		🟨	+
3	A	Dispersal into MDB through lower reaches (via Lake Bungunnia)	🟥	-
	B	Dispersal into MDB through middle reaches (via river capture)	🟨	-
	C	Dispersal into MDB through upper reaches (via river capture)	🟨	-
	D	Both 3A and 3B	🟥	-
	E	Vicariance following patterns of the phylogenetic tree	🟨	-
4	A	Isolation of populations via marine inundation of coast	🟦	-
	B		🟦	+
	C	Isolation of populations via shrinking of Lake Corangamite	🟥	-
	D		🟥	+
	E	Both 4B and 4D	🟪	-
5	A	Divergence between (NauSPR, NauALB and NauGAP) after European settlement	🟥	-
	B	5A but prior to European settlement	🟥	-
	C		🟥	+
	D	Divergence between lower and upper MDB populations after European settlement	🟨	-
	E	5D but prior to European settlement	🟨	-
6	A	Vicariance due to early marine inundation	🟦	-
	B		🟦	+
	C	Dispersal across Bass Strait during lower sea levels	🟨	-
	D		🟨	+

FIGURE 2 Representation of coalescent hypotheses tested using FastSimCoal2. The maps depict the biogeographic scale and patterns of each set of models, with dashed lines indicating vicariance-based models, grey arrows indicating dispersal-based models, and red arrows indicating hybridisation-based models (with associated models listed beside each). Insets depict a representative model (Model 'A') for each set (Model 5 also includes 'D'), which are described in greater detail in the Supporting Information. As some populations were combined within the coalescent analyses, coloured circles within the representative models demonstrate the assignment of localities in the associated map to populations. The table lists the specific hypotheses per model, and whether these relate to eustatic changes (blue), aridification (red), both eustatic changes and aridification (purple) or other biogeographic mechanisms such as tectonics (yellow). Models with post-divergence gene flow are indicated a "+" in the right column

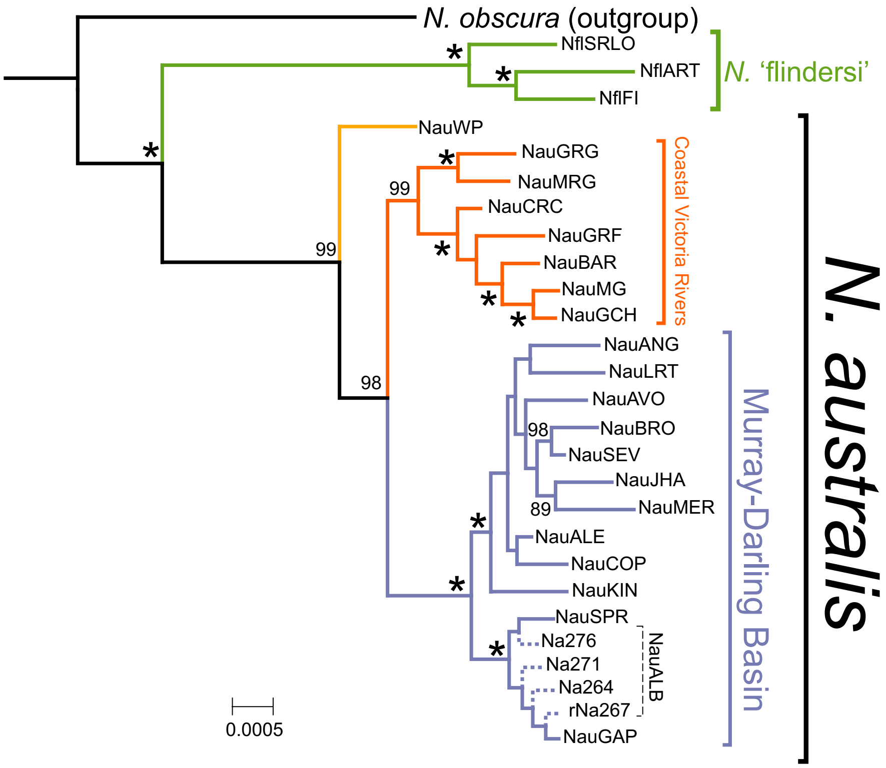


FIGURE 3 Maximum likelihood phylogeny of *N. australis* and *N.* 'flindersi' using 7958 concatenated ddRAD loci containing 45,104 SNPs. As all samples within a population formed clades (excluding NauALB, shown in dashed lines), the phylogeny was collapsed to individual populations. Nodes with 100% bootstrap support are indicated by asterisks. The tree was rooted using *N. obscura* as the outgroup. The full phylogenetic tree with all 119 samples is shown in Figure S8

For models around species divergence, we tested whether vicariance or dispersal (through the absence or presence of a bottleneck, respectively) was associated with the initial divergence of *N.* 'flindersi' and whether post-isolation gene flow had occurred or not (Models 1A–D). For the divergence of the Wilson's Promontory population (see Section 3), we tested various hypotheses of biogeographic causes of lineage isolation by varying the timing of divergence in association with known environmental changes (Model

2A–B). We also tested whether this lineage originated as a hybrid of *N. australis* and *N.* 'flindersi' or as an isolated *N. australis* population (Models 2C–E). For the separation of the MDB and coastal Victoria lineages, we tested colonization pathways from a coastal ancestor by altering the topology of the MDB populations in the model following the expected migratory pathways (Models 3A–C), including a scenario of more than one colonization (Model 3D) and one following the inferred topology of the phylogenetic tree (Model 3E). Within the coastal lineage, we tested whether populations were isolated as a result of marine inundation during sea level rise in the Last Interglacial, the shrinking of Lake Corangamite, or a combination of these factors (Models 4A–E). Within the MDB lineage, we tested whether pre-European settlement (>200 years ago) isolation was evident between either putatively "upper" and "lower" populations, or between the most divergent set of populations (NauGAP, NauALB and NauSPR) and the rest of the MDB populations (Models 5A–E). Within *N.* 'flindersi', we tested whether isolation of populations was associated with early Pleistocene marine inundation of the Bassian Isthmus or as a result of isolated dispersal events, including testing for post-isolation gene flow (Models 6A–D). Specific biogeographic hypotheses for each divergence event, and their predictive impacts on the evolution and demography of southern pygmy perch, are described in the Supporting Information (Table S1 and Figures S1–S6).

2.7 | Species and lineage distribution modelling

The distribution of the species was modelled using MaxEnt 3.4 (Phillips et al., 2017) and 19 BioClim variables from WorldClim v1.4 (Hijmans et al., 2005), summarising precipitation and temperature – two groups of climatic variables known to impact on local adaptation and distribution of southern pygmy perches (Brauer et al., 2016). To account for nonclimatic environmental aspects that may limit the distribution of the species (Paz et al., 2015), elevation (extracted from the Etopo1 combined bathymetry and topography data set; Amante & Eakins, 2009) and topographic wetness index (extracted from the ENVIREM database; Title & Bemmels, 2018) were also included. Species occurrence data was collected from the Atlas of Living Australia (http://www.ala.org.au), with filtering for geographic accuracy and removing outliers based on known distributional limits (6106 retained coordinates). However, this data set did not include some localities within the Murrumbidgee River and mid-Murray River where *N. australis* previously occurred but has recently been extirpated due to post-European settlement habitat modification across the MDB (Beheregaray et al., 2021; Brauer & Beheregaray, 2020; Cole et al., 2016). Duplicates from the same coordinate point were removed to minimise the biasing effect of uneven sampling effort (Elith et al., 2011), reducing the data set to 2528 unique occurrences. Similar tests of spatial autocorrelation were performed for the 19 BioClim variables using a Pearson's pairwise correlation test in SDMToolbox (Table S2; Brown et al., 2017). Highly correlated ($|r| > 0.8$) variables were removed to avoid overfitting of the model (Dormann et al., 2013), reducing the environmental data down to nine bioclimatic variables and the two topographic variables (Table S3). A subset of 25% of occurrence sites were used to train the model.

Climatic data from the Last Glacial Maxima (LGM; 22 Ka) were extrapolated from the WorldClim 1.4 database (Hijmans et al., 2005) to project the historic distribution of the species. To evaluate environmental conditions more reflective of the timing of divergence between the two species, the species distribution model was also projected back to the mid-Pliocene (~3.2 Ma) using a subset of six of the previous nine BioClim variables (excluding variables bio2, bio3 and bio6) from the PaleoClim database (Brown et al., 2018). The fit of each SDM was determined using the area under the receiver operating curve (AUC).

A lineage-specific distribution model (LDM) method described in Rosauer et al., (2015) was used to determine the relative distributions of each lineage over time; this was done with two intraspecific lineages of *N. australis* and *N.* 'flindersi'. A total of 72 site localities ($n = 61$ for *N. australis*; $n = 11$ for *N.* 'flindersi') were used based on genetic assignment to a "species" within this study, as well as based on mitochondrial DNA results (Unmack et al., 2013). We estimated the LDMs for both species across all three time periods (current, LGM, and Pliocene). Although the location of intraspecific lineages is unlikely to remain constant through time, this method allows the inference of possible relative distributions of each 'species' under past climatic conditions.

3 | RESULTS

3.1 | Bioinformatics

A total of 340,950,849 reads resulted in a data set of 7958 ddRAD loci with 45,104 variable sites (SNPs), 30,485 parsimony-informative sites and an average of 2.34% (±3.31% SD) missing data per individual. For coalescent analyses, SNPs were reduced to a single SNP per ddRAD locus, resulting in 7780 biallelic SNPs in the joint site frequency spectrum.

3.2 | Phylogenetic analysis

Partitioning the alignment per RAD locus had negligible impact on the topology and branch lengths across the phylogeny, but improved bootstrap support for several of the more conflicted nodes in the tree (Figure S7). Site concordance factors demonstrated more variable support across gene trees but broadly supported the same major delineations. For simplicity, we focus here on the phylogeny of the concatenated alignment. The maximum likelihood phylogeny (Figure 3; Figure S8) separated *N.* 'flindersi' from the rest of *N. australis*, corroborating previous phylogenetic results (Buckley et al., 2018; Unmack et al., 2013). Within *N. australis*, three major lineages were delineated; one of the Wilson's Promontory population (NauWP), one of coastal Victoria populations and another of populations within the MDB. The coastal Victorian lineage showed relatively greater phylogenetic structure than the MDB lineage, with its easternmost populations diverging more recently compared to westernmost coastal Victorian populations following a longitudinal pattern. The MDB clade, however, generally featured shorter branches and lower bootstrap support. Despite being geographically apart, lower MDB populations (Lake Alexandrina and Angas) and upper Murray populations did not form reciprocally monophyletic clades. The earliest branching clade of the MDB lineage contained populations from the upper Murray River (Spring Creek, Gap Creek, and Albury). Within this group, the Albury population was paraphyletic with the other two; this is expected based on previously described levels of admixture across the populations (Brauer et al., 2016). The breadth of the phylogenetic tree was well supported, with the majority of population-level and above divergences with bootstrap support of >80%.

The StreamTree results did not suggest that contemporary riverine hierarchy alone could explain patterns of historical phylogeographic divergence across the MDB (Figure 4; $R^2 = .464$). Assessing the fit of the StreamTree model by comparing the observed and expected genetic distance for each population individually demonstrated that this was probably driven by several outlier populations (NauANG, NauALE, NauALB and NauCOP) with notably higher modelled genetic distance (Figure S9). Removal of these four populations from the StreamTree model led to much higher correlation ($R^2 = .982$) with similar genetic distance penalties for river segments common to both sets of populations (Figure S10).

3.3 | Ancestral range estimation

Comparison of ancestral range estimations from BioGeoBEARS identified the DIVA-LIKE model as the best supported under both time-stratification scenarios (AIC = 24.16 and 59.1 for models excluding presence in the MDB until 5 Ma and 3 Ma, respectively). This model represents a likelihood approximation of the model implemented in DIVA (Ronquist, 1997) which broadly considers the relative role of dispersal and vicariance (but not sympatric mechanisms) in driving biogeographic patterns (Matzke, 2013). Although patterns were similar across both time-stratification criteria (Table S4), we choose to focus on the more conservative (5 Ma constraint) results given the lack of precision in determining the end of marine inundation into the MDB (McLaren et al., 2011). This DIVA-LIKE model demonstrated strong patterns of vicariance, with weak contributions of dispersal ($d = 1.46 \times 10^{-2}$) and extinction ($e = 1 \times 10^{-12}$) and all major geographic changes associated with vicariance events (Figure 5). Including a parameter for founder event (+J) estimated very weak contributions of founding events and contributed to negligible change in log likelihood across either time-stratification scenario (Table S1). Ancestral states for major nodes were well resolved across the phylogeny.

3.4 | Biogeographic hypothesis testing

Comparison of biogeographic hypotheses under coalescent models clearly supported one model over others for each focal divergence event (Figure 6): these results are detailed in the Table S5, Figure S11. In general, most models including post-isolation gene flow were better supported than those without, and models based on vicariance due to hydrological changes were better supported than those invoking tectonic or dispersal mechanisms.

3.4.1 | Divergence of species

Coalescent models including gene flow between the two species after divergence was better supported than models without, suggesting that secondary contact occurred at some point after their initial divergence (Figure 6; Model 1B). Models including bottlenecks did not have substantially higher support than those without, and inferred bottlenecks were small in magnitude.

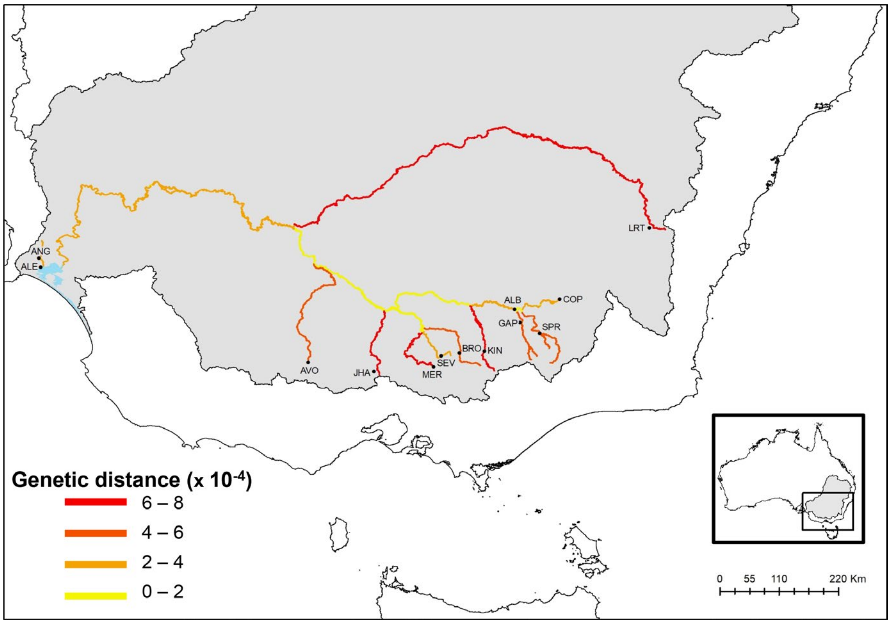


FIGURE 4 Representation of genetic distance across a dendritic riverine network in the Murray-Darling Basin, with streams colour-coded according to the StreamTree model that determines the contribution (as a penalty) of each segment in driving genetic divergence (*p*-distance) across the basin. Segments coloured in yellow confer little penalty (i.e., genetic divergence between populations at either end of the segment is low) whereas red segments confer higher genetic differentiation

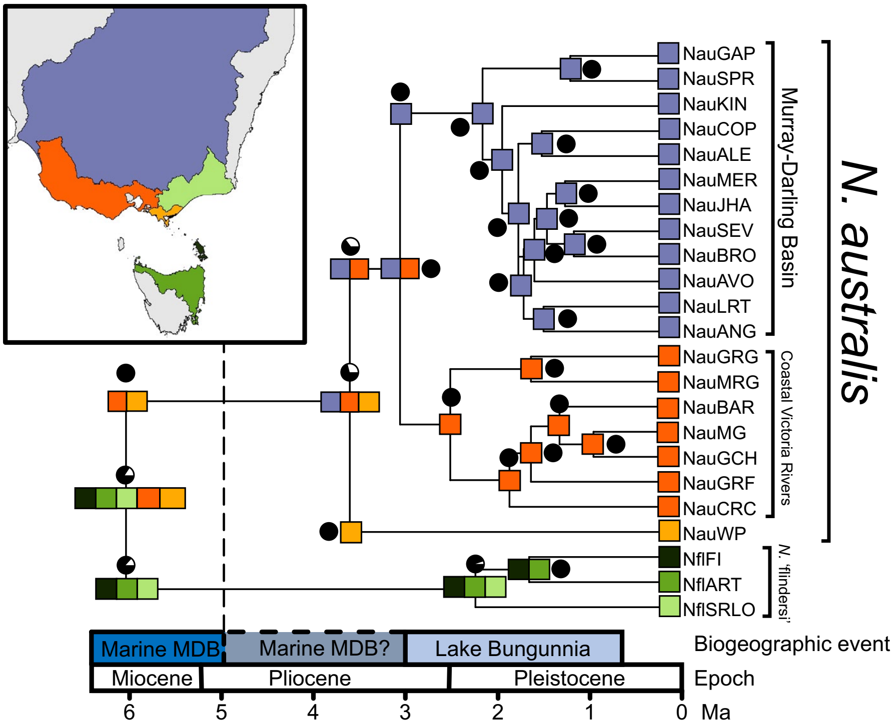


FIGURE 5 Most likely ancestral areas under the best supported model (DIVA-LIKE), with presence in the Murray-Darling Basin (MDB) excluded until 5 Ma (indicated by the dashed line). A biogeographic timeline of major alterations to the MDB is included for reference. Colours denote one of six contemporary areas, or ranges combining more than one area, as described by the legend. Pie charts demonstrate the probability of the most likely state for each node in black

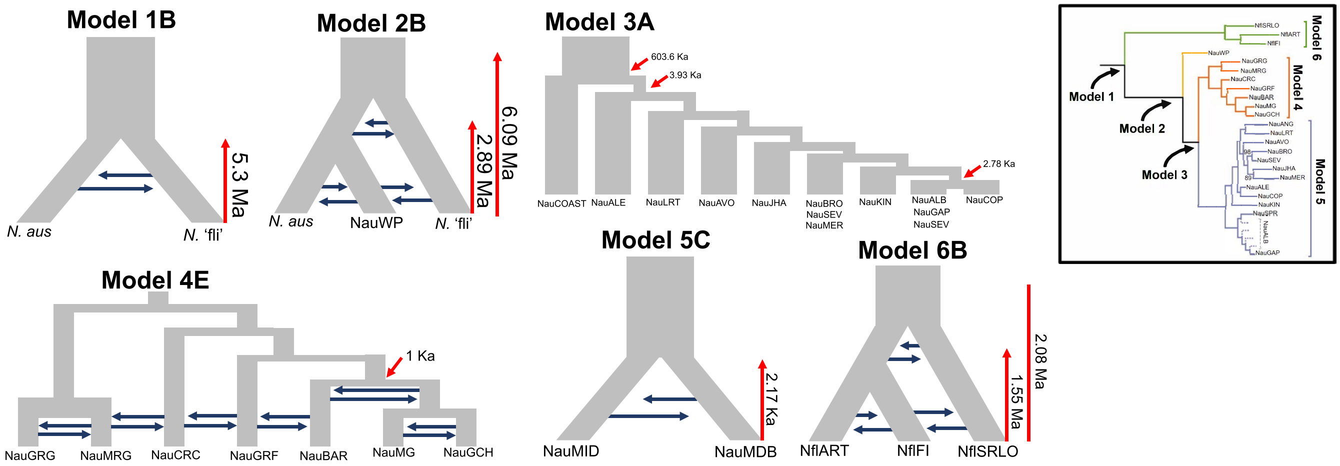


FIGURE 6 Representative diagrams of the best supported coalescent models under each model set. Models are numbered by set and lettered by variant with the set. Inset depicts focal divergence event for each model set within the maximum likelihood phylogenetic tree. The full set of tested models and the biogeographic hypotheses underpinning them are described within Table S1 and Figures S1–S6. Red arrows denote important divergence time parameters whilst blue arrows denote gene flow between populations. All parameter values for each model are demonstrated in Figure S11

3.4.2 | Divergence of Wilson's Promontory population

Coalescent models of the Wilson's Promontory population and the two species separately also suggested likely gene flow across adjacent lineages, with divergence of the Wilson's Promontory population occurring ~3 Ma (Figure 6; Model 2B). However, this gene flow was not indicative of a hybridisation event and models simulating the Wilson's Promontory population as resulting from gene flow from either population coalesced nearly all alleles into the *N. australis* ancestral population. Thus, this population represents an anciently diverged population of *N. australis* that was isolated through vicariance.

3.4.3 | Divergence of major *N. australis* lineages

Testing migration pathways from the ancestral coastal population into the MDB suggested that colonisation most likely occurred through the lower sections of the MDB and upstream into the upper reaches (Figure 6; Model 3A). The timing of this migration event would pre-date ~600 Ka, the estimated time of divergence between the coastal and MDB populations within the best supported model. However, this model had only marginally better likelihood than one estimating the divergence time of the coast and MDB populations at ~1.2 Ma, suggesting that there is large variation around this estimate.

3.4.4 | Divergence within coastal Victoria lineage

The coalescent model accounting for the effects of both sea-level changes leading to isolation of rivers and the shrinking of Lake Corangamite isolating eastern lineages was better supported than models without these factors, or models only considering one (Figure 6; Model 4E).

3.4.5 | Divergence within MDB lineage

Coalescent modelling of the MDB lineage suggested that some phylogeographic structure pre-dated European settlement, with the divergence of the most basal lineage (containing the Spring Creek, Gap Creek and Albury populations) estimated to have occurred ~2 Ka, albeit with low levels of gene flow since divergence (Figure 6; Model 5C). Models partitioning putatively upper and lower populations into single demes did not converge, probably reflecting their paraphyletic nature.

3.4.6 | Divergence within *N.* 'flindersi'

Coalescent models including migration between adjacent populations gave much greater likelihood estimations than models without gene flow, suggesting that migration between lineages had occurred in the past (Figure 6; Model 6B). Including bottlenecks indicative of a single or few dispersal events did not improve likelihoods, supporting a contiguous range expansion and vicariance scenario. The central population of Flinders Island had a much smaller population size than either of the other two populations. Divergence time estimates for between populations suggest a relatively ancient split, with the Snowy River population separating from the other two lineages ~1.5 Ma and the secondary split between Flinders Island and Tasmania at ~1.3 Ma.

3.5 | Species and lineage distribution modelling

Species distribution modelling for southern pygmy perch based on the nine uncorrelated BioClim and two topographic variables effectively predicted the contemporary distribution for the species, showing highest habitat suitability along the Victorian coast, southern MDB and in northern Tasmania (including King and Flinders Island; Figure 7a). However, this SDM probably underestimates presence of *N. australis* within the connective center of the MDB, where downstream migration would have facilitated a mosaic of intermediate populations prior to extensive flow abstraction and regulation over the last 200 years (Cole et al., 2016).

Historic projections of the SDM for both species together highlighted two glacial refugia, one along the western coast of the distribution and another small and isolated refugium closer to the southeast corner of the mainland. Together, these results indicate an overall expansion in suitable habitat following the LGM, despite the loss of habitat across the Bassian Isthmus (Figure 7d). The AUC of the model was estimated at 0.908, indicating a good model fit.

Lineage distribution models under each time period demonstrated the disjunct spatial nature of the two species. Under contemporary conditions, the LDMs showed a geographic break near Wilson's Promontory, albeit with overlap at intermediate localities (Figure 7b,c). An additional delimitation between species distributions occurred approximately halfway across the northern Tasmanian coastline. During the LGM, the disjunct LDMs of the two species in the east and west portion of the range supported their long-term isolation and provided evidence for an environmental barrier preventing contact between the species (Figure 7e, f). The Pliocene projection also showed refugial habitat along the Victorian coast and within the Murrumbidgee River (Figure 7g). Similar to contemporary conditions, the Pliocene LDMs showed a division between species in northern Tasmania and central Victoria (Figure 7h, i). The subset data used for the Pliocene projection had marginally weaker support, with an AUC of 0.887.

4 | DISCUSSION

Establishing how aquatic-dependent lineages responded to past hydroclimatic changes contributes to our understanding about their contemporary ecological requirements and to predicting demographic responses under ongoing climate change. This study demonstrates the overarching impacts of varying hydrology due to Plio-Pleistocene climatic change (e.g., reduction of lake systems and rearrangement of river networks) on the evolution and diversification of a temperate freshwater-dependent fish clade. However, coalescent analyses and species distribution modeling show that the evolutionary consequences of major shifts in sea level and hydroclimatic conditions varied substantially between coastal and inland environments. Aridification altered the demography of populations from inland river systems, whilst eustatic changes and marine inundation were major evolutionary drivers of populations from coastal freshwater landscapes.

4.1 | Aridification drives phylogeographic structure of inland basins

For freshwater species, increasing aridification in Australia since the Pliocene may be responsible for a number of divergent clades, with water availability and river networks being critical for the long-term

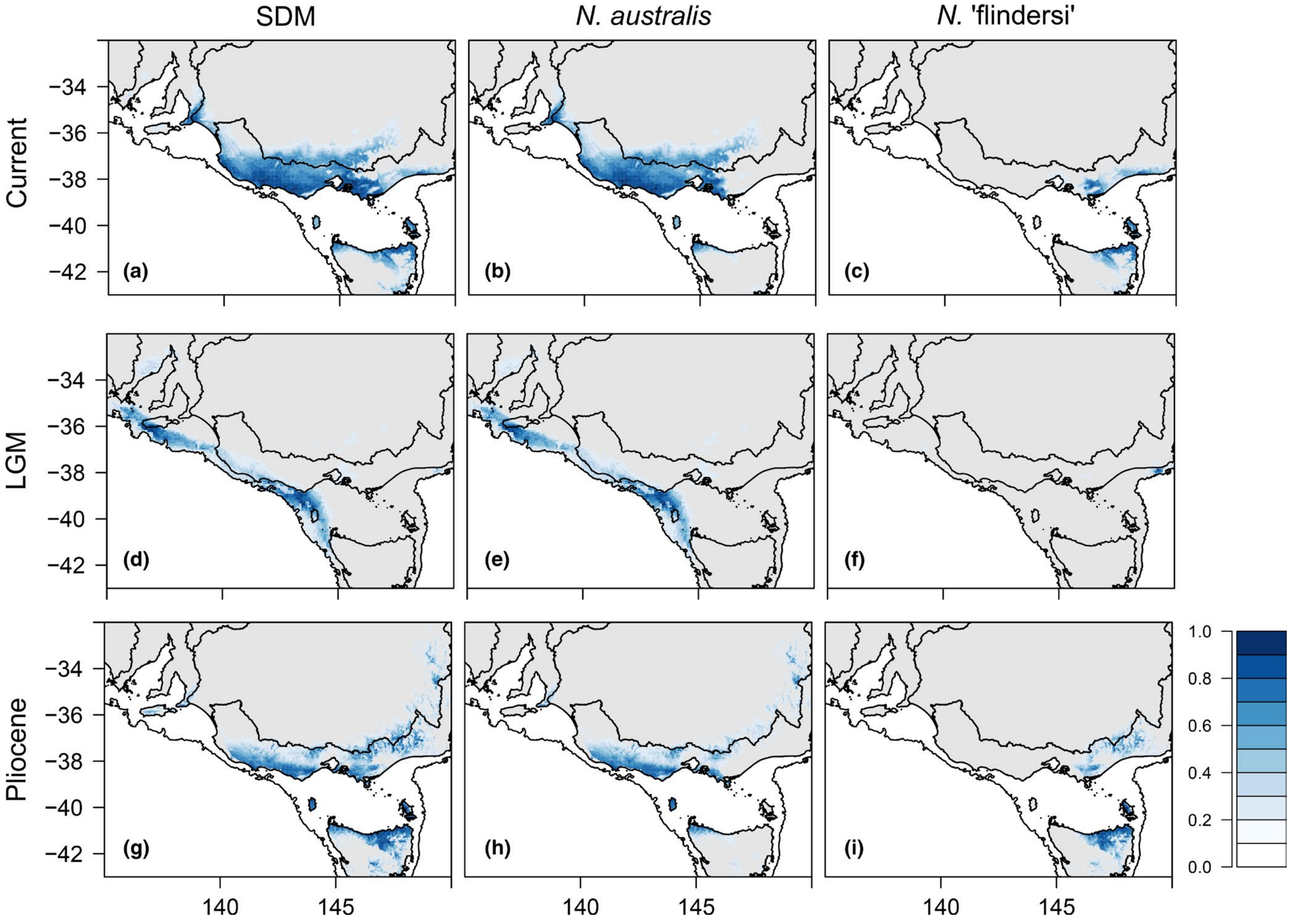


FIGURE 7 Species distribution models for all lineages and lineage-specific distribution models for each putative species based on nine bioclimatic and two topographic variables. Areas shaded in grey indicate landmass under each time period. (a–c) Distributions under contemporary climate conditions. (d–f) Distributions under last glacial maximum (LGM; 22 Ka) climate. (g–i) Distributions under mid Pliocene (3.2 Ma) climate conditions

survival and evolution of freshwater lineages (Beheregaray et al., 2017; Faulks et al., 2010; Hawlitschek et al., 2012). Aridification and tectonics through the formation and demise of paleo-megalake Bungunnia was a major event impacting the evolutionary history of inland lineages of *N. australis* (Figure 6, Model 3A). Lake Bungunnia initially formed ~3 Ma when tectonic shifts along the Padthaway High resulted in major uplift across the region, damming the ancestral Murray River which approximately aligned with the current Glenelg River (McLaren et al., 2011; Waters et al., 2019). For many freshwater taxa across the southeast of the continent, isolation of lineages between the MDB and the southwest Victoria drainages has been associated with this tectonic shift in the Pliocene (Murphy & Austin, 2004; Waters et al., 2019). Similar interpretations of tectonic changes influencing river capture have been proposed for movement across other sections of the Great Dividing Range into the MDB (Cook et al., 2006; Faulks et al., 2010; McGlashan, 2001; Murphy & Austin, 2004). The strong differentiation between clades and their ancient phylogenetic-based divergence time (Figure 5; 3.03 Ma) are probably reflections of the isolating effect of tectonic changes between the two drainages.

Contrasting to the phylogenetic-based divergence estimates, coalescent modelling instead points to more recent divergence (Figure 6, Model 3A; 603 Ka), suggesting the possibility that the edges of Lake Bungunnia at its largest extent could have acted as suitable habitat for southern pygmy perch and potentially facilitated further dispersal into the inland basin. With the eventual demise of the lake ~700 Ka (McLaren et al., 2011), this secondary bout of isolation disconnected the two basins fully, probably accounting for the results of coalescent models. Similar patterns of initial isolation by vicariance during the Pliocene, followed by Pleistocene secondary contact across the Great Dividing Range and into the MDB were observed within mountain galaxias (*Galaxias oliros* and *Galaxias olidus*), which share comparable ecological constraints to southern pygmy perch (Waters et al., 2019). Similar to Lake Bungunnia, the reduction of Lake Corangamite to one seventh of its original size over the course of the Holocene (White, 2000) isolated several eastern coastal Victoria *N. australis* populations (Figure 6, Model 4). The reduced size (~160 km^2) and hypersalinity (>50 g/L) of Lake Corangamite probably prevents connectivity between these populations under contemporary conditions (White, 2000; Williams, 1995).

Within the MDB clade there was weak evidence for historic phylogeographic structure, with coalescent models suggesting divergences dating at ~2 Ka (Figure 6, Model 5C). Correlating contemporary river structure and genetic distance per se did not predict genetic divergence between populations across the MDB. A combination of extensive flow and habitat modification since European colonisation and naturally complex metapopulation dynamics (Brauer et al., 2016) are probably better proxies for contemporary patterns observed within the MDB. The most likely demographic scenarios include multiple waves of dispersal and colonisation, possibly in response to local extinction or during rare environmental events such as flooding, which altered patterns of genetic divergence.

4.2 | Eustatic changes drive phylogeography and speciation along coastal habitats

Sea level changes associated with transitions between glacial periods played a critical role in the divergence of coastal lineages. Marine inundation across the East Gippsland region during the Mio-Pliocene (~6 Ma), prior to the climatic cycles of the Pleistocene, probably drove the initial divergence and speciation of *N.* 'flindersi' (Figure 6, Model 1B). Marine sediments and low elevation of the region indicates that this marine inundation was substantial (Gallagher et al., 2001; Holdgate et al., 2003), and its eventual retreat coincided with the onset of major aridification in the continent (Faulks et al., 2010; Garrick et al., 2004; McLaren & Wallace, 2010). Ancient marine inundation of East Gippsland has been proposed to influence vicariant speciation in various terrestrial species (Chapple et al., 2005; Norgate et al., 2009). This low-lying region forms the interface between the distribution of the two putative study species (Figure 1b) and the timing of this inundation corresponds well with the estimated molecular clock-based divergence time (Figure 5). This period of sea level rise is also associated with inundation of the lower parts of the MDB which probably precluded the presence of pygmy perch (McLaren et al., 2011).

Previous hypotheses of the mechanisms driving the initial divergence of *N.* 'flindersi' have suggested that the separation of drainages by tectonic shifts across the region (Dickinson et al., 2002; Gallagher et al., 2003) isolated populations following a dispersal event facilitated by river capture or flooding (Unmack et al., 2013). Regardless of the mechanism, divergence between Tasmanian and mainland lineages prior to the Pleistocene has been reported for birds (Lamb et al., 2019), lizards (Chapple et al., 2011; Dubey & Shine, 2010; Kreger et al., 2019), butterflies (Norgate et al., 2009) and other freshwater fish (Coleman et al., 2010), suggesting that climatic oscillations during the LGM alone did not drive the speciation of *N.* 'flindersi'.

More recent sea-level changes also probably impacted within-basin phylogeographic patterns. Within *N.* 'flindersi', relatively ancient estimates of divergence times between populations (1.5–2 Ma) suggested that early glacial cycles of the Pleistocene resulted in strong differentiation (Figure 4). However, coalescent models suggested that gene flow across these disparate populations was possible during glacial maxima (Figure 6, Model 6B). At lowered sea levels, river systems occupied by *N.* 'flindersi' all drained eastward towards the continental shelf (Unmack et al., 2013), with shorter overland distances between river mouths than today (Figure 1b). Given the presence of a small glacial refugia in the far eastern extreme of the distribution (Figure 7f), gene flow may have resulted from contraction into a singular locale followed by expansion back across the Bassian Isthmus during more favourable environmental conditions (Lambeck & Chappell, 2001).

Within the coastal *N. australis* lineage, isolation of river catchments during the Pleistocene may have led to the strong structure observed within the phylogenetic tree (Figure 3). During glacial maxima, lowered sea levels substantially increased the extent of the mainland Australian coastline, particularly across the southeast corner (Williams et al., 2018). Although it does not appear that the current rivers of coastal Victoria ever fully connected together before meeting the shoreline (Unmack et al., 2013), climatic modelling has suggested that the low topographic relief and evaporation across this region would have allowed overland networks to form through small lakes and floodplains (Williams et al., 2018). Although eustatic changes appear to have more significantly impacted coastal freshwater lineages than aridity during glacial cycles (e.gDubey & Shine, 2010; Schultz et al., 2008), the contraction of Lake Corangamite during the late Pleistocene–Holocene (1.6 Ma–10 Ka) also probably impacted local-scale connectivity (Coleman et al., 2010; Harrison, 1993; Schultz et al., 2008). Thus, along coastal Victoria aridification and eustatic changes demonstrated interactive effects on phylogeographic patterns. The more historic nature of divergences within the coastal lineage compared to the MDB lineage were corroborated by the well-resolved phylogenetic structure (Figure 3).

4.3 | Identity and maintenance of cryptic species, *N.* 'flindersi'

Although the initial divergence between *N. australis* and *N.* 'flindersi' was associated with older biogeographic events during the Miocene, our results indicated weak post-divergence gene flow between the two species (Figure 6, Model 1B). Distribution modelling indicated potential connectivity between species under contemporaneous conditions across the Victorian and Tasmanian coastlines, with little divergence in environmental ranges between the two species (Figure S12). However, environmental changes during glacial maxima probably caused the two species to retract to isolated refugia. These factors together suggest a history of alternating periods of isolation and connectivity during glacial cycles, with isolated glacial refugia and weak interspecific interglacial gene flow limited to a narrow contact zone. Other studies of terrestrial species diversification across recurrently connected islands suggest patterns of gene flow in accordance with lower sea levels (Jordan & Snell, 2008; Papadopoulou & Knowles, 2017; Parent et al., 2008; Paulay & Meyer, 2002). In this case, gene flow with *N. australis* does not appear to have impeded divergence and our results support the previous denotation of *N.* 'flindersi' as an independent species (Buckley et al., 2018; Unmack, 2001; Unmack et al., 2013).

4.4 | Implications for conservation management

Southern pygmy perch are currently listed as Near Threatened on the International Union for Conservation of Nature Red List (Pearce et al., 2019) and Vulnerable or Endangered within state government management lists (Hammer et al., 2013). Ongoing conservation management has sought to recover their numbers, particularly across the MDB (Attard et al., 2016; Brauer et al., 2016; Cole et al., 2016). Our results suggest that the impact of inland aridification under current climate change may compound the strong and recent fragmentation of the MDB lineage (Brauer & Beheregaray, 2020; Brauer et al., 2016), further threatening these populations. These recent patterns contrast with the results presented here and previous work on the phylogeography of the species which indicated metapopulation dynamics with natural patterns of local extinction, recolonization, and sporadic gene flow in the past (Beheregaray et al., 2021; Cole et al., 2016). Re-establishing historical genetic connectivity within the lineage is critical to improving genetic diversity and evolutionary potential across the system (Frankham et al., 2011; Love Stowell et al., 2017). However, given the relatively ancient isolation between the MDB and coastal lineages, we argue that in situ and ex situ conservation efforts should use a basin-wide context when selecting populations as source for demographic and genetic rescue (e.g., captive breeding and translocations). The relatively restricted current range of *N.* 'flindersi' also argues for a proactive approach to maintaining adaptive potential, especially given the fragmented (i.e., island populations) and predominately coastal habitat for this species.

4.5 | Implications under climate change

Historic climatic fluctuations have often been used to predict future responses to anthropogenic climate change (Dawson et al., 2011; MacDonald et al., 2008). Many bioregions across the world are expected to increase in aridity with ongoing climate change (Christensen et al., 2007), impacting on the stability and structure of freshwater ecosystems globally (Middelkoop et al., 2001; Nijssen et al., 2001), including for the MDB (Cai & Cowan, 2008; Pittock & Finlayson, 2011). Our results demonstrate the strong isolating effect of aridification, particularly in the reduction or loss of major connecting waterbodies. Additionally, projections of sea level rise associated with melting glacial ice predict major inundation of coastal habitats globally (Rotzoll & Fletcher, 2012). Although sea level rises of 1–2 m under contemporary climate change are unlikely to impact many of the coastal *N. australis* populations, associated increases in the frequency and size of storm surges may amplify this threat (McInnes et al., 2011). Increased sea levels are likely to also have other secondary environmental impacts, such as salinisation of freshwater habitats, which may further impact upstream populations (Herbert et al., 2015). Indeed, terrestrial species extinctions have already been directly linked to inundation of island habitats (Waller et al., 2017). Together, these findings demonstrate the interactive effects of hydrological changes resulting from climate change on threatened freshwater ecosystems (Pittock et al., 2008). However, the precise impacts of climatic change on the availability and reliability of water resources are uncertain (Middelkoop et al., 2001), as it is also the case on the influence of hydrological change on the evolution and persistence of species.

Our study highlights how spatial variation in the role and extent of environmental changes may result in variable impacts on the demography, distribution and divergence of populations. Particularly, we show how aridification of inland waterbodies and sea level rise leading to marine inundation impacted different regions across the distribution of a freshwater fish, operating on different timescales and to different extents. These environmental changes caused strong divergence across the clade, resulting in a hierarchy of lineages spanning from a cryptic species to intraspecific clades. While further increases in temperature will directly impact the long-term survival of many species broadly, additional impacts on hydrological systems through aridification will have compounding effects on freshwater species. These findings indicate that ongoing impacts from anthropogenic climate change may be complex in nature and vary across biogeographic regions depending on the role and identity of environmental forces that operate locally. We suggest that future management scenarios should consider this spatial variation in prediction of responses to climate change, particularly in how aridification or eustatic changes may act heterogeneously across species distributions. For example, aridification of inland aquatic systems may further fragment populations and reduce connectivity, highlighting the need for environmental watering and rehabilitation of river reaches. Concordantly, marine inundation of coastal regions may significantly reduce available habitat for terrestrial and freshwater species, making translocations necessary in the immediate future. The combination of these factors should be considered within management frameworks, particularly for species that may be impacted by both simultaneously.

ACKNOWLEDGEMENTS

We acknowledge researchers that provided samples or participated in field expeditions, especially Mark Adams. This work was supported by an Australian Research Council grant (FT130101068 to L.B.B.). This work received logistic support from Flinders University, University of Canberra and the South Australian Museum.

CONFLICT OF INTEREST

The authors have no conflict of interests to declare.

AUTHOR CONTRIBUTIONS

Sean Buckley contributed to all sections of data analysis as well as drafting the manuscript. Luciano B. Beheregaray designed and supervised the study, obtained resources, and helped with manuscript

drafting. Chris Brauer generated the data. Peter Unmack and Michael Hammer contributed with samples and field expertise. All authors contributed to the interpretation of results and critically revised the manuscript.

DATA AVAILABILITY STATEMENT

The sequence alignment and the SNP data set is available in Dryad (https://datadryad.org/stash/share/CnNGh_ualyt97us-yCB-HKX9zOVEg2H8sJvOx7p9FSY).

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REFERENCES

Amante, C., & Eakins, B. W. (2009). ETOPO1 1 Arc-Minute Global Relief Model: procedures, data sources and analysis. NOAA National Centers for Environmental Information. Accessed 07/12/2018.

Ansari, M. H., Cooper, S. J. B., Schwarz, M. P., Ebrahimi, M., Dolman, G., Reinberger, L., Saint, K. M., Donnellan, S. C., Bull, C. M., & Gardner, M. G. (2019). Plio-Pleistocene diversification and biogeographic barriers in southern Australia reflected in the phylogeography of a widespread and common lizard species. *Molecular Phylogenetics and Evolution*, *133*, 107–119. https://doi.org/10.1016/j.ympev.2018.12.014

Attard, C., Möller, L. M., Sasaki, M., Hammer, M. P., Bice, C. M., Brauer, C. J., Carvalho, D. C., Harris, J. O., & Beheregaray, L. B. (2016). A novel holistic framework for genetic-based captive-breeding and reintroduction programs. *Conservation Biology*, *30*(5), 1060–1069. https://doi.org/10.1111/cobi.12699

Balcombe, S. R., Sheldon, F., Capon, S. J., Bond, N. R., Hadwen, W. L., Marsh, N., & Bernays, S. J. (2011). Climate-change threats to native fish in degraded rivers and floodplains of the Murray-Darling Basin, Australia. *Marine and Freshwater Research*, *62*(9), 1099–1114. https://doi.org/10.1071/MF11059

Beheregaray, L. B., Attard, C. R., Brauer, C. J., Whiterod, N. S., Wedderburn, S. D., & Hammer, M. P. (2021). Conservation breeding and reintroduction of pygmy perches in the lower Murray-Darling Basin, Australia: Two similar species, two contrasting outcomes. In P. S. Soorae (Ed.), *Global conservation translocation perspectives: 2021. Case studies from around the globe* (pp. 26–31). IUCN SSC Conservation Translocation Specialist Group, Environment Agency.

Beheregaray, L. B., Pfeiffer, L. V., Attard, C. R. M., Sandoval-Castillo, J., Domingos, F. M. C. B., Faulks, L. K., Gilligan, D. M., & Unmack, P. J. (2017). Genome-wide data delimits multiple climate-determined species ranges in a widespread Australian fish, the golden perch (*Macquaria ambigua*). *Molecular Phylogenetics and Evolution*, *111*, 65–75. https://doi.org/10.1016/j.ympev.2017.03.021

Beheregaray, L. B., Sunnucks, P., & Briscoe, D. A. (2002). A rapid fish radiation associated with the last sea-level changes in southern Brazil: The silverside *Odontesthes perugiae* complex. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *269*(1486), 65–73. https://doi.org/10.1098/rspb.2001.1838

Blom, W. M., & Alsop, D. B. (1988). Carbonate mud sedimentation on a temperate shelf: Bass Basin, southeastern Australia. *Sedimentary Geology*, *60*(1), 269–280. https://doi.org/10.1016/0037-0738(88)90124-8

Blöschl, G., Hall, J., Viglione, A., Perdigão, R. A. P., Parajka, J., Merz, B., Lun, D., Arheimer, B., Aronica, G. T., Bilibashi, A., Boháč, M., Bonacci, O., Borga, M., Čanjevac, I., Castellarin, A., Chirico, G. B., Claps, P., Frolova, N., Ganora, D., … Živković, N. (2019). Changing climate both increases and decreases European river floods. *Nature*, *573*, 108–111. https://doi.org/10.1038/s41586-019-1495-6

Brauer, C. J., & Beheregaray, L. B. (2020). Recent and rapid anthropogenic habitat fragmentation increases extinction risk for freshwater biodiversity. *Evolutionary Applications*, *13*(10), 2857–2869. https://doi.org/10.1111/eva.13128

Brauer, C. J., Hammer, M. P., & Beheregaray, L. B. (2016). Riverscape genomics of a threatened fish across a hydroclimatically heterogeneous river basin. *Molecular Ecology*, *25*(20), 5093–5113. https://doi.org/10.1111/mec.13830

Brauer, C. J., Unmack, P. J., & Beheregaray, L. B. (2017). Comparative ecological transcriptomics and the contribution of gene expression to the evolutionary potential of a threatened fish. *Molecular Ecology*, *26*(24), 6841–6856. https://doi.org/10.1111/mec.14432

Brauer, C. J., Unmack, P. J., Smith, S., Bernatchez, L., & Beheregaray, L. B. (2018). On the roles of landscape heterogeneity and environmental variation in determining population genomic structure in a dendritic system. *Molecular Ecology*, *27*(17), 3484–3497. https://doi.org/10.1111/mec.14808

Brown, J. L., Bennett, J. R., & French, C. M. (2017). SDMtoolbox 2.0: The next generation Python-based GIS toolkit for landscape genetic, biogeographic and species distribution model analyses. *PeerJ*, *5*, e4095. https://doi.org/10.7717/peerj.4095

Brown, J. L., Hill, D. J., Dolan, A. M., Carnaval, A. C., & Haywood, A. M. (2018). PaleoClim, high spatial resolution paleoclimate surfaces for global land areas. *Scientific Data*, *5*(1), 180254. https://doi.org/10.1038/sdata.2018.254

Buckley, S. J., Domingos, F. M. C. B., Attard, C. R. M., Brauer, C. J., Sandoval-Castillo, J., Lodge, R., Unmack, P. J., & Beheregaray, L. B. (2018). Phylogenomic history of enigmatic pygmy perches: Implications for biogeography, taxonomy and conservation. *Royal Society Open Science*, *5*, e172125. https://doi.org/10.1098/rsos.172125

Byrne, M., Yeates, D. K., Joseph, L., Kearney, M., Bowler, J., Williams, M. A. J., Cooper, S., Donnellan, S. C., Keogh, J. S., Leys, R., Melville, J., Murphy, D. J., Porch, N., & Wyrwoll, K.-H. (2008). Birth of a biome: Insights into the assembly and maintenance of the Australian arid zone biota. *Molecular Ecology*, *17*, 4398–4417. https://doi.org/10.1111/j.1365-294X.2008.03899.x

Cai, W., & Cowan, T. (2008). Evidence of impacts from rising temperature on inflows to the Murray-Darling Basin. *Geophysical Research Letters*, *35*(7), L07701. https://doi.org/10.1029/2008gl033390

Carstens, B., Lemmon, A. R., & Lemmon, E. M. (2012). The promises and pitfalls of next-generation sequencing data in phylogeography. *Systematic Biology*, *61*(5), 713–715. https://doi.org/10.1093/sysbio/sys050

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks: An analysis tool set for population genomics. *Molecular Ecology*, *22*(11), 3124–3140. https://doi.org/10.1111/mec.12354

Chapple, D. G., Chapple, S. N. J., & Thompson, M. B. (2011). Biogeographic barriers in south-eastern Australia drive phylogeographic divergence in the garden skink, *Lampropholis guichenoti*. *Journal of Biogeography*, *38*(9), 1761–1775. https://doi.org/10.1111/j.1365-2699.2011.02531.x

Chapple, D. G., Keogh, J. S., & Hutchinson, M. N. (2005). Substantial genetic substructuring in southeastern and alpine Australia revealed by molecular phylogeography of the *Egernia whitii* (Lacertilia: Scincidae) species group. *Molecular Ecology*, *14*(5), 1279–1292. https://doi.org/10.1111/j.1365-294X.2005.02463.x

Christensen, J. H., Hewitson, B., Busuioc, A., Chen, A., Gao, X., Held, I., Jones, R., Kolli, R. K., Kwon, W. T., Laprise, R., Magana Rueda, V., Mearns, L., Menendez, C. G., Raisenen, J., Rinke, A., Sarr, A., & Whetton, P. (2007). Regional climate projections. In S. Solomon, D.

Qin, M. Manning, M. Marquis, K. Averyt, M. M. B. Tignor, H. LeRoy Miller, & Z. Chen (Eds.), *Climate Change 2007: The physical science basis. Contribution of working group 1 to the fourth assessment report of the Intergovernmental Panel on Climate Change* (pp. 847–940). Cambridge, UK: Cambridge University Press. https://www.ipcc.ch/report/ar4/wg1/

Cole, T. L., Hammer, M. P., Unmack, P. J., Teske, P. R., Brauer, C. J., Adams, M., & Beheregaray, L. B. (2016). Range-wide fragmentation in a threatened fish associated with post-European settlement modification in the Murray-Darling Basin, Australia. *Conservation Genetics*, *17*(6), 1377–1391. https://doi.org/10.1007/s10592-016-0868-8

Coleman, R. A., Pettigrove, V., Raadik, T. A., Hoffmann, A. A., Miller, A. D., & Carew, M. E. (2010). Microsatellite markers and mtDNA data indicate two distinct groups in dwarf galaxias, *Galaxiella pusilla* (Mack) (Pisces: Galaxiidae), a threatened freshwater fish from south-eastern Australia. *Conservation Genetics*, *11*(5), 1911–1928. https://doi.org/10.1007/s10592-010-0082-z

Cook, B. D., Baker, A. M., Page, T. J., Grant, S. C., Fawcett, J. H., Hurwood, D. A., & Hughes, J. M. (2006). Biogeographic history of an Australian freshwater shrimp, *Paratya australiensis* (Atyidae): The role life history transition in phylogeographic diversification. *Molecular Ecology*, *15*(4), 1083–1093. https://doi.org/10.1111/j.1365-294X.2006.02852.x

Cooper, S. J. B., Adams, M., & Labrinidis, A. (2000). Phylogeography of the Australian dunnart *Sminthopsis crassicaudata* (Marsupialia : Dasyuridae). *Australian Journal of Zoology*, *48*(5), 461–473. https://doi.org/10.1071/ZO00014

Cutter, A. D. (2013). Integrating phylogenetics, phylogeography and population genetics through genomes and evolutionary theory. *Molecular Phylogenetics and Evolution*, *69*(3), 1172–1185. https://doi.org/10.1016/j.ympev.2013.06.006

Davis, J., Pavlova, A., Thompson, R., & Sunnucks, P. (2013). Evolutionary refugia and ecological refuges: Key concepts for conserving Australian arid zone freshwater biodiversity under climate change. *Global Change Biology*, *19*(7), 1970–1984. https://doi.org/10.1111/gcb.12203

Dawson, T. P., Jackson, S. T., House, J. I., Prentice, I. C., & Mace, G. M. (2011). Beyond predictions: Biodiversity conservation in a changing climate. *Science*, *332*(6025), 53–58. https://doi.org/10.1126/science.1200303

Dickinson, J., Wallace, M., Holdgate, G., Gallagher, S., & Thomas, L. (2002). Origin and timing of the Miocene-Pliocene unconformity in southeast Australia. *Journal of Sedimentary Research*, *72*, 288–303. https://doi.org/10.1306/082701720288

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J. R. G., Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean, C., Osborne, P. E., Reineking, B., Schröder, B., Skidmore, A. K., Zurell, D., & Lautenbach, S. (2013). Collinearity: A review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, *36*(1), 27–46. https://doi.org/10.1111/j.1600-0587.2012.07348.x

Dubey, S., & Shine, R. (2010). Evolutionary diversification of the lizard genus *Bassiana* (Scincidae) across Southern Australia. *PLoS One*, *5*(9), e12982. https://doi.org/10.1371/journal.pone.0012982

Eaton, D. A. (2014). PyRAD: Assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics*, *30*(13), 1844–1849. https://doi.org/10.1093/bioinformatics/btu121

Edwards, S. V., Potter, S., Schmitt, C. J., Bragg, J. G., & Moritz, C. (2016). Reticulation, divergence, and the phylogeography-phylogenetics continuum. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(29), 8025–8032. https://doi.org/10.1073/pnas.1601066113

Elith, J., Phillips, S. J., Hastie, T., Dudík, M., Chee, Y. E., & Yates, C. J. (2011). A statistical explanation of MaxEnt for ecologists. *Diversity and Distributions*, *17*(1), 43–57. https://doi.org/10.1111/j.1472-4642.2010.00725.x

Excoffier, L., Dupanloup, I., Huerta-Sanchez, E., Sousa, V. C., & Foll, M. (2013). Robust demographic inference from genomic and SNP data. *PLOS Genetics*, *9*(10), e1003905. https://doi.org/10.1371/journal.pgen.1003905

Falkenmark, M. (2013). Adapting to climate change: Towards societal water security in dry-climate countries. *International Journal of Water Resources Development*, *29*(2), 123–136. https://doi.org/10.1080/07900627.2012.721714

Faulks, L. K., Gilligan, D. M., & Beheregaray, L. B. (2010). Clarifying an ambiguous evolutionary history: Range-wide phylogeography of an Australian freshwater fish, the golden perch (*Macquaria ambigua*). *Journal of Biogeography*, *37*(7), 1329–1340. https://doi.org/10.1111/j.1365-2699.2010.02304.x

Frankham, R., Ballou, J. D., Eldridge, M. D. B., Lacy, R. C., Ralls, K., Dudash, M. R., & Fenster, C. B. (2011). Predicting the probability of outbreeding depression. *Conservation Biology*, *25*(3), 465–475. https://doi.org/10.1111/j.1523-1739.2011.01662.x

Gallagher, S. J., Greenwood, D. R., Taylor, D., Smith, A. J., Wallace, M. W., & Holdgate, G. R. (2003). The Pliocene climatic and environmental evolution of southeastern Australia: Evidence from the marine and terrestrial realm. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *193*(3–4), 349–382. https://doi.org/10.1016/s0031-0182(03)00231-1

Gallagher, S. J., Smith, A. J., Jonasson, K., Wallace, M. W., Holdgate, G. R., Daniels, J., & Taylor, D. (2001). The Miocene palaeoenvironmental and palaeoceanographic evolution of the Gippsland Basin, Southeast Australia: A record of Southern Ocean change. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *172*(1), 53–80. https://doi.org/10.1016/S0031-0182(01)00271-1

Garrick, R. C., Sands, C. J., Rowell, D. M., Tait, N. N., Greenslade, P., & Sunnucks, P. (2004). Phylogeography recapitulates topography: Very fine-scale local endemism of a saproxylic 'giant' springtail at Tallaganda in the Great Dividing Range of south-east Australia. *Molecular Ecology*, *13*(11), 3329–3344. https://doi.org/10.1111/j.1365-294X.2004.02340.x

Grummer, J. A., Beheregaray, L. B., Bernatchez, L., Hand, B. K., Luikart, G., Narum, S. R., & Taylor, E. B. (2019). Aquatic landscape genomics and environmental effects on genetic variation. *Trends in Ecology & Evolution*, *34*(7), 641–654. https://doi.org/10.1016/j.tree.2019.02.013

Hammer, M. P., Bice, C. M., Hall, A., Frears, A., Watt, A., Whiterod, N. S., Beheregaray, L. B., Harris, J. O., & Zampatti, B. P. (2013). Freshwater fish conservation in the face of critical water shortages in the southern Murray-Darling Basin. *Australia. Marine and Freshwater Research*, *64*(9), 807–821. https://doi.org/10.1071/mf12258

Harrison, S. P. (1993). Late Quaternary lake-level changes and climates of Australia. *Quaternary Science Reviews*, *12*, 211–231. https://doi.org/10.1016/0277-3791(93)90078-Z

Hawlitschek, O., Hendrich, L., Espeland, M., Toussaint, E. F., Genner, M. J., & Balke, M. (2012). Pleistocene climate change promoted rapid diversification of aquatic invertebrates in Southeast Australia. *BMC Evolutionary Biology*, *12*(1), 142. https://doi.org/10.1186/1471-2148-12-142

Herbert, E. R., Boon, P., Burgin, A. J., Neubauer, S. C., Franklin, R. B., Ardón, M., Hopfensperger, K. N., Lamers, L. P. M., & Gell, P. (2015). A global perspective on wetland salinization: Ecological consequences of a growing threat to freshwater wetlands. *Ecosphere*, *6*, 206. https://doi.org/10.1890/ES14-00534.1

Hijmans, R. J., Cameron, S. E., Parra, J. L., Jones, P. G., & Jarvis, A. (2005). Very high resolution interpolated climate surfaces of global land areas. *International Journal of Climatology*, *25*, 1965–1978. https://doi.org/10.1002/joc.1276

Holdgate, G. R., Wallace, M. W., Gallagher, S. J., Smith, A. J., Keene, J. B., Moore, D., & Shafik, S. (2003). Plio-Pleistocene tectonics and eustasy in the Gippsland Basin, southeast Australia: Evidence from magnetic imagery and marine geological data.

Australian Journal of Earth Sciences, *50*(3), 403–426. https://doi.org/10.1046/j.1440-0952.2003.01004.x

Inoue, K., Monroe, E. M., Elderkin, C. L., & Berg, D. J. (2014). Phylogeographic and population genetic analyses reveal Pleistocene isolation followed by high gene flow in a wide ranging, but endangered, freshwater mussel. *Heredity*, *112*(3), 282–290. https://doi.org/10.1038/hdy.2013.104

Jordan, M. A., & Snell, H. L. (2008). Historical fragmentation of islands and genetic drift in populations of Galápagos lava lizards (*Microlophus albemarlensis* complex). *Molecular Ecology*, *17*(5), 1224–1237. https://doi.org/10.1111/j.1365-294X.2007.03658.x

Joseph, L., Dolman, G., Donnellan, S., Saint, K. M., Berg, M. L., & Bennett, A. T. (2008). Where and when does a ring start and end? Testing the ring-species hypothesis in a species complex of Australian parrots. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *275*(1650), 2431–2440. https://doi.org/10.1098/rspb.2008.0765

Kalinowski, S. T., Meeuwig, M. H., Narum, S. R., & Taper, M. L. (2008). Stream trees: A statistical method for mapping genetic differences between populations of freshwater organisms to the sections of streams that connect them. *Canadian Journal of Fisheries and Aquatic Sciences*, *65*(12), 2752–2760. https://doi.org/10.1139/f08-171

Kawakami, T., Butlin, R. K., Adams, M., Saint, K. M., Paull, D. J., & Cooper, S. J. (2009). Re-examination of a proposed case of stasipatric speciation: Phylogeography of the Australian morabine grasshoppers (*Vandiemenella viatica* species group). *Molecular Ecology*, *18*(16), 3429–3442. https://doi.org/10.1111/j.1365-294X.2009.04277.x

Koehn, J. D. (2013). Managing people, water, food and fish in the Murray-Darling Basin, south-eastern Australia. *Fisheries Management and Ecology*, *22*(1), 25–32. https://doi.org/10.1111/fme.12035

Kreger, K., Shaban, B., Wapstra, E., & Burridge, C. P. (2019). Phylogeographic parallelism: Concordance of patterns in closely related species illuminates underlying mechanisms in the historically glaciated Tasmanian landscape. *bioRxiv*, 548446. https://doi.org/10.1101/548446

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Lamb, A. M., Goncalves da Silva, A., Joseph, L., Sunnucks, P., & Pavlova, A. (2019). Pleistocene-dated biogeographic barriers drove divergence within the Australo-Papuan region in a sex-specific manner: An example in a widespread Australian songbird. *Heredity*, *123*, 608–621. https://doi.org/10.1038/s41437-019-0206-2

Lambeck, K., & Chappell, J. (2001). Sea level change through the last glacial cycle. *Science*, *292*(5517), 679–686. https://doi.org/10.1126/science.1059549

Liu, L., Xi, Z., & Davis, C. C. (2015). Coalescent methods are robust to the simultaneous effects of long branches and incomplete lineage sorting. *Molecular Biology and Evolution*, *32*(3), 791–805. https://doi.org/10.1093/molbev/msu331

Love Stowell, S. M., Pinzone, C. A., & Martin, A. P. (2017). Overcoming barriers to active interventions for genetic diversity. *Biodiversity and Conservation*, *26*(8), 1753–1765. https://doi.org/10.1007/s10531-017-1330-z

MacDonald, G. M., Bennett, K. D., Jackson, S. T., Parducci, L., Smith, F. A., Smol, J. P., & Willis, K. J. (2008). Impacts of climate change on species, populations and communities: Palaeobiogeographical insights and frontiers. *Progress in Physical Geography: Earth and Environment*, *32*(2), 139–172. https://doi.org/10.1177/0309133308094081

Matzke, N. (2013). *Probabilistic historical biogeography: New models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing*. PhD Thesis, University of California, Berkeley.

McGlashan, D. J., & 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. https://doi.org/10.1111/j.1095-8649.2001.tb01378.x

McInnes, K. L., Macadam, I., Hubbert, G., & O'Grady, J. (2011). An assessment of current and future vulnerability to coastal inundation due to sea-level extremes in Victoria, southeast Australia. *International Journal of Climatology*, *33*, 33–47. https://doi.org/10.1002/joc.3405

McLaren, S., & Wallace, M. W. (2010). Plio-Pleistocene climate change and the onset of aridity in southeastern Australia. *Global and Planetary Change*, *71*(1–2), 55–72. https://doi.org/10.1016/j.gloplacha.2009.12.007

McLaren, S., Wallace, M. W., Gallagher, S. J., Miranda, J. A., Holdgate, G. R., Gow, L. J., Snowball, I., & Sandgren, P. (2011). Palaeogeographic, climatic and tectonic change in southeastern Australia: The Late Neogene evolution of the Murray Basin. *Quaternary Science Reviews*, *30*(9–10), 1086–1111. https://doi.org/10.1016/j.quascirev.2010.12.016

McLaren, S., Wallace, M. W., & Reynolds, T. (2012). The Late Pleistocene evolution of palaeo megalake Bungunnia, southeastern Australia: A sedimentary record of fluctuating lake dynamics, climate change and the formation of the modern Murray River. *Palaeogeography, Palaeoclimatology, Palaeoecology*, *317–318*, 114–127. https://doi.org/10.1016/j.palaeo.2011.12.020

Middelkoop, H., Daamen, K., Gellens, D., Grabs, W., Kwadijk, J. C. J., Lang, H., Parmet, B. W. A. H., Schädler, B., Schulla, J., & Wilke, K. (2001). Impact of climate change on hydrological regimes and water resources management in the Rhine basin. *Climatic Change*, *49*(1), 105–128. https://doi.org/10.1023/a:1010784727448

Minh, B. Q., Hahn, M. W., & Lanfear, R. (2020). New methods to calculate concordance factors for phylogenomic datasets. *Molecular Biology and Evolution*, *37*(9), 2727–2733. https://doi.org/10.1093/molbev/msaa106

Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New models and efficient methods for phylogenetic inference in the genomic era. *Molecular Biology and Evolution*, *37*(5), 1530–1534. https://doi.org/10.1093/molbev/msaa015

Murphy, N. P., & Austin, C. M. (2004). Phylogeography of the widespread Australian freshwater prawn, *Macrobrachium australiense* (Decapoda, Palaemonidae). *Journal of Biogeography*, *31*(7), 1065–1072. https://doi.org/10.1111/j.1365-2699.2004.01105.x

Nakhleh, L. (2013). Computational approaches to species phylogeny inference and gene tree reconciliation. *Trends in Ecology & Evolution*, *28*(12), 719–728. https://doi.org/10.1016/j.tree.2013.09.004

Nash, S. G. (2000). A survey of truncated-Newton methods. *Journal of Computational and Applied Mathematics*, *124*(1), 45–59. https://doi.org/10.1016/S0377-0427(00)00426-X

Neal, W. C., James, E. A., & Bayly, M. J. (2019). Phylogeography, classification and conservation of pink zieria (*Zieria veronicea*; Rutaceae): Influence of changes in climate, geology and sea level in southeastern Australia. *Plant Systematics and Evolution*, *305*, 503–520. https://doi.org/10.1007/s00606-019-01589-z

Nei, M. (2001). Genetic distance. In S. Brenner, & J. H. Miller (Eds.), *Encyclopedia of genetics* (pp. 828–832). Academic Press.

Nijssen, B., O'Donnell, G. M., Hamlet, A. F., & Lettenmaier, D. P. (2001). Hydrologic sensitivity of global rivers to climate change. *Climatic Change*, *50*(1), 143–175. https://doi.org/10.1023/a:1010616428763

Norgate, M., Chamings, J., Pavlova, A., Bull, J. K., Murray, N. D., & Sunnucks, P. (2009). Mitochondrial DNA indicates late Pleistocene divergence of populations of *Heteronympha merope*, an emerging model in environmental change biology. *PLoS One*, *4*(11), e7950. https://doi.org/10.1371/journal.pone.0007950

Overpeck, J. T., & Weiss, J. L. (2009). Projections of future sea level becoming more dire. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(51), 21461–21462. https://doi.org/10.1073/pnas.0912878107

Pante, E., Puillandre, N., Viricel, A., Arnaud-Haond, S., Aurelle, D., Castelin, M., Chenuil, A., Destombe, C., Forcioli, D., Valero, M., Viard, F., & Samadi, S. (2015). Species are hypotheses: Avoid connectivity assessments based on pillars of sand. *Molecular Ecology*, *24*(3), 525–544. https://doi.org/10.1111/mec.13048

Papadopoulou, A., & Knowles, L. L. (2017). Linking micro- and macroevolutionary perspectives to evaluate the role of Quaternary sea-level oscillations in island diversification. *Evolution*, *71*(12), 2901–2917. https://doi.org/10.1111/evo.13384

Paradis, E., Claude, J., & Strimmer, K. (2004). APE: Analyses of phylogenetics and evolution in R language. *Bioinformatics*, *20*(2), 289–290. https://doi.org/10.1093/bioinformatics/btg412

Parent, C. E., Caccone, A., & Petren, K. (2008). Colonization and diversification of Galápagos terrestrial fauna: A phylogenetic and biogeographical synthesis. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *363*(1508), 3347–3361. https://doi.org/10.1098/rstb.2008.0118

Paulay, G., & Meyer, C. (2002). Diversification in the tropical pacific: Comparisons between marine and terrestrial systems and the importance of founder speciation. *Integrative and Comparative Biology*, *42*(5), 922–934. https://doi.org/10.1093/icb/42.5.922

Paz, A., Ibanez, R., Lips, K. R., & Crawford, A. J. (2015). Testing the role of ecology and life history in structuring genetic variation across a landscape: A trait-based phylogeographic approach. *Molecular Ecology*, *24*(14), 3723–3737. https://doi.org/10.1111/mec.13275

Pearce, L., Bice, C., Whiterod, N., & Raadik, T. (2019). Nannoperca australis. The IUCN Red List of Threatened Species 2019: e.T123358579A123382811. https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T123358579A123382811.en. Downloaded on 26 July 2021.

Pelletier, T. A., Crisafulli, C., Wagner, S., Zellmer, A. J., & Carstens, B. C. (2015). Historical species distribution models predict species limits in western *Plethodon* salamanders. *Systematic Biology*, *64*(6), 909–925. https://doi.org/10.1093/sysbio/syu090

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, *7*(5), e37135. https://doi.org/10.1371/journal.pone.0037135

Phillips, S. J., Anderson, R. P., Dudik, M., Schapire, R. E., & Blair, M. E. (2017). Opening the black box: An open-source release of Maxent. *Ecography*, *40*(7), 887–893. https://doi.org/10.1111/ecog.03049

Pinceel, T., Brendonck, L., Larmuseau, M. H. D., Vanhove, M. P. M., Timms, B. V., & Vanschoenwinkel, B. (2013). Environmental change as a driver of diversification in temporary aquatic habitats: Does the genetic structure of extant fairy shrimp populations reflect historic aridification? *Freshwater Biology*, *58*(8), 1556–1572. https://doi.org/10.1111/fwb.12137

Pittock, J., & Finlayson, C. M. (2011). Australia's Murray-Darling Basin: Freshwater ecosystem conservation options in an era of climate change. *Marine and Freshwater Research*, *62*(3), 232–243. https://doi.org/10.1071/MF09319

Pittock, J., Hansen, L. J., & Abell, R. (2008). Running dry: Freshwater biodiversity, protected areas and climate change. *Biodiversity*, *9*(3–4), 30–38. https://doi.org/10.1080/14888386.2008.9712905

Porter-Smith, R., Lyne, V. D., Kloser, R. J., & Lucieer, V. L. (2012). Catchment-based classification of Australia's continental slope canyons. *Marine Geology*, *303–306*, 183–192. https://doi.org/10.1016/j.margeo.2012.01.008

Razgour, O., Forester, B., Taggart, J. B., Bekaert, M., Juste, J., Ibáñez, C., Puechmaille, S. J., Novella-Fernandez, R., Alberdi, A., & Manel, S. (2019). Considering adaptive genetic variation in climate change vulnerability assessment reduces species range loss projections. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(21), 10418–10423. https://doi.org/10.1073/pnas.1820663116

Ronquist, F. (1997). Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. *Systematic Biology*, *46*(1), 195–203. https://doi.org/10.1093/sysbio/46.1.195

Rosauer, D. F., Catullo, R. A., VanDerWal, J., Moussalli, A., & Moritz, C. (2015). Lineage range estimation method reveals fine-scale endemism linked to Pleistocene stability in Australian rainforest herpetofauna. *PLoS One*, *10*(5), e0126274. https://doi.org/10.1371/journal.pone.0126274

Rotzoll, K., & Fletcher, C. H. (2012). Assessment of groundwater inundation as a consequence of sea-level rise. *Nature Climate Change*, *3*, 477–481. https://doi.org/10.1038/nclimate1725

Sanderson, M. J. (2003). r8s: Inferring absolute rates of molecular evolution and divergence times in the absence of a molecular clock. *Bioinformatics*, *19*(2), 301–302. https://doi.org/10.1093/bioinformatics/19.2.301

Schultz, M. B., Ierodiaconou, D. A., Smith, S. A., Horwitz, P., Richardson, A. M., Crandall, K. A., & Austin, C. M. (2008). Sea-level changes and palaeo-ranges: Reconstruction of ancient shorelines and river drainages and the phylogeography of the Australian land crayfish *Engaeus sericatus* Clark (Decapoda: Parastacidae). *Molecular Ecology*, *17*(24), 5291–5314. https://doi.org/10.1111/j.1365-294X.2008.03996.x

Stamatakis, A. (2014). RAxML version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, *30*(9), 1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Sunnucks, P., & Hales, D. F. (1996). Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). *Molecular Biology and Evolution*, *13*(3), 510–524. https://doi.org/10.1093/oxfordjournals.molbev.a025612

Swofford, D. L. (2002). *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4.0b10 (Vol. Version 4.0)*. Sinauer Associates.

Thomaz, A. T., Malabarba, L. R., & Knowles, L. L. (2017). Genomic signatures of paleodrainages in a freshwater fish along the southeastern coast of Brazil: Genetic structure reflects past riverine properties. *Heredity*, *119*(4), 287–294. https://doi.org/10.1038/hdy.2017.46

Title, P. O., & Bemmels, J. B. (2018). ENVIREM: An expanded set of bioclimatic and topographic variables increases flexibility and improves performance of ecological niche modeling. *Ecography*, *41*(2), 291–307. https://doi.org/10.1111/ecog.02880

Unmack, P. J. (2001). Biogeography of Australian freshwater fishes. *Journal of Biogeography*, *28*(9), 1053–1089. https://doi.org/10.1046/j.1365-2699.2001.00615.x

Unmack, P. J., Hammer, M. P., Adams, M., Johnson, J. B., & Dowling, T. E. (2013). The role of continental shelf width in determining freshwater phylogeographic patterns in south-eastern Australian pygmy perches (Teleostei: Percichthyidae). *Molecular Ecology*, *22*(6), 1683–1699. https://doi.org/10.1111/mec.12204

Waller, N. L., Gynther, I. C., Freeman, A. B., Lavery, T. H., & Leung, L.-K.-P. (2017). The Bramble Cay melomys *Melomys rubicola* (Rodentia: Muridae): A first mammalian extinction caused by human-induced climate change? *Wildlife Research*, *44*(1), 9–21. https://doi.org/10.1071/WR16157

Wallis, G. P., Cameron-Christie, S. R., Kennedy, H. L., Palmer, G., Sanders, T. R., & Winter, D. J. (2017). Interspecific hybridization causes long-term phylogenetic discordance between nuclear and mitochondrial genomes in freshwater fishes. *Molecular Ecology*, *26*(12), 3116–3127. https://doi.org/10.1111/mec.14096

Waters, J. M. (2008). Marine biogeographical disjunction in temperate Australia: Historical landbridge, contemporary currents, or both? *Diversity and Distributions*, *14*(4), 692–700. https://doi.org/10.1111/j.1472-4642.2008.00481.x

Waters, J. M., Burridge, C. P., & Craw, D. (2019). The lasting biological signature of Pliocene tectonics: Reviewing the re-routing of Australia's largest river drainage system. *Journal of Biogeography*, *46*, 1494–1503. https://doi.org/10.1111/jbi.13612

Waters, J. M., Craw, D., Youngson, J. H., & Wallis, G. P. (2001). Genes meet geology: Fish phylogeographic pattern reflects ancient, rather than modern, drainage connections. *Evolution*, *55*(9), 1844–1851. https://doi.org/10.1111/j.0014-3820.2001.tb00833.x

Wedderburn, S. D., Hammer, M. P., & Bice, C. M. (2012). Shifts in small-bodied fish assemblages resulting from drought-induced water level recession in terminating lakes of the Murray-Darling Basin, Australia. *Hydrobiologia*, *691*(1), 35–46. https://doi.org/10.1007/s10750-011-0993-9

White, M. E. (2000). *Running down: Water in a changing land*. Kangaroo Press.

Williams, A. N., Ulm, S., Sapienza, T., Lewis, S., & Turney, C. S. M. (2018). Sea-level change and demography during the last glacial termination and early Holocene across the Australian continent. *Quaternary Science Reviews*, *182*, 144–154. https://doi.org/10.1016/j.quascirev.2017.11.030

Williams, W. D. (1995). Lake Corangamite, Australia, a permanent saline lake: Conservation and management issues. *Lakes & Reservoirs: Science, Policy and Management for Sustainable Use*, *1*(1), 55–64. https://doi.org/10.1111/j.1440-1770.1995.tb00006.x

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Buckley, S. J., Brauer, C., Unmack, P. J., Hammer, M. P., & Beheregaray, L. B. (2021). The roles of aridification and sea level changes in the diversification and persistence of freshwater fish lineages. *Molecular Ecology*, 00, 1–18. https://doi.org/10.1111/mec.16082