Received: 14 August 2020 | Accepted: 22 June 2021

DOI: 10.1111/mms.12851

ARTICLE

Marine Mammal Science

Population genomic structure of killer whales (*Orcinus orca*) in Australian and New Zealand waters

Isabella M. Reeves[1,2] | John A. Totterdell[3] | Andrea Barceló[1,2] | Jonathan Sandoval-Castillo[1] | Kimberley C. Batley[1,2] | Karen A. Stockin[4] | Emma L. Betty[4] | David M. Donnelly[5] | Rebecca Wellard[6,7] | Luciano B. Beheregaray[1] | Luciana M. Möller[1,2]

[1]Molecular Ecology Laboratory, College of Science and Engineering, Flinders University, Bedford Park, South Australia

[2]Cetacean Ecology, Behaviour and Evolution Laboratory, College of Science and Engineering, Flinders University, Bedford Park, South Australia

[3]Cetacean Research Centre (CETREC WA), Esperance, Western Australia

[4]Cetacean Ecology Research Group, School of Natural and Mathematical Sciences, Massey University, Auckland, New Zealand

[5]Killer Whales Australia, Mornington, Victoria, Australia

[6]Project ORCA, Bentley, Western Australia

[7]Centre for Marine Science and Technology, Curtin University, Bentley, Western Australia

Correspondence
Luciana M. Möller, Cetacean Ecology, Behavior and Evolution Laboratory, College of Science and Engineering, Flinders University, Bedford Park, South Australia 5042, Australia.
Email: luciana.moller@flinders.edu.au

Funding information
Cetacean Research Centre (CETREC WA); College of Science and Engineering, Flinders University; Department of Environment and Water of South Australia, Grant/Award Number: NRM RAIN scholarship to I. M. Reeves

Abstract

Population genomic data sets have enhanced power to detect cryptic and complex population structure and generate valuable information for the conservation and management of wildlife species. Globally, killer whales (*Orcinus orca*) are considered to have a complex population structure due to their ability to specialize in a variety of ecological niches. In the Australasian region, they are found year round in New Zealand waters and are sighted in all Australian waters, with seasonal aggregations in the northwest (NWA) and southwest (SWA). Regionally, there is some knowledge regarding killer whale abundance, diet, acoustics, and social structure, but limited information about their population structure. Here, we present a population structure assessment of Australasian killer whales using 17,491 high quality genome-wide single nucleotide polymorphisms (SNPs), combined with sequences of the mitochondrial DNA control region. The results indicate a minimum of three populations: New Zealand, NWA, and SWA. These populations present moderate levels of genomic diversity, negligible levels of inbreeding, small effective population sizes, and low contemporary migration rates among them. Mitochondrial DNA analysis elucidated five closely related haplotypes, suggestive of matrilineal societies, consistent with killer whales elsewhere. This information will assist

conservation management of killer whales in the Australasian region.

KEYWORDS
Bremer Canyon, cetaceans, conservation genomics, ddRAD, Delphinidae, Ningaloo Reef

1 | INTRODUCTION

The spatial distribution of genetic variation among and within populations is a central focus of molecular ecology and conservation biology. Molecular methods allow for inferences of population structure and provide invaluable insights which can assist in the design of conservation and management regimes for at risk wildlife (Frankham et al., 2017). This information can be integrated into species conservation management plans by investigating interactions among genetic drift, mutation, migration, and gene flow at the population level, as well as adaptive variation arising from natural selection (Allendorf et al., 2010; Funk et al., 2012). Moreover, conservation management efforts can be improved or designed considering not only patterns of connectivity within species, but also their predicted adaptability to future environments (Jonas et al., 2018; Ralls et al., 2018). This knowledge is particularly beneficial for marine organisms, as they are often difficult to study and demonstrate cryptic and/or complex population structure, which traditional ecological methods cannot fully account for. The application of genomic approaches to conservation biology is particularly attractive due to its ability to produce thousands of genetic markers, allowing for more reliable estimates of evolutionary relationships and demographic parameters (Gaughran et al., 2018; Grummer et al., 2019). Application of population genomics to conservation has focused on increasing the accuracy of population genetic parameters, including population structure, migration rates, and effective population sizes, as well as identifying loci under selection (Hohenlohe et al., 2018).

Killer whales (*Orcinus orca*) are a globally distributed apex predator found from equatorial regions to the polar pack ice, however, they remain listed as data deficient by the International Union for the Conservation of Nature (Reeves et al., 2017). Thus, conservation and management actions are commonly completed on an ad hoc basis in several regions around the world. This is perhaps a result of both their complex population history (Foote et al., 2016, 2019) and lack of sufficient research effort globally.

Killer whales provide a relatively unique case from both an evolutionary and demographic perspective because geographically they exhibit fine scale variation in their morphology and ecology (reviewed in Foote et al., 2019). Their particular ability to radiate into novel ecological niches around the world's oceans has resulted in distinct ecotype formation, evolving ~220,000–530,000 years ago KYA, which gave rise to the several distinct killer whale clades (Morin et al., 2015). The term ecotype refers here to an ecologically and genetically differentiated population of a species (sensu Turesson, 1922).

Killer whale ecotypes can be characterized by differences in their social structure, morphology, acoustics, physiology, behaviors, spatial range, and feeding ecology (e.g., Baird & Whitehead 2000; Durban & Pitman 2011; Foote et al., 2016; Hoelzel, 1998; Pitman & Ensor 2003; Riesch et al., 2012; Thomsen et al., 2001; Wellard et al., 2018). Killer whale ecotypes are proposed to have originally evolved from small founder groups filling new ecological niches and then maintained by social learning among individuals (Foote et al., 2016). Speciation with the genus *Orcinus* has been heavily debated largely due to some ecotypes forming monophyletic clades (Morin et al., 2015) which, under some species concepts, would suggest they are different species (Barrett-Lennard, 2011). However, demographic processes, including postfounder expansions could lead to a similar signal. Based on recent reconstruction of killer

whale ancestry and demography (Foote et al., 2016, 2019; Hooper et al., 2020) the latter seems more plausible. Two ecotypes in particular have been extensively studied for several decades. These are the sympatric transient mammal-eating and the resident fish-eating ecotypes found in the eastern North Pacific (Bigg, 1982; Ford et al., 1998). They exhibit distinct morphology, different social structures, feeding specializations, and complex population structure, with high genetic differentiation among groups (e.g., Baird & Stacey 1988; Bigg 1987; Ford et al., 1998; Hoelzel & Dover 1991), including in genes associated with diet (Foote et al., 2016). These ecotypes are generally used as sources of general knowledge about killer whale biology globally, with Southern Hemisphere killer whale research somewhat lagging behind. In the Southern Hemisphere, the Antarctic killer whale ecotypes are the best studied, although little published literature exists in comparison to the Northern Hemisphere ecotypes. In Antarctica, there are at least five ecotypes with various specialized diets. Type A, which feeds on marine mammals and penguins, with a preference for minke whales (*Balaenoptera acutorostrata*) and southern elephant seals (*Mirounga leonina*); type B1 (pack ice killer whale), which preys primarily on ice associated seals (e.g., Weddell, *Leptonychotes weddellii*; crab-eater, *Lobodon carcinophagus*, and leopard seals, *Hydrurga leptonyx*), and occasionally minke whales; type B2 (Gerlache killer whales), which feeds on fish or squid, and sporadically on penguins; type C, which preys on fishes (e.g., Antarctic toothfish, *Dissostichus mawsoni*); and sub-Antarctic type D, which also feeds on fish (Jefferson et al., 2015; Pitman, 2011; Pitman & Durban, 2010, 2012; Pitman & Ensor, 2003; Pitman et al., 2007; Travers et al., 2018). Some Antarctic ecotypes appear to be sympatric in range and exhibit unique diets, with parallels to the patterns observed in the eastern North Pacific ecotypes (Foote et al., 2019; Pitman & Ensor, 2003; Pitman et al., 2011). For other regions of the Southern Hemisphere, including Australasia, there is little knowledge about killer whale ecotypes and their population structure.

Killer whales have been sighted throughout Australasia (Atlas Of Living Australia, 2020), primarily in Australia, New Zealand (NZ), and Papua New Guinea (Morrice, 2004; Palmer & Chatto, 2013; Visser, 2000a; Visser & Bonoccorso, 2003). In Australia, two regions with high seasonal use have been discovered recently: one located mostly inshore in the Ningaloo Reef area, northwestern Australia (NWA), and the other in offshore waters of the Bremer Sub-Basin, off southwestern Australia (SWA). Sporadic sightings of killer whales have also been recorded in all states of Australia and the Northern Territory, with perhaps greater numbers in colder waters (Morrice, 2004; Palmer & Chatto, 2013). There is limited knowledge about the biology and ecology of killer whales in this region, with some information about morphology, acoustics (Wellard et al., 2015), abundance (Donnelly et al., 2019; Totterdell, 2015; Wellard & Erbe, 2017), feeding behavior (Cieslak et al., 2021), social structure (Wellard, 2018), habitat use (Salgado Kent et al., 2020), diet (Pitman et al., 2015; Totterdell et al., in review; Wellard et al., 2016), visiting ecotypes (Donnelly et al., 2021) and phylogenetic relationships to other ecotypes around the world (Foote et al., 2019). Observations of predation events and prey remains at the surface suggest they feed upon multiple cetacean species (e.g., coastal dolphins, baleen, and beaked whales) (Pitman et al., 2015; Wellard et al., 2016; J.A.T., personal observation), various teleost and shark species, and also cephalopods (J.A.T., personal observation). Whether these killer whale aggregations represent different ecotypes is yet to be determined, since the lack of a specialist diet and clearly distinct morphological features make it difficult to assign them to a particular ecotype. In NWA, 26 individual killer whales have been photographically identified (Totterdell, 2015), likely representing at least three socially connected groups (Pitman et al., 2015). By contrast, in SWA, more than 140 individuals have been identified (Wellard & Erbe, 2017). From field observations, SWA animals most closely resemble Antarctic type A killer whales (Wellard et al., 2015). This is regarding the coloration and pigmentation patterns, with the saddle and eye patches being in similar positions, size, shape, and pigment strength (J.A.T., personal observation). However, SWA animals are generally smaller in size (J.A.T., personal observation), and have more falcate dorsal fins compared to the Antarctic type A (J.A.T. and R.W., personal observation). From a historical perspective, a recent genomic study based on a single sample per locality indicated that the NWA killer whale had genetic similarities to other tropical killer whales, while the SWA animal was more similar to Southern Ocean killer whales (Foote et al., 2019). This suggests the possibility of distinct contemporary population structure between these two Australian regions.

In NZ, killer whales have been observed around the country (Visser, 2000a; Visser & Cooper 2020) but there is limited published literature on these animals. An early photo identification study suggested a minimum of 115 animals around NZ in the late 1990s and also suggested the possibility of three subpopulations in the area (Visser, 2000a). This was congruent with preliminary mtDNA and acoustic analysis (Visser, 2000a, 2007). They were later shown to exhibit matrilineal structure and local philopatry (Olavarría et al., 2014) and are perhaps historically more genetically similar to Northern Hemisphere populations (Foote et al., 2019). Currently, these killer whales are listed by the NZ government as nationally critical (Baker et al., 2019). These putative NZ subpopulations are thought to exhibit different foraging specializations, with one exclusively preying upon cetaceans, another primarily on rays, and the third exhibiting a more generalist diet (Visser, 1999a, 2000a, 2005, 2007; Visser et al., 2000, 2010). Given that diet specializations may drive ecological speciation (see Riesch et al., 2012), it is possible that the proposed subpopulations in NZ waters are genetically distinct. However, there is still limited knowledge on the relationship between genetic and ecological differentiation within the context of killer whale divergence (e.g., Filatova et al., 2015; Hoelzel & Dover, 1991; Stevens et al., 1989).

Here, we assessed contemporary population genomic structure of killer whales in part of their distribution in the Australasian region. Population structure in Australia and NZ waters was elucidated using a putatively neutral genomic data set. This was used to assess genomic diversity and differentiation, test for the most likely number of populations, and infer levels of gene flow. In addition, sequences of a fragment of the mitochondrial (mtDNA) control region were used to assess matrilineal genetic diversity and differentiation from a maternal perspective. The putative neutral genomic data set was also used to estimate effective population size (N_e) for the inferred populations. Based on previous knowledge about the morphological characteristics and feeding behavior of killer whales in these regions, and the absence of matches between individuals in the two Australian photo-identification catalogs (Totterdell, 2015; Wellard & Erbe, 2017), it was hypothesized that each region represents a small and distinct population. This study generates information about population structure of killer whales in Australasia that can be used for conservation and management of these animals, and also contributes to the global knowledge about this data deficient apex species.

2 | METHODS

2.1 | Sampling and storage

A total of 74 tissue samples from free-ranging and stranded killer whales were obtained through opportunistic sampling between 1997 and 2019 from eight localities across Australia and NZ (Figure 1, Table 1). Tissue samples (skin and blubber) collected from free-ranging animals were obtained using a Barnett crossbow with 150 lb draw weight, 50 cm long darts (designed by F. Larsen, CETA DART, Copenhagen, Denmark), and biopsy tips (length: 40 mm), which provided samples of approximately 25 mm $\times$ 4 mm size ($n = 55$). Samples from strandings were a combination of muscle and/or skin ($n = 19$). Samples were not identified to ecotype level and were collected predominantly from adults and juveniles from several pods, with calf samples only available from strandings. All samples were preserved in 95% ethanol and stored at -80°C until used for laboratory analyses.

2.2 | DNA extractions

Genomic DNA was extracted using a salting-out protocol (Sunnucks & Hales, 1996). DNA quality, integrity, and quantity were assessed using a NanoDrop 1000 spectrophotometer (Thermo Scientific), gel electrophoresis, and a fluorometer (Qubit 2.0, Life Technologies), respectively. If DNA extractions showed the presence of low molecular

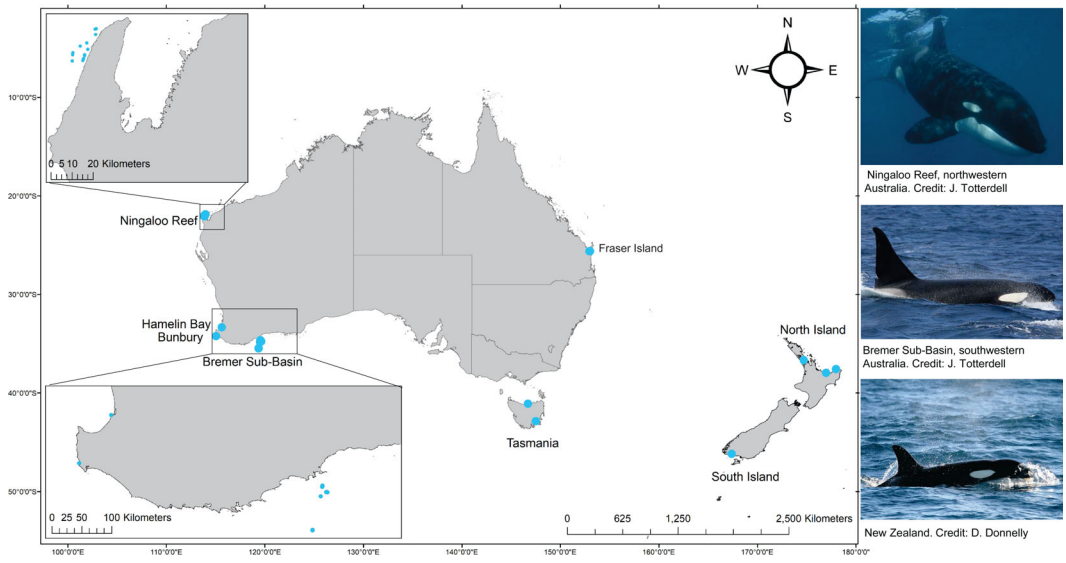

FIGURE 1 Map displaying localities of 74 stranded and biopsied sampled killer whales (*Orcinus orca*) in Australasia from which ddRAD-seq and mtDNA control region sequences were generated. Note that samples were often obtained in very similar geographic coordinates and therefore many appear superimposed in the map. The map was created using ArcMap 10.3.1. To the right of this figure are examples of killer whales from the three sites of high use within Australasia.

TABLE 1 Killer whale (*Orcinus orca*) tissue samples utilized, with their respective sample size locality in Australasia. Note that number of samples varied between the mtDNA and the nuclear SNP data set, see Table S6 for specific dataset details.

Locality	Number of samples	Sample type
Ningaloo Reef, northwestern Australia	14	Biopsy
Hamelin Bay, midwestern Australia	1	Stranding
Bunbury, midwestern Australia	1	Stranding
Bremer Sub-Basin, southwestern Australia	41	Biopsy
Fraser Island, eastern Australia	3	Stranding
Tasmania, southeastern Australia	2	Stranding
South Island, New Zealand	6	Stranding
North Island, New Zealand	6	Stranding

weight molecules, the sample was cleaned with AMPure XP beads (Beckman Coulter Genomics) in attempt to discard them; used as per manufacturer's specifications.

2.3 | Genetic and genomic sequencing

For the mtDNA data set, a fragment of approximately 450 bp of the mtDNA control region was PCR amplified for 68 samples (+10% replicates) using primers DLP 1.5 and DLP 5 (Baker et al., 1993). Six samples were not sequenced due to a lack of sufficient DNA after ddRAD library preparation. The mtDNA control region fragment was sequenced (forward and reverse) using an Applied Biosystems 3730XL Sequencer, with sequences later trimmed and

aligned using Geneious v.6.0.4 (Kearse et al., 2012). Further information about conditions and primers used can be found in the Supplementary material (Mitochondrial DNA Sequencing).

For the sections, "Genetic and genomic diversities" and "Population structure and migration rates" the data used were generated from this study only. In the Supplementary material, we combine this study's mtDNA data set with Australasian killer whale mitogenomes from Morin et al. (2015) and sequences of the mtDNA control regions from Olavarría et al. (2014). For this combined data set, these publicly available sequences were then aligned and trimmed to our shorter mtDNA control region fragments in Geneious v.6.0.4 (Kearse et al., 2012). This analysis resulted in one additional WA (Margaret Cove) and 16 NZ (North, South and Chatham Islands) sequences, with five additional haplotypes (Table S1). Thus, in total, 85 sequences were used for the analysis of this combined data set (hereafter referred to as the combined mtDNA data set),

The SNP data set was generated *in house* using a ddRAD-seq (double digest restriction site associated DNA) method following Peterson et al. (2012), with modifications as in Brauer et al. (2018) and Sandoval et al. (2018). Briefly, restriction enzymes *SbfI* and *MseI* were used to digest 300 ng of genomic DNA per sample. The samples were then multiplexed into eight pools of 12 individuals each and sequenced one lane of the Illumina HiSeq 4000 platform as paired-end 150 bp including ~10% replicates which were randomly chosen.

2.4 | Bioinformatics of the genomic data set

Raw Illumina sequences were demultiplexed (allowing a maximum of two mismatch in the barcodes) using the process_radtags program from STACKS v2.4 (Catchen et al., 2013), which identifies unique barcodes and restriction enzyme recognition sites (RAD tags). These barcodes, RAD tags, Illumina adapters, and low-quality bases were trimmed (LEADING: 23, TRAILING: 23 and SLIDINGWINDOW: 5:20), and then reads with low average base quality (Q > 20) or shorter than 45 bp were removed using Trimmomatic v0.38 (Bolger et al., 2014). The remaining reads were then mapped to the high quality killer whale reference genome publicly available in GenBank (Oorca1.1, GCA_000331955.2), which was recently assembled into chromosome-length scaffolds (Dudchenko et al., 2017, 2018; Foote et al., 2015). Reads were mapped using Bowtie 2 v2.2.7. Indels were then realigned using GATK v3.8 (McKeena et al., 2010), with functions RealignerTargetCreator and IndelRealigner used with default settings to ensure indels were parsimoniously represented and to reduce potential false variants. The SNP data set was identified by calling SNPs from the reference genome alignment using BCFtools v1.19 (Danecek et al., 2016), and then filtered using VCFtools v0.1.15 (Danecek et al., 2011) similar to the approach of Batley et al. (2021). To reduce the calling of false SNPs due to artefacts of the ddRAD-seq approach (i.e., multicopy loci, paralogs, sequencing errors) several filtering steps were applied. Initially, only SNPs called in 80% of individuals with an allele balance of 20%–80% on the heterozygote genotype, and a minor allele frequency of 3% were selected. Minor allele frequency can influence detection of fine scale genomic variation due to the removal of rare alleles (Linck & Battey, 2019). Indels were then removed and loci with a low total depth/read quality ratio (>20%) were discarded (Li, 2014) as well loci with <30 mapping quality. SNPs with high read depth (>mean depth + [2*standard deviation]) were also discarded as this could represent paralogous loci. Additionally, a calling rate filter of 75% was applied to replicates with two errors allowed per loci to reduce false positives. Departures from Hardy–Weinberg equilibrium (HWE) were also filtered at a population level removing SNPs with a significant excess or deficiency of heterozygotes ($p \leq .05$) within two or more populations. The HWE population level filtering was based on preliminary PCA and ADMIXTURE results to gauge potential structure in the data set (ran as per population structure and migration rates section). Additionally, a linkage disequilibrium (LD) filter was applied using VCFtools (Danecek et al., 2011) to calculate the coefficient of determination (R^2) for each SNP pair, with the exponential decay estimated using a spline fitted to the data. The genetic distance at which LD is not significant ($p > .05$) was identified using the Tukey's criteria for anomalies (95% probability distribution: Tukey, 1949). Finally, a custom script was used to filter individuals >15% missing data.

For population structure and demographic analyses, the use of only putatively neutral loci reduces biases and increases accuracy of parameter estimation (Allendorf et al., 2010). Therefore, potentially adaptive loci were removed using an outlier test in BayeScan v2.1 (Foll & Gaggiotti, 2008). This approach uses a Bayesian test, in combination with a logistic regression model of F_{ST}, to detect outliers by decomposing SNPs into locus specific and population specific components (Foll & Gaggiotti, 2008). The program was run for 100,000 iterations with prior odds of 5,000. Outlier loci were identified with Q < 0.1 (i.e., false recovery rate <10%). These putatively adaptive loci were removed to obtain a putatively neutral SNP data set (hereafter referred to as the neutral data set), which was then used for downstream analyses. We intentionally did not explore further the putatively adaptive data set identified here because of well-known issues related to using genome scans in nonmodel species, such as detection of false positive signals of adaptation (Grummer et al., 2019).

2.5 | Genetic and genomic diversities

Genetic diversity for the mtDNA control region sequences was estimated using ARLEQUIN v3.5.2.2 (Excoffier et al., 2005), with haplotype and nucleotide diversities calculated. Additionally, genomic diversity was estimated for the neutral data set by estimating observed (H_o) and expected (H_e) heterozygosities per population. These measures were calculated using GenoDive v2.0b27, with 10,000 permutations (Meirmans & Van Tienderen, 2004). ARLEQUIN was also used to estimate F_{IS} (the population level inbreeding coefficient) and the percentage of polymorphic loci.

2.6 | Population structure and migration rates

Population genetic structure using the mtDNA data set was investigated in ARLEQUIN by estimating genetic differentiation using F_{ST} (10,000 permutations), which incorporates haplotype frequency, and Φ_{ST}, which also considers frequency and sequence divergence.

Jmodeltest2 (Darriba et al., 2012; Guindon & Gascuel, 2003) identified the Juke and Cantor (1969) model as the best fit for the data set and this was therefore used to estimate Φ_{ST}.

Relationships among the Australasian mtDNA control region haplotypes were investigated using a minimum spanning haplotype network, with a 95% cut off in PopART v1.7 (Leigh & Bryant, 2015). Sequences were blasted against GenBank to check if they were regionally or globally unique. Australasian haplotypes were compared (from this study) to the global data set of Morin et al. (2015) and additional NZ animals from Olavarría et al. (2014) through a phylogenetic analysis. We used one representative of each clade and ecotype, and any different NZ haplotypes that were not already represented (Accession codes: Table S1). A maximum likelihood phylogenetic tree was created using Mega X with 10,000 bootstraps (Kumar et al., 2018).

The neutral SNP data set was initially used to estimate relatedness between individuals with the triadic likelihood estimator (Wang, 2007) in COANCESTRY v1.0.1.9 (Wang, 2011). This estimator was chosen as it appeared to best reflect true relatedness values for cetaceans, (e.g., Zanardo et al. (2018)). This test was conducted to identify potential duplicate samples and closely related individuals for exclusion from the population analyses. Population genomic structure based on the SNP data set was first examined using a principal component analysis (PCA) in RStudio v1.1.453 (Rstudio Team, 2016) using package FactoMineR v2.3 (Lê et al., 2008), as it makes few assumptions and allows for individuals to cluster based on the overall variance (Reich et al., 2008; Rodríguez Ramilo & Wang, 2012). An individual assignment and nonhierarchical clustering analysis was then performed in ADMIXTURE v1.3.0 (Alexander et al., 2009). ADMIXTURE infers ancestry proportions from population allele frequencies to model the probability of observed genotypes using maximum likelihood estimates to assign the mostly likely number of K (i.e., populations) in the data set (Alexander et al., 2009). Cross validation of K estimates was completed with three independent replicate runs of K = 1–10 (Alexander et al., 2009; Alexander & Lange, 2011).

Genomic differentiation based on the SNP data set from the different geographic localities was initially examined using F_{ST} (Weir & Cockerham, 1984) in Genodive with 10,000 permutations, and the Benjamini-Yekutiel method correction to the significance value (Narum, 2006), excluding localities with small sample size ($n < 5$). F_{ST} was subsequently estimated for the putative populations identified by PCA and ADMIXTURE. F_{ST} heatmaps were then created with RStudio, using the ggplot2 package (Wickham, 2016). For localities with small sample sizes (i.e., Hamelin Bay, Fraser Island, and Tasmania), assignment tests were also performed using a Bayesian method (Rannala & Mountain, 1997) available in GENECLASS2 (Piry et al., 2004). This test is based on multilocus genotypes and allows for assignment or exclusion of an individual to a reference population as its possible origin (Piry et al., 2004). The method used the entire SNP data set, with 1,000 simulations and an exclusion rate set at 5%. Contemporary migration rates between identified populations were estimated in BayesAss v3.0.4 (SNP version), which allows a maximum of 30,000 SNPs, 20 populations and 2,000 individuals (Rannala, 2020; Wilson & Rannala, 2003). This method uses a Markov Chain Monte Carlo (MCMC) approach to estimate asymmetric rates of recent migration (i.e., first two generations), allowing for HWE deviations and possible genotypes for individuals with missing data (Wilson & Rannala, 2003). Contemporary migration rates were estimated with 10 million iterations, one million iterations as burn-in, and mixing parameters (allele frequencies, inbreeding coefficients, and migration rates), which were adjusted for ideal acceptance rates (i.e., 20%–40%) (Wilson & Rannala, 2003). The cumulative log likelihoods of the iterations were plotted to confirm convergence using TRACER 1.7.1 (Rambaut et al., 2018), with three separate runs performed to check for consistency among runs (data not shown) The results obtained were plotted in Rstudio using the package circlize v0.4.13 (Gu et al., 2014).

2.7 | Effective population size

Effective population size (N_e) was estimated based on the SNP data set for the identified populations using the LD method in NeEstimator V2.1 (Do et al., 2014). This is based on the nonrandomized associations of alleles at several loci within a population with consistent size and isolated structure (Hill, 1981). It assumes populations are closed, loci are selectively neutral, generations are discrete (Waples & Do, 2008), and loci recombine randomly (Waples & England, 2011). This method was used due to consistently producing more accurate and unbiased estimates compared to other methods (Álvarez et al., 2015), owing to its robustness to assumption violations (Waples, 2016). Within the analysis, mating system was selected as random, singletons excluded, and confidence values were based on 95% confidence intervals of the jackknife method (Do et al., 2014).

3 | RESULTS

3.1 | SNP genotyping and relatedness (r)

Approximately 900 million raw sequence reads were retained. Following demultiplexing and trimming, the sequencing reads totaled approximately 176 million, with 90% of the paired-end reads being >130 bp. On average, 90.17% (± *SD* 0.02) of the reads mapped to the killer whale reference genome, a result attesting to the quality of the data. From these, 1,160,129 RAD loci were identified, and after strict filtering the data set was reduced to 17,491 high quality SNPs (Table S2). The genotyping error was estimated at 0.04 (± *SD* 0.20), indicating high accuracy of the genotype calling. The outlier test identified 52 SNPs putatively under selection, and these were subsequently removed to obtain the neutral data set. The SNP data set (neutral and adaptive) were represented in all chromosome length scaffolds (Figure S1), suggesting a lack of bias to chromosomes. The mean depth per SNP per sample was 28 (± *SD* 15). The estimation of LD exponential decay became nonsignificant at 702 bp (Figure S2). Several samples were removed due to low quality, or >15% missing data; this excluded the singleton sample from Bunbury, Tasmania,

and several from SWA. The remaining 57 samples and 17,491 SNPs used for population analyses showed an average of 1.6% missing data. Six samples were also removed due to having high relatedness. Three were duplicates (r ~ 1), and the remaining were individuals closely related to several others (r = 0.46–0.51). Therefore, 51 samples were used for the population analyses based on the SNP data set.

3.2 | Genetic and genomic diversities

The final mitochondrial control region sequences (generated from this study) were 434 bp in length, with no sequencing error observed in the replicate samples ($n = 7$). These sequences revealed five unique haplotypes among 68 Australasian killer whale samples from the eight geographic localities. One haplotype (Fraser Island haplotype) was novel for the species, while the remaining four were novel for each locality as they were not previously found in the region. A single haplotype was found in each locality; with one of them shared between SWA, NZ (North and South Islands) and Tasmania (Figure 2, Figure S3; see Figure S4 for global phylogenetic relationships). Since all localities displayed predominantly one haplotype, Australasian diversity was estimated by combining all samples, resulting in a haplotype diversity of 0.392 (± *SD* 0.065) and nucleotide diversity of 0.0025 (± *SD* 0.002). See Supplementary material for diversity estimates and haplotype network (Figure S3) of combined mtDNA data set in "Combined mtDNA data set genetic diversity." Neutral genomic diversity was relatively similar across localities (0.185–0.327), with negligible levels of inbreeding estimated ($F_{IS} = \leq 0.0001$) and a mean of 31.5% (± *SD* 10.4) polymorphic loci between localities (Table 2).

3.3 | Population structure, genetic differentiation, and migration rates

Given that only one mtDNA haplotype was found in each of the three main localities (NWA, SWA, and NZ), results based on fixation indices were not informative (combined mtDNA data set; see Table S3). Network analyses revealed that haplotypes are closely related, with between two and nine mutations among them at five nucleotide sites (Figure 2). The Fraser Island haplotype was more related to SWA-Tasmania-NZ, whereas others from strandings along western WA were more related to the NWA haplotype (Figure 2).

Levels of F_{ST} between these localities based on the SNP data set ranged from relatively low between NWA and SWA ($F_{ST} = 0.08$) to moderate between NWA and NZ ($F_{ST} = 0.17$) (all pairwise comparisons [$p \leq .05$] were significant at $\alpha = 0.018$; Figure 3a, b). These were congruent with the ADMIXTURE results, indicating that Australian aggregations are less divergent from each other than either is to NZ. The PCA delineated three groups within Australasia, represented by NWA, SWA, and NZ (North and South Island combined; Figure 4). The moderate F_{ST} estimates (all pairwise comparisons were significant [$p \leq .05$] at $\alpha = 0.002$) based on these three groupings reinforces considerable genetic differentiation between them (Figure 3b). ADMIXTURE identified $K = 2$ as the most supported number of clusters, separating Australia from NZ animals, but three genetic clusters were also highly supported and can be visually observed (Figure 5a; Table S4). The results from the multiple methods indicate a minimum of three populations of killer whales in the Australasian region, represented by NWA, SWA, and NZ. Further subdivision was investigated within the major regions using ADMIXTURE; however, they did not support any additional structure (data not shown). The strandings from Hamelin Bay, Tasmania, and Fraser Island ($n < 5$) were all rejected from the NWA and NZ populations ($p \leq .05$), but not from the SWA population at the threshold level used (Table 3). Low contemporary migration rates (<3%) were estimated between the three inferred populations (Figure 6, Table S5) within recent generations, further suggesting that these are relatively isolated populations. Convergence between runs suggested accuracy of the MCMC, providing statistical confidence to these estimates (data not shown).

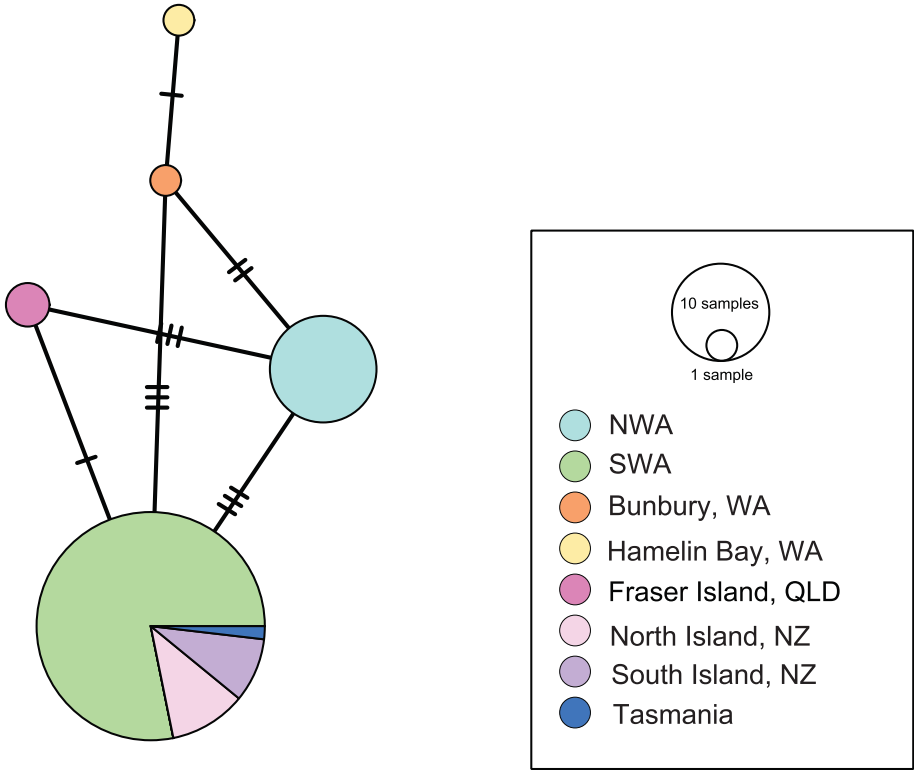

FIGURE 2 Minimum spanning network of 68 Australasian killer whales (*Orcinus orca*) generated based on a 434 bp fragment of the mtDNA control region, showcasing phylogenetic relationships among haplotypes. Each individual circle represents a haplotype, with the size of the circle proportional to the number of samples that share the same haplotype, with each locality represented by a different color. There are five haplotypes with a maximum of five variable sites, with two to nine nucleotide changes among them. Abbreviations for localities and populations are as follows: NWA (northwestern Australia), SWA (southwestern Australia), QLD (Queensland) and NZ (New Zealand).

TABLE 2 Genomic diversity measures based on 17,491 high quality, putatively neutral SNPs from Australasian killer whales (*Orcinus orca*), excluding resamples and closely related individuals r = 0.46–0.52). This was calculated only for sites with >10 samples and combining the South and North Islands of New Zealand due to no disclosed population substructure. Singleton samples and localities with <5 were not included. H_o observed heterozygosity; H_e, expected heterozygosity, PL, polymorphic loci.

Population	Number of samples	H_o	H_e	F_{IS}	%PL
Northwestern Australia	14	0.231	0.223	≤0.0001	31.70%
Southwestern Australia	26	0.327	0.324	0	41.8%
New Zealand	11	0.185	0.184	≤0.0001	21.04%

3.4 | Effective population sizes

The N_e was estimated using the full neutral, unlinked data set, excluding duplicate samples. The N_e for the NWA population was 22 (95% CI [9, 7165]), for SWA it was 77 (95% CI [48, 161]), and for NZ it was 12 (95% CI [6, 36]). All populations exhibited relatively small effective population sizes, although the upper bound for the confidence interval of the NWA population was high.

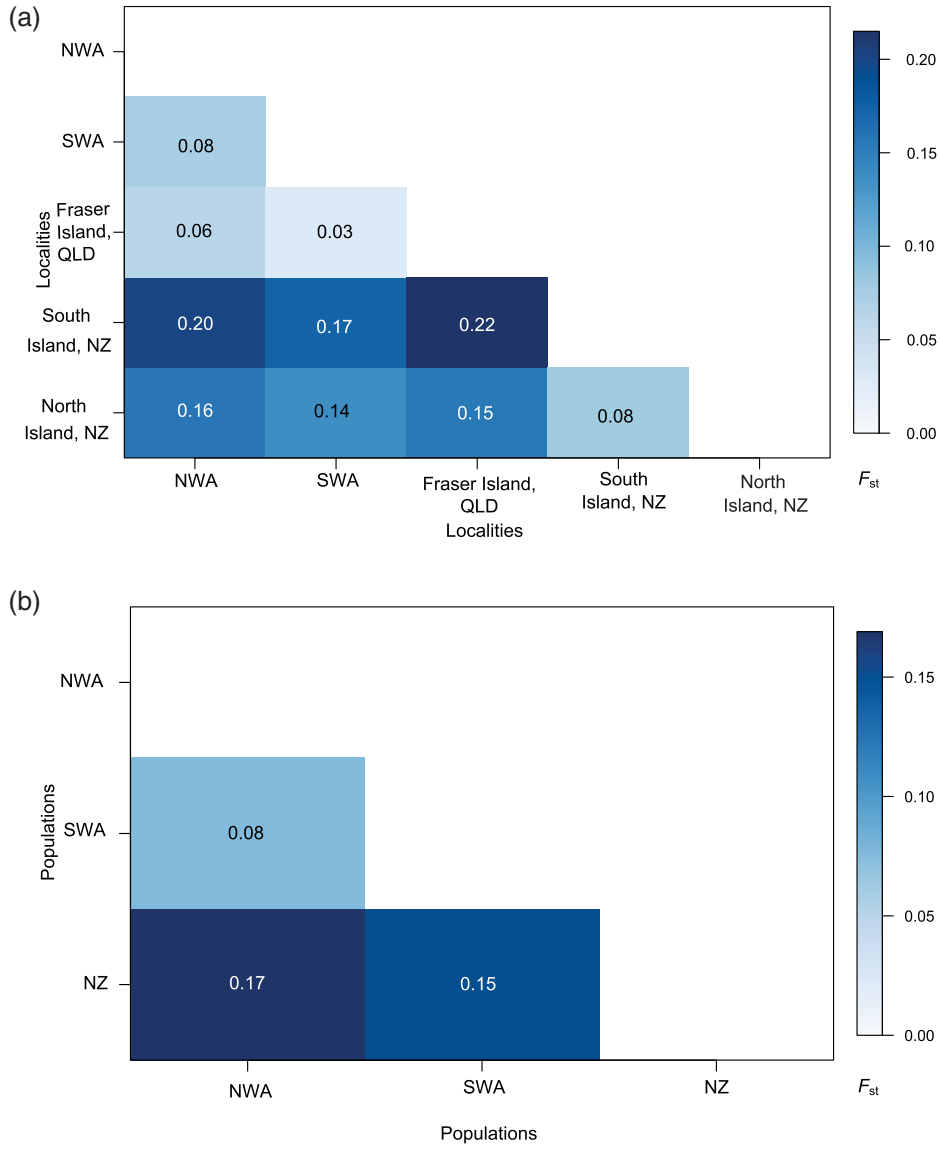

FIGURE 3 Pairwise F_{ST} based on 17,491 putatively neutral SNPs for killer whales (*Orcinus orca*) from Australasia based on (a) localities with ≥3 samples, (b) putative populations suggested by the model-free population approaches (PCA and ADMIXTURE). All F_{ST} comparisons were significant ($p \leq 0.05$). Abbreviations for localities and populations are as follows: NWA (northwestern Australia), SWA (southwestern Australia), QLD (Queensland), and NZ (New Zealand).

4 | DISCUSSION

This study provides evidence for the presence of population structure in killer whales in the Australasian region. It revealed at least three differentiated populations characterized by low mitochondrial and moderate genome wide diversity, low contemporary migration between populations, and small effective population sizes. The populations seasonally inhabit NWA and SWA and occur year around in NZ. These findings are congruent with previous photo identification records in the region that suggested small and potentially differentiated populations due to the relatively small number of individuals identified in each

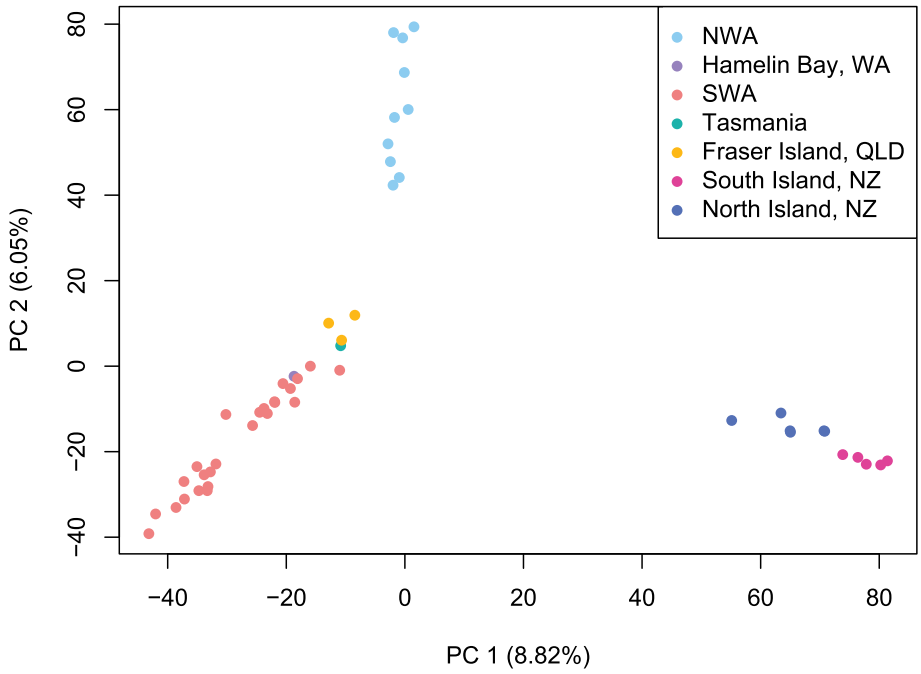

FIGURE 4 Population structure analysis based on a principal component analysis (PCA) of 17,491 putatively neutral SNPs for killer whales (*Orcinus orca*) from Australasia. The *x*-axis explained 8.82% of the variation, while *y*-axis explained 6.02%, demonstrating three genetic clusters. Abbreviations for localities are as follows: NWA (northwestern Australia), SWA (southwestern Australia), QLD (Queensland) and NZ (New Zealand).

location (Donnelly et al., 2019; Totterdell, 2015; Visser, 2000a; Visser & Cooper 2020; Wellard & Erbe, 2017), and due to the absence of photographic matches between areas.

4.1 | Low mitochondrial and moderate genome wide diversity

Genetic diversity plays a significant role in a population's ability to adapt and persist in novel and/or unfavorable conditions. Demographic analyses have recently shown that high latitude killer whales, as well as Australian animals, exhibit signals of a Holocene bottleneck, and therefore the low genomic diversity of these contemporary populations may be reflective of postglacial founder events (Hooper et al., 2020). Australasian killer whales showed particularly low diversity at the mtDNA control region in comparison to other cetaceans (e.g., common dolphins, *Delphinus delphis*, Barceló et al., 2021; common bottlenose dolphins, *Tursiops truncatus*, Gaspari et al., 2015; Cuvier's beaked whales, *Ziphius cavirostris*, Dalebout et al., 2005), but has similar diversity to killer whale populations elsewhere (e.g., Foote et al., 2009; Hoelzel et al., 2002; Parsons et al., 2013). This is likely exacerbated by the characteristic matrilineal social structure of this species (Hoelzel et al., 1998, 2002, 2007).

While only a few genome-wide SNP data sets of killer whales have been published so far, these did not report heterozygosity levels to be used as a comparison here (Foote & Morin, 2016; Moura et al., 2014). However, compared to other cetaceans, Australasian killer whales showed similar levels of genome wide heterozygosity to eastern Indian Ocean pygmy blue whales (Attard et al., 2018), and slightly lower than in other Australasian delphinids (Indo-Pacific bottlenose dolphins, Pratt, 2020; common dolphins, Barceló et al., 2021). Levels of inbreeding in the three populations identified were found to be negligible. In killer whales, high levels of inbreeding have only been reported for two populations, one that is endangered in the North Pacific (Ford et al., 2018), and another along the west coast

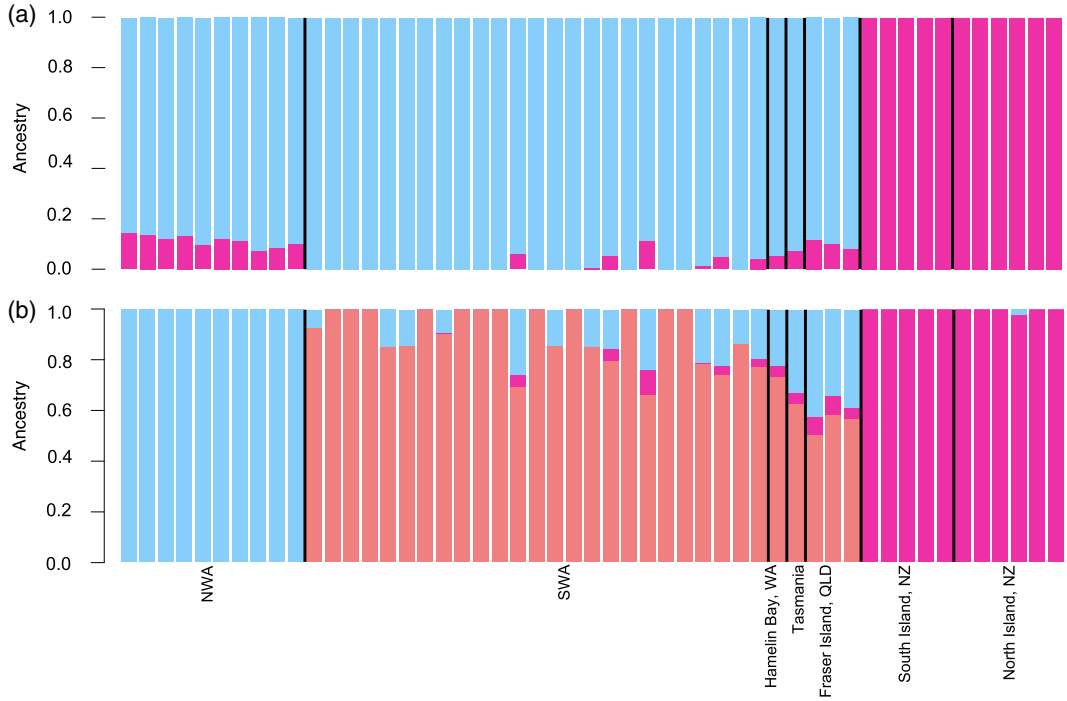

FIGURE 5 Population structure based on ADMIXTURE analysis of 17,491 putatively neutral SNPs for killer whales (*Orcinus orca*) from Australasia, labelled by locality and separated by a black line, and with each sample represented by a single column. Plots depict (a) K = 2, and (b) K = 3, with K = 2 being the most supported K, followed by K = 3 as also highly supported. Abbreviations for localities are as follows: Western Australia (WA), NWA (northwestern Australia), SWA (southwestern Australia), QLD (Queensland) and NZ (New Zealand).

TABLE 3 Results of assignment tests for killer whales (*Orcinus orca*) from localities with less than five samples in Australasia, displaying the probability of an individual belonging to a population, with a 5% exclusion criterion considered.

Sample locality (ID)	Northwestern Australia	Southwestern Australia	New Zealand
Tasmania (A4183)	0.006	0.577	0.096
Fraser Island (QLD1)	0.043	0.272	0.157
Fraser Island (QLD2)	0.041	0.291	0.149
Fraser Island (QLD 3)	0.039	0.307	0.164
Hamelin Bay (Hamelin Bay)	0.042	0.585	0.127

of Scotland (Hooper et al., 2020). While killer whales exhibit a matrilineal social structure, males are known to temporarily disperse between groups for mating (Baird & Whitehead, 2000; Foote et al., 2011; Hoelzel et al., 2007). As such, these male biased movements likely represent a strategy to avoid inbreeding between close relatives, as also reported for other delphinids (Möller & Beheregaray, 2004).

4.2 | Differentiated populations of killer whales in Australasia

Results from analyses of the mtDNA and genomic data sets highlighted strict matrilineal lineages within Australasian killer whale populations. Based on the mtDNA data set, the haplotypes are possibly fixed to each area, with close

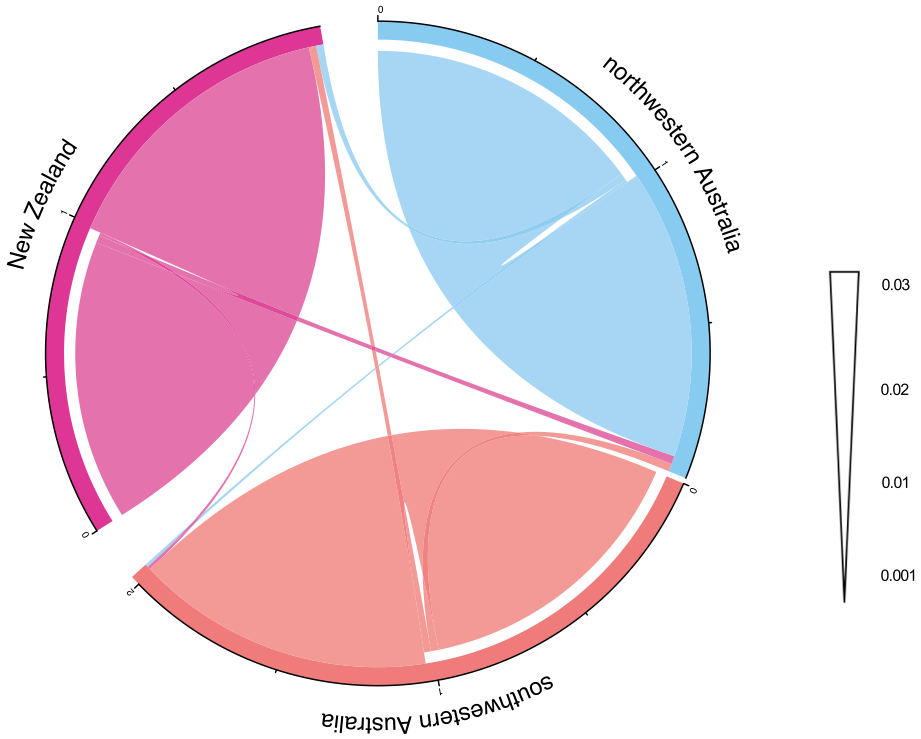

FIGURE 6 Circos plots of contemporary (per generation) migration rates within the last few generations between identified populations of killer (*Orcinus orca*) from Australasia based on 17,491 putatively neutral SNPs. Migration estimates are represented by the thickness of the curves between the distinct populations, while the scale bar unit is proportional to the migration. See Table S5 for estimate values and 95% CIs.

phylogenetic relationships among them. In Australia, it is possible that a single founding matrilineal event occurred at each aggregation site (e.g., NWA and SWA). However, relatively short fragments of the mtDNA control region can be misleading and provide low resolution for analysis of phylogenetic relationships (Hoelzel et al., 2002).

Based on the SNP data sets and multiple population analyses, three contemporary genomic clusters (i.e., populations) were revealed. Moderate genomic differentiation was observed between Australasian populations. The two Australian populations (NWA and SWA) exhibited similar levels of genomic differentiation to those between Antarctic type B (Foote et al., 2016) and Atlantic populations (Morin et al., 2015), while differentiation between the Australian and NZ populations was more similar to those reported between Antarctic type B1 and C, and between transient and offshore ecotypes in the eastern North Pacific (Morin et al., 2015). All three populations exhibit a low level of contemporary gene flow between them, perhaps because of intermittent gene flow. Gene flow between distinct killer whale populations may not be uncommon, with evidence of migrants present between different regional populations across oceans (Pilot et al., 2010). Additionally, low level gene flow between Australasian animals is likely reinforced by their typical matrilineal societies. These societies are built on the premise of social learning (Guinet, 1991; Hoelzel, 1991, 1993) and cultural transmission between kin (e.g., Deecke et al., 2000; Riesch et al., 2012; Yurk et al., 2002). In Australasia, killer whales also appear to form these types of societies (Wellard, 2018; J.A.T., personal observation), and this is also corroborated by the lack of diversity at the mtDNA control region at each site.

Within Australia, ongoing killer whale photo identification studies indicate no matching of individuals between the two aggregation areas in WA, nor to animals opportunistically sighted in the eastern Australia (Donnelly et al., 2019; Totterdell, 2015; Wellard & Erbe, 2017). However, killer whales are observed in WA only on a seasonal

basis and sporadically elsewhere, and little information is available on their movements outside these aggregation areas. It is therefore possible that they come into contact in other parts of their distribution, but if that is the case our results indicate that only limited, if any, gene flow may occur while in spatial contact. Alternatively, the low levels of admixture observed in the population analyses may reflect remnants of ancestral polymorphisms, or of other populations not sampled in this study (e.g., Antarctic killer whale ecotypes; Foote et al., 2019).

For killer whales in NZ, early studies suggested possibility of three subpopulations (Visser, 2000a, 2007); however, our genome wide study did not support this subdivision. Interestingly, a previous regional mtDNA study found four haplotypes within 11 samples taken predominantly from the North Island (Olavarría et al., 2014), while our study found only one, and had a similar sample size, but with individuals represented from both North and South Islands. This could be due to a change over time in the composition of killer whale groups utilizing NZ waters (e.g., Eisert et al., 2015), as the sampling periods do not overlap (1994–2005 versus 2010–2017). Perhaps this could also be due to random sampling of matrilines through both standings and biopsies, or lower sequence quality yielding multiple haplotypes or due to differences between the sizes of fragments used. An additional potential factor relates to some NZ samples used in our study originating from mass strandings, although individuals from four separate strandings were represented here. Importantly, biopsy samples from the two Australian sites showed a unique haplotype to each site, with a haplotype shared between SWA and NZ, suggesting that the low diversity in Australasia is a true finding. Further studies of killer whale genetic diversity in this region, including the collection of biopsy samples from additional sites, would be important to confirm the patterns observed here.

The number of Australasian killer whale populations reported here should be considered as a minimum since there are gaps in the distribution of samples in areas where killer whales are known to occur. Due to the offshore and large scale distribution of killer whales, only the two known WA aggregations have had dedicated biopsy effort, and in NZ the effort has been limited in the past (note biopsy samples from NZ were unavailable for this study). Information about killer whales in eastern Australia, NZ, and Papua New Guinea rely mainly on sightings gathered through citizen science and opportunistic stranding events. The Killer Whales Australia catalog currently contains >450 sightings and 62 photographically identified individuals from the eastern Australia (Donnelly et al., 2019). Killer whales in southeastern Australia seem to range from Sydney (New South Wales; 33.8°S), all the way to Hobart (Tasmania; 42.8°S) and as far west as Portland (Victoria; 38.3°S), and to the north of perhaps Sydney or Byron Bay (New South Wales; 28.6°S) (D.D., personal observation). In this study, the samples from eastern Australia's Fraser Island (Queensland) had different mtDNA haplotypes to the other sites, while the Tasmania sample had the same haplotype as SWA and NZ. Based on the SNP data set these animals could not be rejected as belonging to the SWA population, although their assignment value to this population was low to moderate. A larger number of samples from eastern Australia and beyond would be required to test for the presence of additional killer whale populations in Australasian waters.

4.3 | Small effective population sizes

Effective population size has only recently been readily estimated for wildlife populations due to the availability and refinement of single sample (point) genetic estimators (Waples, 2016). However, wildlife populations can often violate model assumptions, causing some biases to the estimates. Killer whales, like most k-selected species, violate the assumption of no overlapping generations because samples were not selected from a single age cohort, though the LD method tends to be robust to this type of violation (Waples & Do, 2010; Waples et al., 2014). The estimates showed very small effective population sizes (most with small confidence intervals) for the three populations identified. These corroborate the sightings data and small number of photo-identified individuals in the two Australian aggregations, which suggest small populations of killer whales in the areas. While there is a paucity of recent photo identification published on the NZ population, reported group sizes recorded from platforms of opportunity in the North Island (Hupman et al., 2015) suggest these estimates may be a conservative reflection of the true N_e. As

highlighted here, and previously by Visser (2000a), the NZ population of killer whales is small, emphasizing the need for regional conservation attention. Continuing photo identification and mark-recapture studies in all regions will allow for accurate population estimates, from which a ratio of effective size to census size could be potentially obtained. This is a vital parameter in wildlife management to determine a population's viability into the future (Frankham, 1995).

4.4 | Management and conservation implications

Population genomic data sets allow the detection of cryptic and complex population structure with great power and resolution, which can generate information to address issues in wildlife conservation and management. Such knowledge is particularly important when species are exposed to anthropogenic impacts that can negatively affect populations. Anthropogenic pressures can be exacerbated in small, genetically differentiated cetacean populations (Pratt et al., 2018) with low genome wide diversity (Attard et al., 2018) and small N_e. This study found a minimum of three genomically distinct populations of killer whales in the Australasian region, with low mitochondrial and moderate genome wide diversity, low migration rates between them, and small effective population sizes. We suggest that given these characteristics, the populations need to be cautiously considered as at least three distinct management units. However, due to gaps in the geographic distribution of available samples, the number of populations should be considered to represent a minimum for the region. Marine reserves provide the opportunity for the conservation of killer whales in Australasia, however, currently they do not encompass a large section of their distribution (Bouchet et al., 2018; CALM & MPRA, 2005; Donnelly et al., 2019; Ministry for the Environment, 2008; Salgado Kent et al., 2020; Visser, 2000a; Willis, 2013). Thus, Australasian killer whales are still exposed to anthropogenic pressures, such as overlap with commercial fisheries, vessel activity, pollution, oil and gas drilling, bycatch, and shootings (Abraham et al., 2017; Lusseau et al., 2009; Slooten & Dawson, 1995; Taylor & Smith, 1997; Visser, 1999b, 2000a, 2000b; Williams et al., 2006). These populations would benefit from comprehensive marine reserves, particularly in areas of biological significance that considers their low to moderate levels of genetic/genomic diversity and small population sizes, to reduce the potential of future declines and local extinctions.

4.5 | Future directions

This study provides the first population genomic analysis of killer whales in the Australasian region. Acquiring a broader distribution of samples should now be a priority for investigating the presence of additional populations in the region. In addition, comparisons between Australasian killer whales and global samples would allow for the investigation of contemporary and historical gene flow at larger scales. Finally, population ecological data, such as abundance estimates, ecotype characterization (e.g., morphometrics and feeding ecology), and social structure studies, including dispersal patterns, are generally still lacking. Such studies would complement genomic and genetic data, allowing for a more comprehensive understanding about the population history of these killer whales.

ACKNOWLEDGMENTS

Biopsy samples were collected under approval of the Flinders University Animal Ethics Committee (Approval number: E460/17), Department of Agriculture, Environment (Biological resources permit approval number: AU-COM2016-317 / AU-COM 2019-459; Cetacean research: Approval number CP-2013-0003 / CP 2018-0017) and western Australian Department of Environment and Conservation (Approval number: DPAW 08-000870-1). We would like to thank those that helped for the collection, processing and storage of samples, including volunteers in the field. This includes, the Tasmanian Museum and Art Gallery (specimen A4183) the Queensland Parks and Wildlife Service, the Department of Primary Industries, Parks, Water and Environment, Tasmania and their Marine Conservation program, Cetacean Ecology and Acoustics Laboratory at the University of Queensland and the Australian

Antarctic Division. New Zealand tissue samples were collected and examined under NZ Department of Conservation (DOC) permits RNW/NO/2009/06, 39239-MAR and 78364-MAR issued to Massey University. We acknowledge the full support of all DOC staff and mana whenua who facilitated and supported this work. We are particularly indebted to Ōraka-Aparima Rūnaka and Te Rūnanga o Te Whānau ā Apanui for entrusting the examination of their mass stranded Tohora as part of this wider Australasian study, and to Ramari Stewart for her support and facilitation of this and related mahi. We also would like to thank DEW, for awarding NRM RAIN scholarship to I.M.R. to partially fund this project, the College of Science and Engineering at Flinders University for partially covering sequencing costs and to CETREC WA for funding WA fieldwork seasons from 2013 to 2019. Lastly, we thank the reviewers for their time and suggestions that improved this paper.

AUTHOR CONTRIBUTIONS

Isabella Reeves: Conceptualization; data curation; formal analysis; funding acquisition; investigation; project administration; validation; visualization; writing - original draft; writing-review & editing. **John Totterdell:** Conceptualization; funding acquisition; investigation; project administration; resources; writing-review & editing. **Andrea Barcelo:** Formal analysis; investigation; visualization; writing-review & editing. **Jonathan Sandoval-Castillo:** Formal analysis; investigation; software; validation; visualization; writing-review & editing. **Kimberley Batley:** Formal analysis; investigation; writing-review & editing. **Karen Stockin:** Investigation; resources; writing-review & editing. **Emma Betty:** Investigation; resources; writing-review & editing. **David Donnelly:** Investigation; resources; writing-review & editing. **Rebecca Wellard:** Investigation; resources; writing-review & editing. **Luciano Beheregaray:** Conceptualization; data curation; investigation; project administration; resources; supervision; writing - original draft; writing-review & editing. **Luciana Moller:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; project administration; resources; supervision; validation; writing - original draft; writing-review & editing.

DATA ACCESSIBILITY

The SNP data sets and mtDNA sequences generated from this study are available on figshare at https://figshare.com/s/5b608d500c4a6691d0c1. Table S6 provides details of samples used in this study.

ORCID

Isabella M. Reeves https://orcid.org/0000-0003-0640-333X
John A. Totterdell https://orcid.org/0000-0003-4857-3134
Andrea Barceló https://orcid.org/0000-0002-4748-9953
Jonathan Sandoval-Castillo https://orcid.org/0000-0002-8428-3495
Kimberley C. Batley https://orcid.org/0000-0003-2084-0288
Karen A. Stockin https://orcid.org/0000-0002-2981-3983
Emma L. Betty https://orcid.org/0000-0002-9187-1957
Rebecca Wellard https://orcid.org/0000-0001-8427-564X
Luciano B. Beheregaray https://orcid.org/0000-0003-0944-3003
Luciana M. Möller https://orcid.org/0000-0002-7293-5847

REFERENCES

Abraham, E., Neubauer, P., Berkenbusch, K., & Richard, Y. (2017). *Assessment of the risk to New Zealand marine mammals from commercial fisheries*. New Zealand Aquatic Environment and Biodiversity Report No. 189. http://fs.fish.govt.nz/Page.aspx?pk=113&dk=24554

Alexander, D. H., & Lange, K. (2011). Enhancements to the ADMIXTURE algorithm for individual ancestry estimation. *BMC Bioinformatics*, *12*, 246. https://doi.org/10.1186/1471-2105-12-246

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*(9), 1655–1664. http://doi.org/10.1101/gr.094052.109

Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. *Nature Reviews Genetics*, *11*(10), 697. https://doi.org/10.1038/nrg2844

Álvarez, D., Lourenço, A., Oro, D., & Velo-Antón, G. (2015). Assessment of census (*N*) and effective population size (N_e) reveals consistency of N_e single-sample estimators and a high N_e/N ratio in an urban and isolated population of fire salamanders. *Conservation Genetics Resources*, *7*(3), 705–712. https://doi.org/10.1007/s12686-015-0480-0

Atlas of Living Australia. (2020). *Killer Whale record occurrences in Australasia*. https://doi.org/10.26197/5f2ce45bd9ab6

Attard, C. R., Beheregaray, L. B., Sandoval-Castillo, J., Jenner, K. C. S., Gill, P. C., Jenner, M.-N. M., Morrice, M.G., & Möller, L. M. (2018). From conservation genetics to conservation genomics: a genome-wide assessment of blue whales (*Balaenoptera musculus*) in Australian feeding aggregations. *Royal Society Open Science*, *5*, 170925. https://doi.org/10.1098/rsos.170925

Baird, R. W., & Stacey, P. J. (1988). Variation in saddle patch pigmentation in populations of killer whales (*Orcinus orca*) from British Columbia, Alaska, and Washington State. *Canadian Journal of Zoology*, *66*(11), 2582–2585. https://doi.org/10.1139/z88-380

Baird, R. W., & Whitehead, H. (2000). Social organization of mammal-eating killer whales: group stability and dispersal patterns. *Canadian Journal of Zoology*, *78*(12), 2096–2105. https://doi.org/10.1139/z00-155

Baker, C. S., Boren, L., Childerhouse, S., Constantine, R., van Helden, A., Lundquist, D., Rayment, W., & Rolfe, J. R. (2019). Conservation status of New Zealand marine mammals, 2019. *New Zealand Threat Classification Series*, *29*, 1–18.

Baker, C., Perry, A., Bannister, J., Weinrich, M., Abernethy, R. B., Calambokidis, J., Lien, J., Lambertsen, R. H., Ramírez, J. U., & Vasquez, O. (1993). Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proceedings of the National Academy of Sciences of the United States of America*, *90*(17), 8239–8243. https://doi.org/10.1073/pnas.90.17.8239

Barceló, A., Sandoval-Castillo, J., Stockin, K. A., Bilgmann, K., Attard, C. M., Zanardo, N., Parra, G. J., Hupman, K., Reeves, I. M., Betty, M. L., Tezanos-Pinto, G., Beheregaray, L. B., & Möller, L. M. (2021). A matter of scale: Population genomic structure and connectivity of fisheries at-risk common dolphins (*Delphinus delphis*) From Australasia. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.616673

Barrett-Lennard, L. (2011). Killer whale evolution: Populations, ecotypes, species, Oh my. *Whalewatcher: Journal of the America Cetacean Society*, *40*, 48–53.

Batley, K., Sandoval-Castillo, J., Kemper, C., Zanardo, N., Tomo, I., Beheregaray, L., & Möller, L. (2021). Whole genomes reveal multiple candidate genes and pathways involved in the immune response of dolphins to a highly infectious viral disease. *Molecular Ecology*. https://doi.org/10.1111/mec.15873

Bigg, M. A. (1982). An assessment of killer whale (*Orcinus orca*) stocks off Vancouver Island, British Columbia. *Reports of the International Whaling Commission*, *32*, 625–666.

Bigg, M. A. (1987). *Killer whales: A study of their identification, genealogy, and natural history in British Columbia and Washington State*. Phantom Press.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30* (15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bouchet, P., Meeuwig, J., Erbe, C., Wellard, R., Pattiaratchi, C., & Salgado-Kent, C. (2018). *Bremer Canyon emerging priorities project EP2: final report: Occurrence and distribution of marine wildlife in the Bremer Bay region*. University of Western Australia. https://research-repository.uwa.edu.au/en/publications/bremer-canyon-emerging-priorities-project-ep2-final-report-occurr

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

CALM & MPRA. (2005). *Management Plan for the Ningaloo Marine Park and Muiron Islands Marine Management Area 2005–2015*. Western Australian Government, Perth. https://www.dpaw.wa.gov.au/images/documents/parks/management-plans/decarchive/ningaloo_mp_01_2005_withmaps.pdf

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

Cieslak, M., Tixier, P., Richard, G., Hindell, M., Arnould, J. P., & Lea, M.-A. (2021). Acoustics and photo-identification provide new insights on killer whale presence and movements when interacting with longline fisheries in South East Australia. *Fisheries Research*, *233*, 105748. https://doi.org/10.1016/j.fishres.2020.105748

Dalebout, M. L., Robertson, K. M., Frantzis, A., Engelhaupt, D., Mignucci Giannoni, A. A., Rosario Delestre, R. J., & Baker, C. S. (2005). Worldwide structure of mtDNA diversity among Cuvier's beaked whales (*Ziphius cavirostris*): implications for threatened populations. *Molecular Ecology*, *14*(11), 3353–3371. https://doi.org/10.1111/j.1365-294X.2005.02676.x

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., Depristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., Durbin, R., 1000 Genomes Project Analysis Group, & Sherry, S. T. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Danecek, P., Schiffels, S., & Durbin, R. (2016). *Multiallelic calling model in bcftools (−m)*. https://samtools.github.io/bcftools/call-m.pdf.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, *9*(8), 772–772. https://doi.org/10.1038/nmeth.2109

Deecke, V. B., Ford, J. K., & Spong, P. (2000). Dialect change in resident killer whales: implications for vocal learning and cultural transmission. *Animal Behaviour*, *60*(5), 629–638. https://doi.org/10.1006/anbe.2000.1454

Do, C., Waples, R. S., Peel, D., Macbeth, G., Tillett, B. J., & Ovenden, J. R. (2014). NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (*Ne*) from genetic data. *Molecular Ecology Resources*, *14*, 209–214. https://doi.org/10.1111/1755-0998.12157

Donnelly, D. M., McInnes, J. D., Jenner, K. C. S., Jenner, M.-N. M. & Morrice, M. (2021). The first records of antarctic type B and C killer whales (*Orcinus orca*) in Australian coastal waters. *Aquatic Mammals*, *47*(3), 292–302. https://doi.org/10.1578/AM.47.3.2021.292

Donnelly, D., McInnes, J., Morrice, M. G., & Andrews, C. (2019). *Killer whales of Eastern Australia*. Killer Whales Australia, Victoria, Australia. https://fliphtml5.com/psby/rmzq/basic

Dudchenko, O., Batra, S. S., Omer, A. D., Nyquist, S. K., Hoeger, M., Durand, N. C., Shamim, M. S., Machol, I., Lander, E. S., Presser Aiden, A., & Aiden, E. L. (2017). De novo assembly of the *Aedes aegypti* genome using Hi-C yields chromosome-length scaffolds. *Science*, *356*(6333), 92–95. https://doi.org/10.1126/science.aal3327

Dudchenko, O., Shamim, M. S., Batra, S., Durand, N. C., Musial, N. T., Mostofa, R., Pham, M., St Hilaire, B. G., Yao, W., Stamenova, E., Hoeger, M., Nyquist, S. K., Korchina, V., Pletch, K., Flanagan, J. P., Tomaszewicz, A., McAloose, D., Pérez Estrada, C., … Aiden, E. L. (2018). The Juicebox Assembly Tools module facilitates de novo assembly of mammalian genomes with chromosome-length scaffolds for under $1000. *BioRxiv*. https://doi.org/10.1101/254797

Durban, J., & Pitman, R. (2011). Antarctic killer whales make rapid, round-trip movements to subtropical waters: evidence for physiological maintenance migrations? *Biology Letters*, *8*(2), 274–277. https://doi.org/10.1098/rsbl.2011.0875

Eisert, R., Ovsyanikova, E., Visser, I., Ensor, P., Currey, R., & Sharp, B. (2015). *Seasonal site fidelity and movement of type-C killer whales between Antarctica and New Zealand*. Paper SC/66a/SM/69 presented to the Scientific Committee of the International Whaling Commission, San Diego, CA.

Excoffier, L., Laval, G., & Schneider, S. (2005). Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, 47-50. https://doi.org/10.1177/117693430500100003

Filatova, O., Borisova, E., Shpak, O., Meschersky, I., Tiunov, A., Goncharov, A., Fedutil, I. D., & Burdin, A. (2015). Reproductively isolated ecotypes of killer whales *Orcinus orca* in the seas of the Russian Far East. *Biology Bulletin*, *42*(7), 674–681. https://doi.org/10.1134/S1062359015070043

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics*, *180*(2), 977–993. https://doi.org/10.1534/genetics.108.092221

Foote, A. D., & Morin, P. A. (2016). Genome-wide SNP data suggest complex ancestry of sympatric North Pacific killer whale ecotypes. *Heredity*, *117*(5), 316. https://doi.org/10.1038/hdy.2016.54

Foote, A. D., Liu, Y., Thomas, G. W., Vinař, T., Alföldi, J., Deng, J., Dugan, S., van Elk, C. E., Hunter, M. E., Joshi, V., Khan, Z., Kovar, C., Lee, S. L., Joshi, V. Lindblad-Toh, K., Mancia, A., Nielsen, R., Qin, X., Qu, J., … Gibbs, R. A. (2015). Convergent evolution of the genomes of marine mammals. *Nature Genetics*, *47*, 272–275. https://doi.org/10.1038/ng.3198

Foote, A. D., Martin, M., Louis, M., Pacheco, G., Robertson, K., Sinding, M.-H., Amaral, A. R., Baird, R. W., Baker, C. S., Ballance, L., Barlow, J., Brownlow, A., Collins,T., Constantine, R., Dabin, W., Dalla Rosa, L., Davison, N. J., Durban, J. W., Esteban, R., … Morin, P. (2019). Killer whale genomes reveal a complex history of recurrent admixture and vicariance. *Molecular Ecology*, *28*(14), 3427–3444. https://doi.org/10.1111/mec.15099

Foote, A. D., Newton, J., Piertney, S. B., Willerslev, E., & Gilbert, M. T. P. (2009). Ecological, morphological and genetic divergence of sympatric North Atlantic killer whale populations. *Molecular Ecology*, *18*(24), 5207–5217. https://doi.org/10.1111/j.1365-294X.2009.04407.x

Foote, A. D., Vijay, N., Ávila-Arcos, M. C., Baird, R. W., Durban, J. W., Fumagalli, M., Gibbs, R. A., Bradley Hanson, M., Korneliussen, T. S., Martin, M. D., Robertson, K. M., Sousa, V. C., Vieira, F. G., Vina, T., Wade, P., Worley, K. C., Excoffier, L., Morin, P. A., Gilbert, M. T. P., & Martin, M. D. (2016). Genome-culture coevolution promotes rapid divergence of killer whale ecotypes. *Nature Communications*, *7*, 11693. https://doi.org/10.1038/ncomms11693

Foote, A. D., Vilstrup, J. T., De Stephanis, R., Verborgh, P., Abel Nielsen, S. C., Deaville, R., Kleivane, K., Martín, V., Miller, P. J. O., Øien, N., Pérez-Gil, M., Rasmussen, M., Reid, R. J., Robertson, K. M., Rogan, E., Similä, T., Tejedor, M. L., Vester, H., Víkingsson, G. A., … Piertney, S. B. (2011). Genetic differentiation among North Atlantic killer whale populations. *Molecular Ecology*, *20*(3), 629–641. https://doi.org/10.1111/j.1365-294X.2010.04957.x

Ford, J. K., Ellis, G. M., Barrett-Lennard, L. G., Morton, A. B., Palm, R. S., & Balcomb, K. C., III. (1998). Dietary specialization in two sympatric populations of killer whales (*Orcinus orca*) in coastal British Columbia and adjacent waters. *Canadian Journal of Zoology*, *76*(8), 1456–1471. https://doi.org/10.1139/z98-089

Ford, M. J., Parsons, K., Ward, E., Hempelmann, J., Emmons, C. K., Bradley Hanson, M., Balcomb, K. C., & Park, L. K. (2018). Inbreeding in an endangered killer whale population. *Animal Conservation*, *21*(5), 423–432. https://doi.org/10.1111/acv.12413

Frankham, R. (1995). Effective population size/adult population size ratios in wildlife: a review. *Genetics Research*, *66*(2), 95–107. https://doi.org/10.1017/S0016672300034455

Frankham, R., Ballou, J. D., Ralls, K., Eldridge, M., Dudash, M. R., Fenster, C. B., Lacy, R. C., & Sunnucks, P. (2017). *Genetic management of fragmented animal and plant populations*. Oxford University Press.

Funk, W. C., Mckay, J. K., Hohenlohe, P. A., & Allendorf, F. W. (2012). Harnessing genomics for delineating conservation units. *Trends in Ecology & Evolution*, *27*(9), 489–496. https://doi.org/10.1016/j.tree.2012.05.012

Gaspari, S., Scheinin, A., Holcer, D., Fortuna, C., Natali, C., Genov, T., Frantzis, A., Chelazzi, G., & Moura, A. E. (2015). Drivers of population structure of the bottlenose dolphin (*Tursiops truncatus*) in the Eastern Mediterranean Sea. *Evolutionary Biology*, *42*(2), 177–190. https://doi.org/10.1007/s11692-015-9309-8

Gaughran, S. J., Quinzin, M. C., Miller, J. M., Garrick, R. C., Edwards, D. L., Russello, M. A., Poulakakis, N., Ciofi, C., Beheregaray, L. B., & Caccone, A. (2018). Theory, practice, and conservation in the age of genomics: The Galápagos giant tortoise as a case study. *Evolutionary Applications*, *11*(7), 1084–1093. https://doi.org/10.1111/eva.12551

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

Guindon, S., & Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, *52*(5), 696–704. https://doi.org/10.1080/10635150390235520

Guinet, C. (1991). Intentional stranding apprenticeship and social play in killer whales (*Orcinus orca*). *Canadian Journal of Zoology*, *69*(11), 2712–2716. https://doi.org/10.1139/z91-383

Gu, Z., Gu, L., Eils, R., Schlesner, M., & Brors, B. (2014). *circlize* implements and enhances circular visualization in R. *Bioinformatics*, *30*(19), 2811–2812. https://doi.org/10.1093/bioinformatics/btu393

Hill, W. G. (1981). Estimation of effective population size from data on linkage disequilibrium. *Genetics Research*, *38*(3), 209–216. https://doi.org/10.1093/bioinformatics/btu393

Hoelzel, A. R. (1993). Foraging behaviour and social group dynamics in Puget Sound killer whales. *Animal Behaviour*, *45*(3), 581–591. https://doi.org/10.1006/anbe.1993.1068

Hoelzel, A. R. (1998). Genetic structure of cetacean populations in sympatry, parapatry, and mixed assemblages: implications for conservation policy. *Journal of Heredity*, *89*(5), 451–458. https://doi.org/10.1093/jhered/89.5.451

Hoelzel, A. R., & Dover, G. A. (1991). Genetic differentiation between sympatric killer whale populations. *Heredity*, *66*(2), 191–195. https://doi.org/10.1038/hdy.1991.24

Hoelzel, A., Dahlheim, M., & Stern, S. (1998). Low genetic variation among killer whales (*Orcinus orca*) in the eastern North Pacific and genetic differentiation between foraging specialists. *Journal of Heredity*, *89*(2), 121–128. https://doi.org/10.1093/jhered/89.2.121

Hoelzel, A. R., Hey, J., Dahlheim, M. E., Nicholson, C., Burkanov, V., & Black, N. (2007). Evolution of population structure in a highly social top predator, the killer whale. *Molecular Biology and Evolution*, *24*(6), 1407–1415. https://doi.org/10.1093/molbev/msm063

Hoelzel, A. R., Natoli, A., Dahlheim, M. E., Olavarria, C., Baird, R. W., & Black, N. A. (2002). Low worldwide genetic diversity in the killer whale (*Orcinus orca*): implications for demographic history. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1499), 1467–1473. https://doi.org/10.1098/rspb.2002.2033

Hohenlohe, P. A., Hand, B. K., Andrews, K. R., & Luikart, G. (2018). Population genomics provides key insights in ecology and evolution. In O. P. Rajora (Ed.) *Population genomics concepts*, *approaches and applications* (pp. 483–510). Springer International Publishing.

Hooper, R., Excoffier, L., Forney, K., Gilbert, M. T. P., Martin, M. D., Morin, P. A., Wolf, J. B. W., & Foote, A. (2020). Runs of homozygosity in killer whale genomes provide a global record of demographic histories. *BioRxiv* [preprint]. https://doi.org/10.1101/2020.04.08.031344

Hupman, K., Visser, I., Martinez, E., & Stockin, K. (2015). Using platforms of opportunity to determine the occurrence and group characteristics of orca (*Orcinus orca*) in the Hauraki Gulf, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, *49*, 132–149. https://doi.org/10.1080/00288330.2014.980278

Jefferson, T. A., Webber, M. A., Pitman, R., & Jarrett, B. (2015). *Marine mammals of the world: A comprehensive guide to their identification*. Elsevier Science Publishing.

Jonas, E., Fikse, F., Rönnegård, L., & Mouresan, E. F. (2018). Genomic selection. In P. O. Rajora (Ed.), *Population genomics concepts*, *approaches and applications* (pp. 427–480). Springer International Publishing.

Jukes, T. H., & Cantor, C. R. (1969). Evolution of protein molecules. *Mammalian Protein Metabolism*, *3*, 21–132.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C., Thierer, T., Ashton, B., Meintjes, P., & Drummond, A. (2012). Geneious Basic: an integrated and extendable desktop

software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, *35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software*, *25* (1), 1–18. https://doi.org/10.18637/jss.v025.i01

Leigh, J. W., & Bryant, D. (2015). POPART: full feature software for haplotype network construction. *Methods in Ecology and Evolution*, (9)*6*, 1110–1116. https://doi.org/10.1111/2041-210X.12410

Li, H. (2014). Toward better understanding of artifacts in variant calling from high-coverage samples. *Bioinformatics*, *30*(20), 2843–2851. https://doi.org/10.1093/bioinformatics/btu356

Linck, E., & Battey, C. (2019). Minor allele frequency thresholds strongly affect population structure inference with genomic data sets. *Molecular Ecology Resources*, *19*(3), 639–647. https://doi.org/10.1111/1755-0998.12995

Lusseau, D., Bain, D. E., Williams, R., & Smith, J. C. (2009). Vessel traffic disrupts the foraging behavior of southern resident killer whales *Orcinus orca*. *Endangered Species Research*, *6*(3), 211–221. https://doi.org/10.3354/esr00154

Mckenna, A., Hanna, M., Banks, E., Sivachenko, A., Cibulskis, K., Kernytsky, A., Garimella, K., Altshuler, D., Gabriel, S., Daly, M., & Daly, M. (2010). The Genome Analysis Toolkit: A MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Research*, *20*(9), 1297–1303. http://doi.org/10.1101/gr.107524.110

Meirmans, P. G., & Van Tienderen, P. H. (2004). GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. *Molecular Ecology Notes*, *4*(4), 792–794. https://doi.org/10.1111/j.1471-8286.2004.00770.x

Ministry for the Environment. (2008). *Environmental report card*: *Marine areas with legal protection*. https://environment.govt.nz/publications/environmental-report-card-marine-areas-with-legal-protection/

Möller, L. M., & Beheregaray, L. B. (2004). Genetic evidence for sex biased dispersal in resident bottlenose dolphins (*Tursiops aduncus*). *Molecular Ecology*, *13*(6), 1607–1612. https://doi.org/10.1111/j.1365-294X.2004.02137.x

Morin, P. A., Parsons, K. M., Archer, F. I., Ávila Arcos, M. C., Barrett-Lennard, L. G., Dalla Rosa, L., Duchêne, S., Durban, J. W., Ellis, G. M., Ferguson, S. H., Ford, J. K., Ford, M. J., Garilao, C., Gilbert, M. T. P., Kaschner, K., Matkin, C. O., Petersen, S. D., Robertson, K. M., Visser, I. N., … Foote, A. D. (2015). Geographic and temporal dynamics of a global radiation and diversification in the killer whale. *Molecular Ecology*, *24*(15), 3964–3979. https://doi.org/10.1111/mec.13284

Morrice, M. G. (2004). *Killer whales* (Orcinus orca) *in Australian territorial waters* [Techinical paper]. Deakin University.

Moura, A. E., Kenny, J. G., Chaudhuri, R., Hughes, M. A., Welch, A. J., Reisinger, R. R., Nico de Bruyn, P. J., Dahlheim, M. E., Hall, N., & Hoelzel, A. R. (2014). Population genomics of the killer whale indicates ecotype evolution in sympatry involving both selection and drift. *Molecular Ecology*, *23*(21), 5179–5192. https://doi.org/10.1111/mec.12929

Narum, S. R. (2006). Beyond Bonferroni: less conservative analyses for conservation genetics. *Conservation Genetics*, *7*(5), 783–787. https://doi.org/10.1007/s10592-005-9056-y

Olavarría, C., Baker, C. S., & Tezanos-Pinto, G. (2014). Low mtDNA genetic diversity among killer whales around New Zealand. *New Zealand Journal of Marine and Freshwater Research*, *48*, 147–153. https://doi.org/10.1080/00288330.2013.844721

Palmer, C., & Chatto, R. (2013). First confirmed sighting of the Killer Whale *Orcinus orca* in Northern Territory coastal waters. *Northern Territory Naturalist*, *24*(6), 65–67.

Parsons, K. M., Durban, J. W., Burdin, A. M., Burkanov, V. N., Pitman, R. L., Barlow, J., Barrett-Lennard, L. G., LeDuc, R. G., Robertson, K. M., Matkin, C. O., & Wade, P. R. (2013). Geographic patterns of genetic differentiation among killer whales in the northern North Pacific. *Journal of Heredity*, *104*(6), 737–754. https://doi.org/10.1093/jhered/est037

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

Pilot, M., Dahlheim, M. E., & Hoelzel, A. R. (2010). Social cohesion among kin, gene flow without dispersal and the evolution of population genetic structure in the killer whale (*Orcinus orca*). *Journal of Evolutionary Biology*, *23*, 20–31. https://doi.org/10.1111/j.1420-9101.2009.01887.x

Piry, S., Alapetite, A., Cornuet, J.-M., Paetkau, D., Baudouin, L., & Estoup, A. (2004). GENECLASS2: a software for genetic assignment and first-generation migrant detection. *Journal of Heredity*, *95*(6), 536–539. https://doi.org/10.1093/jhered/esh074

Pitman, R. (2011). Antarctic killer whales: top of the food chain at the bottom of the world. *Journal of the American Cetacean Society*, *40*, 39–45. https://doi.org/10.1098/rsbl.2011.0875

Pitman, R. L., & Durban, J. W. (2010). Killer whale predation on penguins in Antarctica. *Polar Biology*, *33*(11), 1589–1594. https://doi.org/10.1007/s00300-010-0853-5

Pitman, R. L., & Durban, J. W. (2012). Cooperative hunting behavior, prey selectivity and prey handling by pack ice killer whales (*Orcinus orca*), type B, in Antarctic Peninsula waters. *Marine Mammal Science*, *28*, 16–46. https://doi.org/10.1111/j.1748-7692.2010.00453.x

Pitman, R. L., Durban, J. W., Greenfelder, M., Guinet, C., Jorgensen, M., Olson, P. A., Plana, J., Tixier, P., & Towers, J. R. (2011). Observations of a distinctive morphotype of killer whale (*Orcinus orca*), type D, from subantarctic waters. *Polar Biology*, *34*(2), 303–306. https://doi.org/10.1007/s00300-010-0871-3

Pitman, R. L., & Ensor, P. (2003). Three forms of killer whales (*Orcinus orca*) in Antarctic waters. *Journal of Cetacean Research and Management*, *5*(2), 131–140.

Pitman, R. L., Perryman, W. L., Leroi, D., & Eilers, E. (2007). A dwarf form of killer whale in Antarctica. *Journal of Mammalogy*, *88*, 43–48. https://doi.org/10.1644/06-MAMM-A-118R1.1

Pitman, R. L., Totterdell, J. A., Fearnbach, H., Ballance, L. T., Durban, J. W., & Kemps, H. (2015). Whale killers: Prevalence and ecological implications of killer whale predation on humpback whale calves off Western Australia. *Marine Mammal Science*, *31*(2), 629–657. https://doi.org/10.1111/mms.12182

Pratt, E. A. (2020). *The genomic basis of adaptation in bottlenose dolphins* (*genus* Tursiops) [Doctoral dissertation], Flinders University, Adelaide.

Pratt, E. A., Beheregaray, L. B., Bilgmann, K., Zanardo, N., Diaz-Aguirre, F., & Möller, L. M. (2018). Hierarchical metapopulation structure in a highly mobile marine predator: the southern Australian coastal bottlenose dolphin (*Tursiops* cf. *australis*). *Conservation Genetics*, *19*(3), 637–654. https://doi.org/10.1007/s10592-017-1043-6

Ralls, K., Ballou, J. D., Dudash, M. R., Eldridge, M. D., Fenster, C. B., Lacy, R. C., Sunnucks, P., & Frankham, R. (2018). Call for a paradigm shift in the genetic management of fragmented populations. *Conservation Letters*, *11*(2), e12412. https://doi.org/10.1111/conl.12412

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *67*(5), 901–904. https://doi.org/10.1093/sysbio/syy032

Rannala, B. (2020). *BayesAss Version 3.0.4.2* [Computer software]. https://github.com/brannala/BA3/releases

Rannala, B., & Mountain, J. (1997). Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(17), 9197–9201. https://doi.org/10.1073/pnas.94.17.9197

Reeves, R., Pitman, R., & Ford, J. K. (2017). *Orcinus orca*. *The IUCN Red List of Threatened Species 2017*, *e. T15421A50368125*. https://doi.org/10.2305/IUCN.UK.2017-3.RLTS.T15421A50368125.en

Reich, D., Price, A. L., & Patterson, N. (2008). Principal component analysis of genetic data. *Nature Genetics*, *40*(5), 491–492. https://doi.org/10.1038/ng0508-491

Riesch, R., Barrett-Lennard, L. G., Ellis, G. M., Ford, J. K., & Deecke, V. B. (2012). Cultural traditions and the evolution of reproductive isolation: ecological speciation in killer whales? *Biological Journal of the Linnean Society*, *106*, 1–17. https://doi.org/10.1111/j.1095-8312.2012.01872.x

Rodríguez Ramilo, S. T., & Wang, J. (2012). The effect of close relatives on unsupervised Bayesian clustering algorithms in population genetic structure analysis. *Molecular Ecology Resources*, *12*(5), 873–884. https://doi.org/10.1111/j.1755-0998.2012.03156.x

RStudio Team. (2016). *RStudio*: *Integrated development for R* [Computer software]. RStudio, Inc., Boston, MA.

Salgado Kent, C., Bouchet, P., Wellard, R., Parnum, I., Fouda, L., & Erbe, C. (2020). Seasonal productivity drives aggregations of killer whales and other cetaceans over submarine canyons of the Bremer Sub-Basin, south-western Australia. *Australian Mammalogy*, *43*(2), 168–178. https://doi.org/10.1071/AM19058

Sandoval Castillo, J., Robinson, N. A., Hart, A. M., Strain, L. W., & Beheregaray, L. B. (2018). Seascape genomics reveals adaptive divergence in a connected and commercially important mollusc, the greenlip abalone (*Haliotis laevigata*), along a longitudinal environmental gradient. *Molecular Ecology*, *27*(7), 1603–1620. https://doi.org/10.1111/mec.14526

Slooten, E., & Dawson, S. M. (1995). Conservation of marine mammals in New Zealand. *Pacific Conservation Biology*, *2*, 64–76.

Stevens, T. A., Duffield, D. A., Asper, E. D., Hewlett, K. G., Bolz, A., Gage, L. J., & Bossart, G. D. (1989). Preliminary findings of restriction fragment differences in mitochondrial DNA among killer whales (*Orcinus orca*). *Canadian Journal of Zoology*, *67*(10), 2592–2595. https://doi.org/10.1139/z89-365

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

Taylor, R., & Smith, I. (1997). *The state of New Zealand's enviroment*. GP Publications.

Thomsen, F., Franck, D., & Ford, J. (2001). Characteristics of whistles from the acoustic repertoire of resident killer whales (*Orcinus orca*) off Vancouver Island, British Columbia. *Journal of the Acoustical Society of America*, *109*(3), 1240–1246. https://doi.org/10.1121/1.1349537

Totterdell, J. (2015). *Ningaloo killer whales*: *ID Catalogue 2015*. Marine Information and Research Group (MIRG), Esperance, Australia. https://doi.org/10.13140/RG.2.1.3072.5208

Totterdell, J., Wellard, R., Reeves, I. M., Elsdon, B., Markovic, P., Yoshida, M., Fairchild, A., Sharp, G., & Pitman, R. (in review). From Australia: The first three records of killer whale predation on blue whales [Manuscript submitted for publication].

Travers, T., Van Den Hoff, J., Lea, M.-A., Carlyon, K., Reisinger, R., De Bruyn, P. N., & Morrice, M. (2018). Aspects of the ecology of killer whale (*Orcinus orca* Linn.) groups in the near-shore waters of Sub-Antarctic Macquarie Island. *Polar Biology*, *41*(11), 2249–2259. https://doi.org/10.1007/s00300-018-2361-y

Tukey, J. W. (1949). Comparing individual means in the analysis of variance. *Biometrics*, *5*, 99–114. https://doi.org/10.2307/3001913

Turesson, G. (1922). The species and the variety as ecological units. *Hereditas*, *3*, 100–113. https://doi.org/10.1111/j.1601-5223.1922.tb02727.x

Visser, I. (1999a). Benthic foraging on stingrays by killer whales (*Orcinus orca*) in New Zealand waters. *Marine Mammal Science*, *15*, 220–227. https://doi.org/10.1111/j.1748-7692.1999.tb00793.x

Visser, I. N. (1999b). Propeller scars on and known home range of two orca (*Orcinus orca)* in New Zealand waters. *New Zealand Journal of Marine and Freshwater Research*, *33*(4), 635–642. https://doi.org/10.1080/00288330.1999.9516906

Visser, I. N. (2000a). *Orca* (Orcinus orca) *in New Zealand waters* [Doctoral dissertation]. University of Auckland.

Visser, I. N. (2000b). Killer whale (*Orcinus orca*) interactions with longline fisheries in New Zealand waters. *Aquatic Mammals*, *26*(3), 241–252.

Visser, I. N. (2005). First observations of feeding on thresher (*Alopias vulpinus*) and hammerhead (*Sphyrna zygaena*) sharks by killer whales (*Orcinus orca*) specialising on elasmobranch prey. *Aquatic Mammals*, *31*, 83–88. https://doi.org/10.1578/AM.31.1.2005.83

Visser, I. N. (2007). Killer whales in New Zealand waters: status and distribution with comments on foraging. Paper SC/59/-SM19 *presented to the Scientific Committee of the International Whaling Commission*, Anchorage, AK.

Visser, I. N., & Bonoccorso, F. J. (2003). New observations and a review of killer whale (*Orcinus orca*) sightings in Papua New Guinea waters. *Aquatic Mammals*, *29*, 150–172.

Visser, I. N., & Cooper, T. E. (2020). *Orca Research Trust Guide to New Zealand Orca*. Black & White Fish Publications, Tutukaka.

Visser, I. N., Berghan, J., Van Meurs, R., & Fertl, D. (2000). Killer whale (*Orcinus orca*) predation on a shortfin mako shark (*Isurus oxyrinchus*) in New Zealand waters. *Aquatic Mammals*, *26*(3), 229–231.

Visser, I. N., Zaeschmar, J., Halliday, J., Abraham, A., Ball, P., Bradley, R., Daly, S., Hatwell, T., Johnson., T., Johnson, W., Kay, L., Maessen, T., McKay, V., Peters, T., Turner, N., Umuroa, B., & Pace, D. S., & Johnson, W. (2010). First record of predation on false killer whales (*Pseudorca crassidens*) by killer whales (*Orcinus orca*). *Aquatic Mammals*, *36*(2), 195–204. https://doi.org/10.1578/AM.36.2.2010.195

Wang, J. (2007). Triadic IBD coefficients and applications to estimating pairwise relatedness. *Genetics Research*, *89*(3), 135–153. https://doi.org/10.1017/S0016672307008798

Wang, J. (2011). COANCESTRY: a program for simulating, estimating and analysing relatedness and inbreeding coefficients. *Molecular Ecology Resources*, *11*, 141–145. https://doi.org/10.1111/j.1755-0998.2010.02885.x

Waples, R. S. (2016). Making sense of genetic estimates of effective population size. *Molecular Ecology*, *25*, 4689–4691. https://doi.org/10.1111/mec.13814

Waples, R. S., & Do, C. (2008). LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources*, *8*(4), 753–756. https://doi.org/10.1111/j.1755-0998.2007.02061.x

Waples, R. S., & Do, C. (2010). Linkage disequilibrium estimates of contemporary *Ne* using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evolutionary Applications*, *3*(3), 244–262. https://doi.org/10.1111/j.1752-4571.2009.00104.x

Waples, R. S., & England, P. R. (2011). Estimating contemporary effective population size on the basis of linkage disequilibrium in the face of migration. *Genetics*, *189*(2), 633–644. https://doi.org/10.1534/genetics.111.132233

Waples, R. S., Antao, T., & Luikart, G. (2014). Effects of overlapping generations on linkage disequilibrium estimates of effective population size. *Genetics*, *197*(2), 769–780. https://doi.org/10.1534/genetics.114.164822

Weir, B. S., & Cockerham, C. C. (1984). Estimating *F*-statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370. https://doi.org/10.2307/2408641

Wellard, R. (2018). *Vocal repertoire, social structure and feeding preferences of Australian and Antarctic killer whales* (Orcinus orca) [Doctoral dissertation]. Curtin University.

Wellard, R., & Erbe, C. (2017). *Killer whales of the Bremer Sub-Basin: A photo-id catalogue*. Centre for Marine Science and Technology, Curtin University.

Wellard, R., Erbe, C., Fouda, L., & Blewitt, M. (2015). Vocalisations of killer whales (*Orcinus orca*) in the Bremer Canyon, Western Australia. *PLoS ONE*, *10*(9), e0136535. https://doi.org/10.1371/journal.pone.0136535

Wellard, R., Lightbody, K., Fouda, L., Blewitt, M., Riggs, D., & Erbe, C. (2016). Killer whale (*Orcinus orca*) predation on beaked whales (*Mesoplodon* spp.) in the Bremer Sub-Basin, Western Australia. *PLoS ONE*, *11*(12), e0166670. https://doi.org/10.1371/journal.pone.0166670

Wellard, R., Pitman, R., Durban, J., & Erbe, C. (2018). Antarctic type C killer whale (*Orcinus orca*) call repertoire from McMurdo Sound, Ross Sea. *Journal of the Acoustical Society of America*, *144*(3), 1978–q978. https://doi.org/10.1121/1.5068635

Wickham, H. (2016). *ggplot2: Elegant graphics for data analysis*. Springer.

Williams, R., Lusseau, D., & Hammond, P. S. (2006). Estimating relative energetic costs of human disturbance to killer whales (*Orcinus orca*). *Biological Conservation*, *133*(3), 301–311. https://doi.org/10.1016/j.biocon.2006.06.010

Willis, T. J. (2013). *Scientific and biodiversity values of marine reserves*: *a review* (DOC Research and Development series 340). Department of Conservation, Wellington, New Zealand.

Wilson, G. A., & Rannala, B. (2003). Bayesian inference of recent migration rates using multilocus genotypes. *Genetics*, *163*(3), 1177–1191. https://doi.org/10.1093/genetics/163.3.1177

Yurk, H., Barrett-Lennard, L., Ford, J., & Matkin, C. (2002). Cultural transmission within maternal lineages: vocal clans in resident killer whales in southern Alaska. *Animal Behaviour*, *63*(6), 1103–1119. https://doi.org/10.1006/anbe.2002.3012

Zanardo, N., Parra, G. J., Diaz-Aguirre, F., Pratt, E. A., & Möller, L. M. (2018). Social cohesion and intra-population community structure in southern Australian bottlenose dolphins (*Tursiops* sp.). *Behavioral Ecology and Sociobiology*, *72*(9), 156. https://doi.org/10.1007/s00265-018-2557-8

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Reeves, I. M., Totterdell, J. A., Barceló, A., Sandoval-Castillo, J., Batley, K. C., Stockin, K. A., Betty, E. L., Donnelly, D. M., Wellard, R., Beheregaray, L. B., & Möller, L. M. (2021). Population genomic structure of killer whales (*Orcinus orca*) in Australian and New Zealand waters. *Marine Mammal Science*, 1–24. https://doi.org/10.1111/mms.12851