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Genome-wide data delimits multiple climate-determined species ranges in a widespread Australian fish, the golden perch (*Macquaria ambigua*)

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ABSTRACT

Species range limits often fluctuate in space and time in response to variation in environmental factors and to gradual niche evolution due to changes in adaptive traits. We used genome-wide data to investigate evolutionary divergence and species range limits in a generalist and highly dispersive fish species that shows an unusually wide distribution across arid and semi-arid regions of Australia. We generated ddRAD data (18,979 filtered SNPs and 1.725 million bp of sequences) for samples from 27 localities spanning the native range of golden perch, *Macquaria ambigua* (Teleostei; Percichthyidae). Our analytical framework uses population genomics to assess connectivity and population structure using model-based and model-free approaches, phylogenetics to clarify evolutionary relationships, and a coalescent-based Bayesian species delimitation method to assess statistical support of inferred species boundaries. Addressing uncertainties regarding range limits and taxonomy is particularly relevant for this iconic Australian species because of the intensive stocking activities undertaken to support its recreational fishery and its predicted range shifts associated with ongoing climate change. Strong population genomic, phylogenetic, and coalescent species delimitation support was obtained for three separately evolving metapopulation lineages, each lineage should be considered a distinct cryptic species of golden perch. Their range limits match the climate-determined boundaries of main river basins, despite the ability of golden perch to cross drainage divides. We also identified cases suggestive of anthropogenic hybridization between lineages due to stocking of this recreationally important fish, as well as a potential hybrid zone with a temporally stable pattern of admixture. Our work informs on the consequences of aridification in the evolution of aquatic organisms, a topic poorly represented in the literature. It also shows that genome-scale data can substantially improve and rectify inferences about taxonomy, hybridization and conservation management previously proposed by detailed genetic studies.

1. Introduction

Understanding historical and contemporary factors that influence species range limits is an important topic in evolution and ecology, and is also becoming increasingly relevant for the conservation management of biodiversity. The study of geographic ranges involves multiple aspects of species distribution and abundance, including how spatial variation in the fit between phenotype and environment, and how adaptation to past and novel environments, result in population-level differences, niche evolution and evolutionary diversification (reviewed in Sexton et al., 2009). From a conservation perspective, we are currently witnessing multiple shifts in the distribution of marine, freshwater, and terrestrial groups, which suggest that climate change is driving a global biological response in biogeographic patterns (Parmesan, 2006; Brown et al., 2016). Therefore, clarifying past- and present-day patterns of species distribution, and improving predictions about future range shifts (e.g. Burrows et al., 2014), can contribute to informing effective conservation management under different environmental scenarios.

Species range limits are often not fixed, they fluctuate substantially in space and time (MacArthur, 1972). These fluctuations might reflect spatial niche tracking due to variation in environ-

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mental factors (e.g. climate), or they might be related to gradual niche evolution due to changes in adaptive traits (e.g. dispersal) caused by natural selection (Sexton et al., 2009). These non-mutually exclusive possibilities delineate species abilities to persist during climatic shifts and to disperse and colonize new environments (Sexton et al., 2009; Auer and King, 2014). Ecologically generalist species usually show low physiological limitations and high potential for dispersal, colonization of new habitats and population persistence (DeWitt et al., 1998; Auer and King, 2014). Here we use genome-wide data to investigate species range limits and evolutionary divergence in a generalist and highly dispersive fish taxon that shows an unusually wide distribution across river basins of Australia.

River basins are fundamental units in aquatic biogeography that define spatial and temporal scopes of habitat and evolutionary opportunities available for obligate aquatic organisms since they are only able to move via direct connections between habitats (e.g. Unmack, 2014). Australian river basins provide an ideal setting for assessing the influence of historical and contemporary factors – including changes in climate – in shaping biogeographic history. The sedimentary basins of Australia have an ancient geomorphological history, with the main contemporary river basins formed over 50 million years ago (Ma) (Veevers, 1991). Although Australia has the lowest relief of any continent, it has probably experienced less tectonic activity than other major land masses during the last 80 My (Unmack, 2001). Therefore, drainage rearrangements resulting in changed catchment boundaries and physical connectivity between drainage divides are not expected. Alternatively, obligate aquatic organisms in Australia could move across low drainage divides during high rainfall and flooding periods. Today, most of Australia's freshwater ecosystems are in arid or semi-arid environments, but environmental conditions were very different in the past (reviewed in Byrne et al., 2008). During most of the Cenozoic the climate was warm and moist, drainages were connected more often, and the continent was dominated by temperate and tropical rainforests. However, in the late Miocene (~6–10 Ma) Australia began to experience a gradual transition towards arid and fluctuating climate conditions, with fully arid landforms appearing in central Australia around 1–4 Ma. The continent then experienced highly cyclical warm/moist and cool/dry conditions typical of the periodic Pleistocene (~2 Ma) and progressive drying. Although unpredictable floods are still a major feature of Australia's climate, its aridification resulted in loss of large inland freshwater lakes and overall reductions in riverine connectivity (Byrne et al., 2008).

Obligate freshwater organisms have probably experienced unique biogeographic constraints associated with the intensive drying and fragmentation of aquatic habitats in Australia. A conflicting perspective to the climatic-driven isolation of river basins in Australia and their freshwater faunas is provided by the few species naturally found across drainage divides (reviewed in Unmack, 2014). Establishing the mechanism(s) underpinning these unusual species range limits is challenging, especially when species are found across multiple biogeographical provinces and river basins, as is the case of our study system, the golden perch *Macquaria ambigua*.

The native range of golden perch spans multiple drainage divides across over 2.2 million km^2 of central-eastern Australia, including all of its inland river basins and its largest coastal basin (Allen et al., 2002; Fig. 1). This medium-large sized migratory species is naturally found in three main river basins, namely the Murray-Darling (MDB), Lake Eyre (LEB), and Fitzroy (FITZ) basins, and also in the Bulloo-Bancannia (BULL) – a small basin located between the MDB and LEB. Because of its preeminent importance in recreational fisheries, translocated populations exist in various catchments and the species is intensively stocked into impoundments and rivers, particularly in the MDB (Lintermans, 2007; Faulks et al., 2010a,b). It has an opportunistic diet and shows broad physiological constraints, being found across several climatic environments and in a variety of arid and semi-arid habitats (Pusey et al., 2004). Golden perch has high dispersal abilities during all life history stages, with records of individual movements larger than 2000 km (Reynolds, 1983). Reproductive migration upstream and spawning are triggered by increasing water temperatures and flow levels during spring (Roberts et al., 2008). Eggs are released and fertilized during aggregation spawning events in the upper reaches of rivers and then drift downstream (Lake, 1967). A riverscape genetics study (Faulks et al., 2010b) supports the key role of hydrological variability in shaping dispersal and persistence of golden perch. It showed that contemporary genetic variation is positively correlated with spring-time flow volume, which stimulates recruitment booms, population size and gene flow over seasonally disconnected regions. Because of its generalist life-history and high dispersal, golden perch is one of the few freshwater species in Australia predicted to experience region-wide increases in abundance and range expansion associated with climate change (Bond et al., 2011).

Although golden perch from different river basins are currently classified as a single taxon (*M. ambigua*), its taxonomy is controversial. Population level studies based on ten morphological characters (Musyl, 1990), six variable allozyme loci (Musyl and Keenan, 1992), and eight microsatellite loci (Faulks et al., 2010b) detected moderate to high differentiation among populations from different river basins, with one study suggesting that golden perch from the LEB represents a cryptic species (Musyl and Keenan, 1992). However, a phylogenetic analysis based on mitochondrial DNA (mtDNA) (Faulks et al., 2010a) provided a different view about evolutionary relationships. It detected two reciprocally monophyletic clades: one represented by a coastal FITZ population and another including all inland populations (MDB, LEB and BULL).

Here, we explore features of the golden perch system and Australian river basins to assess range limits and evolutionary history in a widespread arid and semi-arid zone fish. We used a genotype-by-sequencing method to generate genome-wide data for individuals spanning the native range of golden perch. Our final genomic datasets (18,979 single nucleotide polymorphisms (SNPs) and 1.725 million bp of sequences) are several orders of magnitude larger than previous golden perch genetic datasets and we expect them to deliver greater power for clarifying population differentiation, genealogical divergence and species boundaries (Luikart et al., 2003; Pante et al., 2015). Our analytical framework uses population genomics to assess population structure and connectivity, phylogenetics to clarify evolutionary relationships, and a coalescent-based Bayesian species delimitation method to assess statistical support of inferred species boundaries. We integrate results across these three approaches to test if the spatial distribution of genome-wide variation in this taxon is better explained by isolation associated with climate-determined drainage basin evolution, or by high dispersal. The climate-determined hypothesis predicts that aridification and associated loss of direct connections between habitats would result in multiple separately evolving metapopulation lineages. On the other hand, the high dispersal hypothesis predicts that flood events would often connect low relief areas and result in a wide-ranging metapopulation lineage. Addressing uncertainties regarding range limits and taxonomy is particularly relevant for this iconic Australian species because of the intensive stocking activities undertaken to support its recreational fishery and its predicted range shifts associated with ongoing climate change. To the best of our knowledge, this represents the first genome-wide population study of a widespread arid zone fish, and as such it makes an original contribution to our general

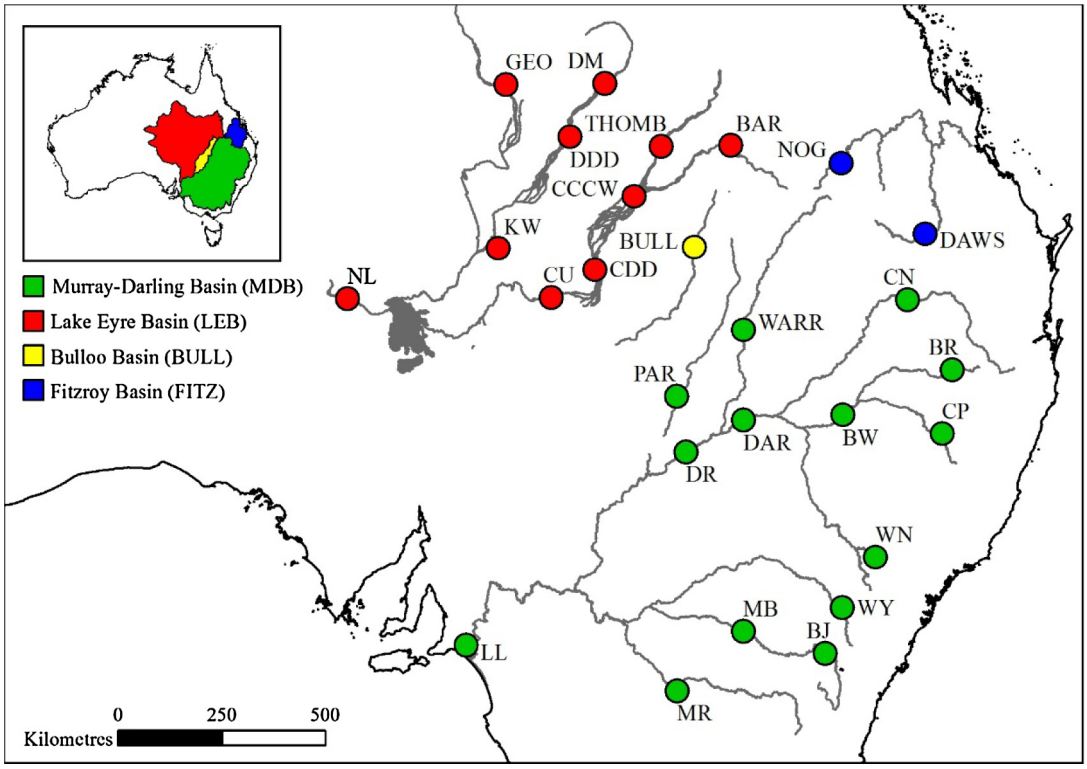

Fig. 1. Sampled localities from across the natural range of golden perch in central, eastern and southeastern Australia. Locality abbreviations follow Table 1.

understanding of the consequences of aridification on evolutionary diversification in aquatic biotas.

2. Material and methods

2.1. Sampling and DNA extraction

Caudal fin tissues from 603 golden perch were collected from across the species range (Fig. 1) using electrofishing, gill-, fyke- or seine-netting. These encompass 28 populations from 27 localities (Table 1). Samples include all fish used in Faulks et al. (2010a,b) plus three key novel populations: a sample from the Lower Lakes representing the population from the terminal region of the Murray River in the MDB, a sample from the Dawson River that enables assessment of structure and stocking within the FITZ basin, and a 2014 sample from the Bulloo River locality originally sampled in 2006. Samples were stored at −80 °C at the South Australian Museum, or in 99% ethanol at Flinders University. Four individuals and one replicate for each of the two closest living relatives of golden perch, Macquarie perch (*Macquaria australasica*) and Bloomfield River cod (*Guyu wujalwujalensis*), were used as outgroups (Jerry et al., 2001) for phylogenetics and species delimitation. Total DNA was extracted using a modified salting-out method (Sunnucks and Hales, 1996) or a Qiagen DNeasy kit (Qiagen Inc., Valencia, CA, USA). Genomic DNA was checked for quality using a spectrophotometer (NanoDrop, Thermo Scientific), integrity using 2% agarose gels, and quantity using a fluorometer (Qubit, Life Technologies).

2.2. ddRAD library preparation and bioinformatics

From the 603 fish, a subset of 105 was selected for genomic analysis. These were samples that passed stringent requirements of DNA quality and quantity for library preparation, while also including 2–3 individuals per locality to cover the range of golden perch and all previously identified populations (Faulks et al., 2010a,b) and 8 outgroup samples. This resulted in an average of 24 (16–39) individuals sequenced per basin. Quality and reproducibility of libraries and sequencing was assessed by running 15 individual duplicates (14% of all samples); these covered all basins, 13 localities and outgroups (Table 1).

Double-digest Restriction-site Associated DNA (ddRAD) sequencing libraries were prepared in house following Peterson et al. (2012), with modifications as described in Brauer et al. (2016). This involved digestion using *Sbf*I and *Mse*I and ligation of DNA adapters to fragments. We size selected for fragments between 300 and 700 bp with a 1.5% Pippin prep electrophoresis gel (Sage Science). Libraries were checked for fragment size range using electrophoresis and flow cytometry (Bioanalyzer, Agilent), quantity using real-time PCR (StepOne, Applied Biosystems), and quality using a spectrophotometer (NanoDrop, Thermo Scientific). Libraries were multiplexed with 48 samples randomly assigned to each lane and sequenced on four HiSeq2000 lanes as paired-end, 100 bp reads at Génome Québec, Canada.

Raw sequences were demultiplexed and trimmed using 'pro cess_radtags.pl' in the STACKS 1.19 pipeline (Catchen et al., 2013). Reads with ambiguous barcodes and/or restriction sites were eliminated allowing up to two mismatches. Remaining reads were trimmed to 80 bp (forward and reverse reads) by removing barcodes (forward reads only), restriction sites and the last 8 bp (forward reads) and 17 bp (reverse reads). Demultiplexed reads were processed using the pyRAD 3.0 pipeline (Eaton, 2014). First, bases with a Phred quality score below 30 were replaced with N, and sequences having more than 4 Ns (5%) were discarded. Filtered reads were clustered by 80% threshold similarity, and just clusters with >10× coverage per individual, <10% missing data, and <0.6 observed heterozygosity were retained as loci. These ddRAD loci were used to generate a concatenated sequence matrix for phylogenomics. Heterozygous sites were coded with standard ambigu-

Table 1
The 27 sampling localities from across the natural range of golden perch. The locality in the Bulloo River includes two temporal samples. *n* is the number of individuals used for data analysis after passing all ddRAD filtering steps. Asterisk denotes samples where one individual was replicated to assess reproducibility of genomic libraries and Illumina sequencing. NA: not available. BULL: Bulloo Basin; FITZ: Fitzroy Basin; LEB: Lake Eyre Basin; MDB: Murray-Darling Basin. Macquarie perch and Bloomfield River cod were used as outgroups.

Basin	Locality (abbreviation)	*n*	Latitude	Longitude	Date sampled
BULL	Bulloo R (BULL)	7*	−26.3822	144.2986	October-2006
BULL	Bulloo R (BULL)	7*	−26.3842	144.2985	May-2014
FITZ	Dawson R (DAWS)	6*	−25.5744	149.8622	September-2012
FITZ	Nogoa R (NOG)	10*	−24.2417	147.645	October-2006
LEB	Barcoo R (BAR)	2	−24.0833	144.9667	October-2006
LEB	Georgina R (GEO)	2	−23.0717	139.5128	October-2006
LEB	Neales R (NL)	2*	−27.8922	135.8222	April-2002
LEB	Thomson R (THOMB)	2*	−24.2256	143.2997	October-2006
LEB	Diamantina R (DM)	2*	−22.9314	141.8633	October-2006
LEB	Davenport Downs (DDD)	2	−24.156	141.1007	May-2014
LEB	Warburton R (KW)	2	−26.6883	139.5055	May-2014
LEB	Cooper Ck (CU)	2*	−27.7047	140.8692	May-2014
LEB	Cooper Ck (CCCW)	2*	−25.37	142.7446	May-2014
LEB	Cooper Ck (CDD)	2	−27.0382	141.9029	May-2014
MDB	Lower Lakes (LL)	11*	−35.4167	139.1167	April-2015
MDB	Condamine R (CN)	2	−27.05	149.6333	May-2006
MDB	Murray R (MR)	2	−36.1221	144.8078	March-2005
MDB	Murrumbidgee R (MB)	2	−34.7035	146.4203	September-2004
MDB	Burrinjuck Dam (BJ)	2	−34.9833	148.6333	NA
MDB	Wyangala Dam (WY)	2	−33.95	148.9333	May-2006
MDB	Windamere Dam (WN)	2	−32.7667	149.6333	NA
MDB	Copeton Dam (CP)	2*	−29.8833	150.9167	NA
MDB	Border R (BR)	2	−28.459	150.9553	April-2005
MDB	Barwon R (BW)	2	−29.7531	148.3782	February-2005
MDB	Darling R (DR)	2	−30.8951	144.5165	May-2005
MDB	Warrego R (WARR)	1*	−28.117	145.6861	May-2014
MDB	Paroo R (PAR)	2	−29.6975	144.1589	September-2013
MDB	Darling R (DAR)	2*	−30.0868	145.8930	May-2014
Outgroup	Macquarie perch	1*	−33.8681	150.2607	May-2005
Outgroup	Macquarie perch	1	−36.1595	149.0044	April-2012
Outgroup	Macquarie perch	1	−34.0878	149.5510	May-2008
Outgroup	Macquarie perch	1	−36.8796	145.6198	April-2009
Outgroup	Bloomfield River cod	4*	NA	NA	NA

ity codes. For population genomic analyses only one SNP per locus was selected; for a locus with multiple SNPs the site with the least missing data across samples was selected, if several SNPs have the same missing data, we selected the first SNP in the locus. Despite the potential caveats of concatenation in phylogenetic inferences (Edwards, 2009), recent simulations have shown that concatenation and species tree methods can produce very similar results under a range of different conditions (Tonini et al., 2015). Topological congruence has also been found for concatenated and species tree inferences in empirical studies using next-generation sequencing data (Domingos et al., 2017; Tucker et al., 2016). Furthermore, phylogenomic species trees appear to be highly influenced by topological incongruent gene trees, while concatenation is not particularly affected (Manthey et al., 2016; Pyron et al., 2014).

2.3. Genome-wide diversity and population genomic structure

For each basin sample and basin sub-region (Supplementary Table S1), the SNPs were used to calculate nucleotide diversity (π), expected heterozygosity (He) and percentage of polymorphic SNPs (% poly) in ARLEQUIN 3.5 (Excoffier and Lischer, 2010). Population differentiation was calculated between basins and sub-regions using F_{ST} in ARLEQUIN. An Analysis of Molecular Variance based on F_{ST} (AMOVA) was performed in ARLEQUIN to assess hierarchical population structure among river basins and between sub-regions within basins. Significance for all tests was assessed using 10,000 permutations.

Genetic structure was also explored using the model-based fastSTRUCTURE (Raj et al., 2014) and the model-free Discriminant Analysis of Principal Components (DAPC) using the package ADEGENET 2.0 (Jombart, 2008) in R 3.1.0 (R Core Team 2014). FastSTRUCTURE was run with 10 replicates of 1 million iterations for each value of the number of populations (K), with 'simple prior'. This uses a variational Bayesian framework under a model assuming Hardy-Weinberg equilibrium and linkage equilibrium. The program 'chooseK.py' was used to determine the value of K that best explained structure underlying the data. In contrast, DAPC makes no assumptions about population models (Jombart, 2008). It defines synthetic variables in which genetic variation is maximized between clusters of individuals (K) and minimized within clusters. K-means clustering and the Bayesian Information Criterion were used to identify the best-supported number of clusters.

2.4. Phylogenomics and divergence time estimates

The concatenated ddRAD sequences were used to reconstruct phylogenetic relationships using a maximum likelihood (ML) method in RAxML 8.1.1 (Stamatakis, 2014). A thorough search for the best-scoring ML tree was done using rapid hill-climbing and rapid bootstrapping with a GTRGAMMA model and 1000 replicates.

Although it would be informative to obtain a temporal perspective for inferred genealogical divergences, the lack of dated biogeographic events and fossils does not enable a calibration of the phylogenomic tree. Moreover, we are not aware of precise information about a general mutation rate for teleost ddRAD loci. To tentatively estimate divergence times, we used previous divergence estimates among golden perch populations (Faulks et al., 2010a) to calibrate the nodes of a ddRAD ML tree using r8s

(Sanderson, 2003). Robust branch length estimates were obtained after 20 independent ML tree searches run on 20 randomized maximum parsimony starting trees. The tree with the best GAMMA-based likelihood score was selected to do a ML search. Analyses were run using RAxML 8.1.1 and parameters estimated as above. Two nodes were constrained: the most recent common ancestor (MRCA) of FITZ and remaining lineages (maximum age 1.7 Ma and minimum 0.6 Ma), and the MRCA of MDB and LEB (maximum 0.28 Ma and minimum 0.1 Ma). The age of the root was free to vary. r8s was run using the penalized likelihood algorithm after selecting an optimal smoothing parameter by cross-validation. We acknowledge the importance of coalescent estimations in phylogenomic studies (Edwards et al., 2016); however, our large dataset makes it intractable to estimate divergence times using a full coalescent analysis (e.g., *Beast). Given that external calibrations have been successfully used in recent phylogenomic studies (Gottscho et al., 2017), that branch length estimations are robust when a large independent set of loci are used (Blom et al., 2016; Manthey et al., 2016), and considering that our coalescent species tree had the same topology of the concatenated ML tree (see Results), we believe that our methods are sufficiently robust in relation to tree inference and divergence times estimates.

2.5. Coalescent species delimitation

BPP 3.2 (Yang and Rannala, 2010, 2014) was used to test the hypothesis that golden perch from each of the three main basins (MDB, LEB, FITZ) and the intermediate BULL correspond to different species. BBP 3 simultaneously estimates a species tree while running the reversible-jump MCMC species delimitation algorithm, eliminating concerns about over-estimating species limits (Leaché and Fujita, 2010). Briefly, BPP estimates a species tree using a subtree pruning and regrafting algorithm (Rannala and Yang, 2015), while species hypotheses are tested by collapsing branches of different species trees and comparing their posterior probabilities.

BPP requires independent loci alignments as input, so each cleaned and trimmed RAD alignment was used as a locus. Because of computational constraints, four individuals were selected to represent each basin (Supplementary Figs. S1 and S2). Previous studies have shown that correct species delimitation can be achieved even with one individual per species, given that enough loci are sampled (Zhang et al., 2011). Two *Macquaria australasica* and one *Guyu wujalwujalensis* samples were used as outgroups. After initial trials using different priors, a gamma prior of ~G (1100) for population size (θs) and ~G (2100) for the age of the root in the species tree (τ_0) were used, and the Dirichlet prior (Yang and Rannala, 2010) for other divergence time parameters. The gamma prior G (α, β) has mean α/β, so the theta prior ~G (1100) corresponds to one difference per mega base (0.01), while the tau prior ~G (2100) corresponds to 2% sequence divergence (0.02). Priors correspond to a relatively small population size and shallow divergence times, a meaningful scenario for the taxon (Faulks et al., 2010a). Moreover, these are empirically consistent: evaluating convergence on BPP depends on how similar the results from independent runs are (Yang, 2015). Other priors delivered very dissimilar results in independent runs, while our chosen priors returned consistent results in almost every run. Analyses were run for 5×10^5 MCMC generations, taking samples every five, and using 1×10^4 burn-in generations. Both available species delimitation algorithms were used (Yang and Rannala, 2010). Ambiguous sites were excluded (cleandata = 1) to conform with the multi-species coalescent (MSC) model. Each analysis was done using at least three independent runs starting at random tree models.

Population-level analyses indicated that Bulloo Basin (BULL) potentially represents a hybrid zone (Results). This can negatively impact phylogenomic and species delimitation analyses. Since the MSC model implemented in BPP does not account for migration, BULL was sometimes retrieved as a full species, sometimes forming a species with LEB, and less often forming a species with LEB and MDB. Thus, all phylogenomic and species delimitation analyses were repeated after excluding BULL from the alignment.

3. Results

3.1. Sequence characteristics

A total of ~148 billion bp of data were obtained. Ninety-seven percent of sequenced reads passed quality filtering, with an average of 5.9 million reads retained per individual. After alignment, 20,391 ddRAD loci were retained in >90% of individuals. A subset of filtered 18,979 putatively unlinked SNPs was retained for population genomic analyses. For phylogenomics, the final concatenated dataset yielded 1.725 million bp (from the 20,391 loci), with 113,698 variable and 85,606 parsimony-informative sites (Supplementary Table S2).

3.2. Marked population differentiation among basins

Genetic diversity measured as nucleotide diversity and heterozygosity varied little across basins, except for MDB which showed the lowest levels despite having the largest sample (Supplementary Table S1). The Dawson River (FITZ) showed higher heterozygosity, which might be due to hybridization (below).

Golden perch shows high population differentiation across its distribution, with high F_{ST} detected among river basins populations (0.261–0.589; all $P < 0.001$; Supplementary Table S3). Accordingly, the F_{ST}-based AMOVA indicated that a much larger proportion of genetic variation is explained by differences among (46.6%) rather than within (4.7%) basins (Table 2). Low to moderate, but yet statistically significant differentiation was also detected between sub-regions within the LEB ('western' Diamantina versus 'eastern' Cooper groups) and within the FITZ basin (Dawson River versus Nogoa River) (Supplementary Table S4).fastSTRUCTURE identified three population clusters that correspond to the three major river basins: LEB, MDB and FITZ (Fig. 2). All sampled golden perch showed a clear membership (i.e. typically 100%) to the basin where they were sampled, but there were a few exceptions. All BULL individuals showed intermediate ancestry to LEB and MDB, a finding that suggests admixture in the BULL region. The other distinct patterns likely relate to human-induced movement of MDB fish into the Dawson River (a sub-basin of FITZ) and to very low levels of admixture between basins seen in six individuals. The DAPC results were in marked agreement with those from fastSTRUCTURE, with three clearly distinct population clusters corresponding to LEB, MDB and FITZ. They were also generally consistent with the above-mentioned cases of admixture (Fig. 3).

3.3. Phylogenomic distinctiveness among recently diverged lineages

The ML phylogenetic reconstruction identified three reciprocally monophyletic clades, each restricted to one of the three major river basins and supported by 100% bootstrap (Fig. 4). The membership for individuals from each clade matched exactly their SNP assignments to the FITZ, LEB and MDB groups. The first split in the phylogeny separated the coastal basin (FITZ clade) from the inland basins (LEB and MDB), whereas the next split separated the MDB and LEB clades. As expected due to their admixtured nature (above), individuals from BULL were not reciprocally monophyletic but were more related to the LEB clade (Supplementary Fig. S1).

Table 2
Analysis of Molecular Variance (AMOVA) for golden perch based on 18,979 SNPs shared among all sampled localities.

	d.f.	Sum of squares	Variance component	% of variation		
Among basins	3	27722.2	186.21644	46.58	F_{CT} = 0.466	p = 0.023
Among subdivisions within basins	2	1112.99	18.87775	4.72	F_{SC} = 0.088	p < 0.001
Among individuals	184	35819.9	194.67341	48.7		
Total	189	64655.1	399.7676	100	F_{ST} = 0.513	p < 0.001

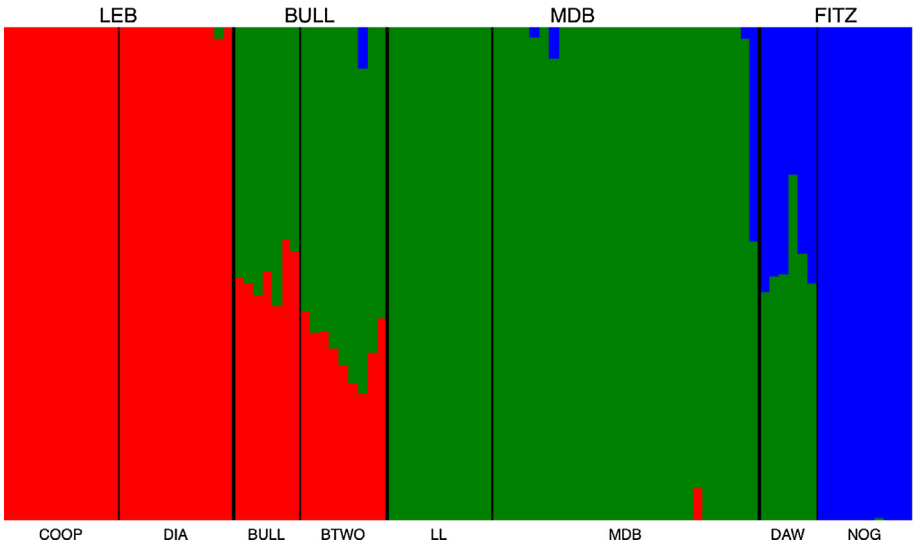

Fig. 2. Plots of cluster membership estimated by fastSTRUCTURE for golden perch from four basins based on 18,979 SNPs. The basins shown above the plot are Lake Eyre (LEB), Bulloo (BULL), Murray-Darling (MDB) and Fitzroy (FITZ). The basin subdivisions are Cooper (COOP) and Diamantina (DIA) and Dawson (DAW) and Nogoa (NOG). Also shown are the two temporal samples from BULL (i.e. BULL and BTWO) and the Lower Lakes (LL) region in the MDB.

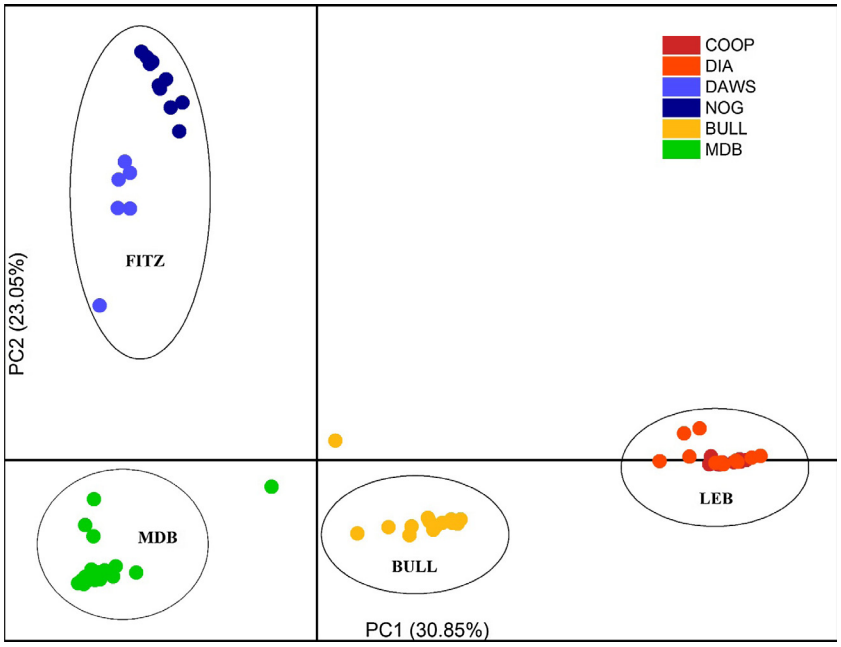

Fig. 3. Discriminant Analysis of Principal Components (DAPC) for golden perch from four basins based on 18,979 SNPs. The basins shown are Lake Eyre (LEB), Bulloo (BULL), Murray-Darling (MDB) and Fitzroy (FITZ). The basin subdivisions are Cooper (COOP) and Diamantina (DIA) in the LEB, and Dawson (DAW) and Nogoa (NOG) in the FITZ.

Further, there was phylogeographic structure within the LEB, with two distinct and well supported clades (96% and 99% bootstrap; Fig. 4): one with eastern catchment (Cooper, Barcoo and Thomson) and the other with western catchment samples (Diamantina, Georgina, Neales and Warburton). In the FITZ basin, structure was evident between fish from the Dawson (DAWS) and Nogoa (NOG) rivers (Supplementary Fig. S1). In contrast, lower divergence was detected within the MDB. Although some MDB localities often clustered together (particularly the Lower Lakes

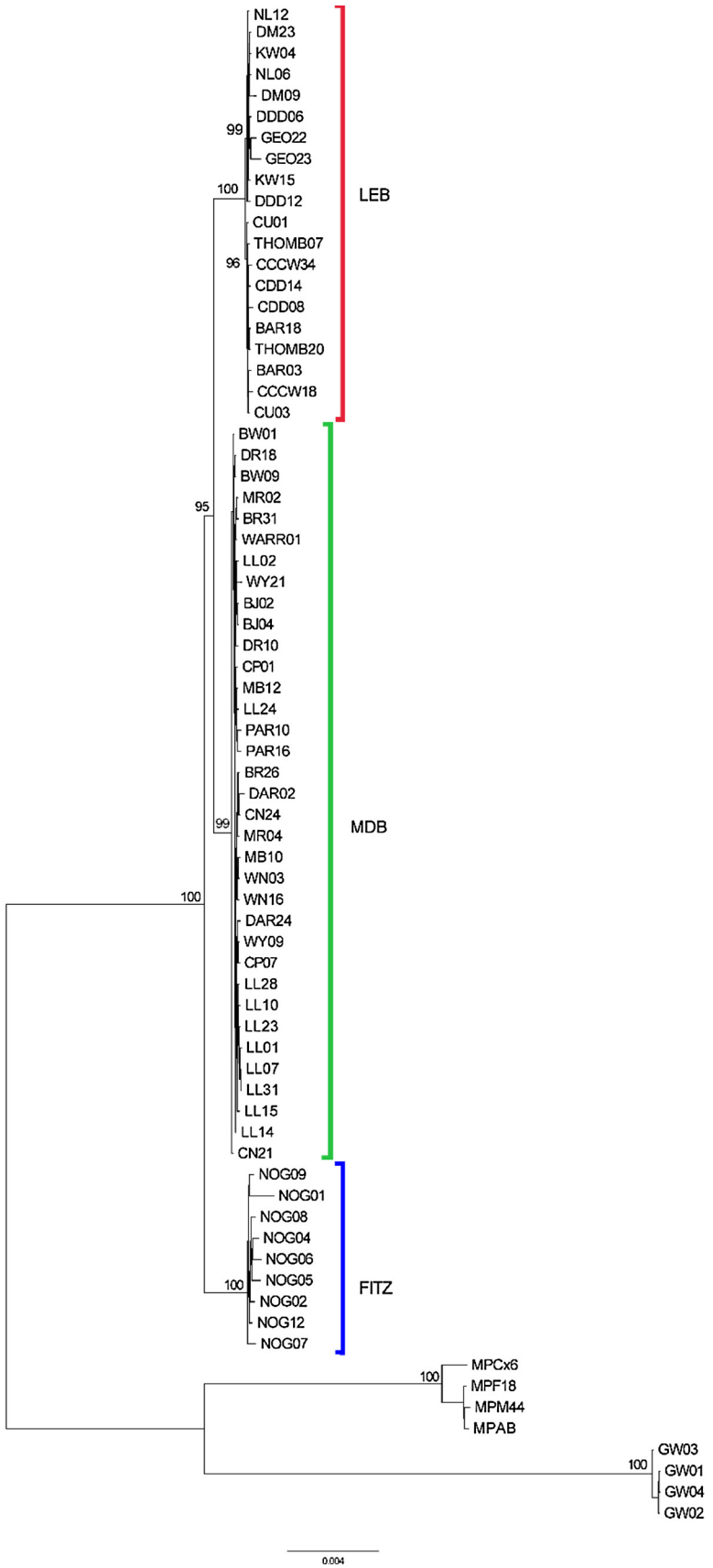

Fig. 4. Maximum likelihood tree of golden perch based on 1.725 million bp of ddRAD sequences. The basins shown are Lake Eyre (LEB), Murray-Darling (MDB) and Fitzroy (FITZ). The Macquarie perch (*Macquaria australasica*, samples abbreviated MP) and the Bloomfield River cod (*Guyu wujalwujalensis*, samples abbreviated GW) were used as outgroups. The Bulloo Basin was excluded from this analysis (see Methods); see Supporting Information Fig. S1 for the tree containing Bulloo Basin and Dawson River samples.

samples), the overall pattern is suggestive of much greater connectivity across this basin.

The three inferred clades had low sequence divergence (0.15–0.47% across the entire dataset, 2.3–7.2% across variable sites only; Supplementary Table S5). The tentative divergence time estimated between FITZ and remaining lineages was 600 ka and 280 ka between MDB and LEB. The divergence between golden perch and the outgroups was estimated at 5.3 Ma.

3.4. Coalescent species delimitation

After excluding BULL from the alignment, different BPP runs using both algorithms consistently returned the same result, corroborating the hypothesis that MDB, LEB and FITZ each correspond to a different lineage. The BPP runs recovered five species with a posterior probability of 1: two being the outgroups, and the remaining three being the MDB, LEB and FITZ groups. The best species tree estimated by BPP (higher posterior probability in all runs) had a slightly different topology compared to the concatenated phylogenetic analysis because in the latter *G. wujalwujalensis*, and not *M. australasica*, was recovered as sister-species of golden perch (Fig. 5).

4. Discussion

We investigated genome-wide signatures of species range limits in the generalist and high-dispersive golden perch (*Macquaria ambigua*), a taxon with a vast distribution across several currently isolated river basins of Australia's arid and semi-arid zones. We tested if the spatial distribution of genome-wide variation is better explained by differences between than within river basins. This enabled an assessment of the relative influence of life-history and past climatic changes in shaping biogeographic history. Our study provides insights about the role of aridification on the evolution of aquatic organisms and showcases the benefits of genome-wide SNPs for resolving taxonomy and hybridization and for informing conservation management.

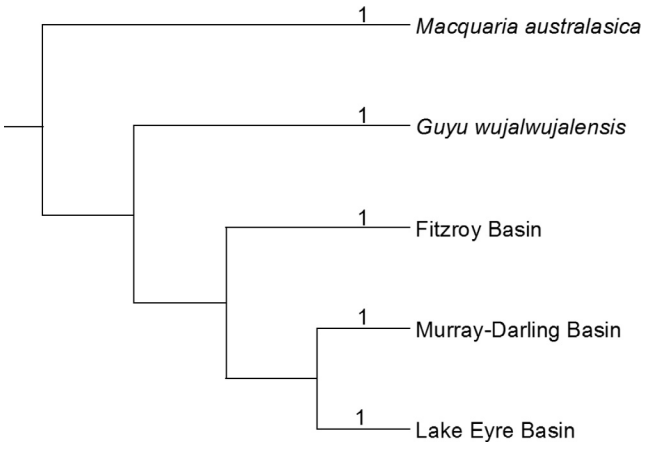

Fig. 5. Species tree recovered by BPP joint coalescent species tree reconstruction and species delimitation analyses for golden perch based on 1.725 million bp of ddRAD sequences. Numbers above branches are posterior probabilities for each species from the species delimitation analyses, indicating the full support for species status of golden perch found in each of the three main river basins (Fitzroy, Murray-Darling and Lake Eyre). Macquarie perch (*Macquaria australasica*) and Bloomfield River cod (*Guyu wujalwujalensis*) were used as outgroups.

4.1. Species range limits match climate-determined boundaries of river basins

Strong population genetic, phylogenetic, and coalescent species delimitation support was obtained for three separate lineages within golden perch, each endemic to a different river basin. One is endemic to the coastal FITZ basin and the other two to the inland basins, LEB and MDB. Individuals were largely assigned (based on 18,979 SNPs) to each of the three lineage clusters that exactly matched the three inferred reciprocally monophyletic clades (based on 1.725 million bp). The exceptions relate to a few cases of admixture and to fish found in the small and intermediate BULL basin (discussed below). In addition, the coalescent-based Bayesian species delimitation method provided maximum statistical support to the hypothesis of three divergent lineages, each with a range delimited by the main drainage boundaries. The evidence thus overwhelmingly supports the conclusion that golden perch is represented by three separately evolving metapopulation lineages and that each should be considered a distinct cryptic species. This agrees with known morphological differences between the three groups, which will form the basis of forthcoming species descriptions (Unmack et al., unpublished). Our conclusion is consistent with several different properties used by biologists to delineate species and satisfies operational criteria used for empirical applications of the biological (Mayr, 1942; Dobzhansky, 1950), phylogenetic (Nelson and Platnick, 1981), and unified (de Queiroz, 2007) species concepts. As such, it is insensitive to biases and concepts that might drive taxonomic inflation (reviewed by Isaac et al., 2004 and de Queiroz, 2007, respectively) and is probably reflective of a true discovery of species delimitation.

The low physiological limitation and high potential for inter-basin dispersal of golden perch do not account for our main findings. Rather, contemporary differences in species ranges among the cryptic species probably reflects spatial niche tracking associated with past climatic changes, and in particular with aridification of the continent during late Miocene and Pleistocene (Byrne et al., 2008). The onset of aridity likely intensified the effects of biogeographic barriers represented by drainage divides. Drainage divides are thought to account for the relatively high endemism of fish assemblages across river basins of Australia (Unmack, 2001). Based on current taxonomy, golden perch's wide distribution is unusual compared to other Australian fish species, a peculiarity attributed to their distinctive breeding biology, migratory ability, large size and prolonged age. Instead, our proposal for multiple species with range limits matching the configuration of the main river basins agrees with general biogeographic patterns of Australian fishes (Unmack, 2001). This also corroborates the notion that, despite being found in a continent with low relief, Australian river basins represent fundamental biogeographic units to study spatial and climatic determinants of evolutionary processes (Beheregaray, 2008; Unmack, 2014).

Major splits within fish clades of Australia are likely of Miocene and Oligocene age; however, several examples exist of diversification linked with climatic changes of the last 2 My (reviewed in Faulks et al., 2015). This includes a phylogeographic reconstruction of golden perch that combined mtDNA-based coalescent analyses with palaeoclimatic information (Faulks et al., 2010a). On one hand, the mtDNA phylogeography showed signals of demographic and range expansion events (including drainage crosses and inter-basin colonizations) associated with higher freshwater connectivity during moister Pleistocene conditions. On the other hand, divergence estimates suggested that the two major inferred mitochondrial lineages (i.e. coastal FITZ and inland MDB and LEB) diverged during Pleistocene periods of arid climate (Faulks et al., 2010a). The genome-wide tree supports this hypothesis showing that the major split in golden perch separates coastal from inland

lineages, an event tentatively dated to upper-middle Pleistocene. In terms of colonization route, the topology of our tree is not inconsistent with previous proposals for a coastal origin of golden perch, followed by crossing of the Great Dividing Range (possibly during floods in areas of low relief) and range expansion and establishment in inland basins (Faulks et al., 2010a, 2015). Nonetheless, we cannot rule out that golden perch were once found across other coastal river basins and have gone extinct as climate changed during the Pleistocene. That scenario impacts on the ability to infer biogeographic history from extant genealogies. Future studies should analyze genome-wide datasets using robust coalescent simulations of both present and ancient genealogies. Such approaches (e.g. Excoffier et al., 2013) can handle complex evolutionary scenarios with multiple events of migration, admixture and demographic changes, enabling the statistical testing of a range of competing phylogeographic hypotheses.

Connectivity in arid zone fishes results from the interaction between physical connections of habitats and life-history attributes of species. A recent review (Murphy et al., 2015) revealed that desert aquatic taxa with high dispersal ability often showed high (i.e. between-basin) connectivity. Nonetheless, the same review demonstrated that some species showed less connectivity than expected given their dispersal ability. This was attributed to reduced hydrological connectivity and to the possibility that dispersal traits vary across regions due to adaptation to local environmental conditions (Murphy et al., 2015). We hypothesize that contrasting hydroclimatic selective environments across the range of golden perch have driven adaptive divergence between populations, either in isolation or in the presence of gene flow (e.g. Beheregaray and Sunnucks, 2001). This is consistent with a recent riverscape genomics study of another Australian percichthyid that showed strong adaptive divergence associated with hydroclimatic heterogeneity (Brauer et al., 2016), and with findings that the greater aridity in western LEB has influenced the distribution of putatively adaptive diversity in golden perch (Beheregaray and Attard, 2015).

4.2. Genome-wide SNPs resolve taxonomic uncertainty and inform conservation management

This study provides a tangible example of the benefits of genome-wide datasets (and in particularly ddRAD SNPs) for resolving taxonomy, clarifying admixture and informing conservation management. Regarding taxonomy, previous morphological- (Musyl, 1990), allozyme- (Musyl and Keenan, 1992), mtDNA- (Faulks et al., 2010a) and microsatellite-based (Faulks et al., 2010b) studies did not resolve the true extent of cryptic species diversity within golden perch. They provided support for cryptic species but the evidence was conflicting across different datasets. Studies based on allozymes and morphology suggested one species in the LEB and another in remaining basins (Musyl and Keenan, 1992). Microsatellites and mtDNA studies indicated, respectively, population differentiation (Faulks et al., 2010b) and reciprocal monophyly (Faulks et al., 2010a) between coastal and inland clades, with a possible third but much less-divergent inland group. On the other hand, genome-wide SNPs provided unprecedented high resolution and support for three species, each endemic to a different river basin and showing reciprocal monophyly and strong population divergence. The modest resolving power of previous studies cannot be accounted by insufficient sampling (e.g. the two DNA-based studies included 590 samples covering the range of golden perch). Instead, we attribute the different outcomes to the substantial increase in number of loci enabled by the ddRAD method. A larger number of loci allows better sampling of different demographic histories and accommodates for problems associated with inherent locus-to-locus stochasticity in levels of polymorphism and the extent of lineage sorting (Knowles, 2004; Garrick et al., 2015). In addition, it reduces the probability of errors in coalescent species delimitation (Zhang et al., 2011). Our suggestion agrees with other empirical RAD studies that not only supported earlier species delimitation hypotheses based on mtDNA but also discovered previously undetected cryptic species (e.g. Pante et al., 2015). Our work adds to a rapidly growing list of DNA-based studies that have exposed high cryptic diversity, including within large and commercially important teleosts (Beheregaray and Caccone, 2007; Cooke et al., 2012).

This study also disclosed potential cases of human-induced hybridization undetected by genetic studies. All Dawson River fish showed ancestry to both their native FITZ and to the MDB lineage in a pattern suggestive of first-generation admixture: most fish had around 50% membership to each parental lineage (Fig. 2) and unusually high heterozygosity (Supplementary Table S1). Additionally, five of the 39 MDB golden perch had low levels of mixed ancestry with either FITZ or LEB. Very low levels of MDB and FITZ ancestry were also identified in one LEB and in one BULL fish, respectively (Fig. 2). These cases are likely due to stocking across basins. Golden perch is arguably the most heavily stocked native freshwater fish in Australia, with government hatcheries from the state of New South Wales alone accounting for stocking of over half a million golden perch per year to support recreational fisheries (NSW DPI 2005). Current government regulations prevent exchange of golden perch across river basins, and in some cases regionalize broodstock collection and fingerling release within each state (NSW DPI 2005). It is thus conceivable that stocking practices carried out by the various private hatcheries operating in Australia account for the hybridization reported here. Anthropogenic hybridization is a serious treat to many species since it might lead to swamping of locally adapted gene pools and can contribute to species extinctions (Allendorf et al., 2010). We recommend that all stocking, restocking and translocation of golden perch be done exclusively within the confines of each river basin using locally sourced fish. We also encourage an urgent SNP-based population survey in the FITZ to assess patterns of MDB introgression in that basin, and the discontinuation of breeding or translocation programs that use fish from the Dawson River.

Finally, an intriguing pattern suggestive of natural admixture was detected in BULL, with fish showing intermediate ancestry to both LEB and MDB lineages. Golden perch from BULL were previously proposed as a subspecies of the LEB lineage (Musyl and Keenen, 1992). Our study instead indicates that BULL probably represents a stable hybrid zone. The inferred pattern of admixture appeared as temporally stable because it remained remarkably similar between different cohorts sampled at the same BULL locality (i.e. BULL in 2006 and BTWO in 2014, Fig. 2). We propose that periodic extreme flooding events promote recurrent population connectivity and admixture between LEB and MDB lineages in the BULL basin. From a management perspective, BULL golden perch should only be used for stocking activities within the Bulloo River.

5. Conclusion

In spite of the high resolution provided by genome-wide datasets to clarify evolutionary history, species boundaries and key population parameters for management (Allendorf et al., 2010), considerable debate exists about the current uptake of genomics in biodiversity research and its potential for improving traditional genetic inferences in conservation management and taxonomy (e.g. Shafer et al., 2014). Our study shows that genome-scale data can substantially improve and rectify inferences from traditional genetic studies. This includes clarification of complex issues about

taxonomy and hybridization essential for developing sound management policies and practices. Our work also provides insights into the relative roles of spatial niche tracking associated with climatically-determined drainage basin evolution, and adaptive niche evolution associated with the selection of generalist and highly dispersive traits. It contributes to understanding the consequences of aridification on the connectivity and evolution of aquatic biomes, a topic poorly represented in the literature needed to guide climate adaptation policy and planning in arid zones (Murphy et al., 2015).

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2017.03.021.

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