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# Ancestry testing of "Old Tom," a killer whale central to mutualistic interactions with human whalers

**Isabella M. Reeves[1,2,*], John A. Totterdell[2], Emma L. Betty[3], David M. Donnelly[4], Angela George[5], Steven Holmes[5], Luciana Moller[1,6,7], Karen A. Stockin[3], Rebecca Wellard[8], Charlie White[1,6], Andrew D. Foote[9,10]**

[1]Flinders University, College of Science and Engineering, Bedford Park, Adelaide,South Australia, Australia
[2]Cetacean Research Centre (CETREC WA), Esperance, Perth, Western Australia, Australia
[3]Cetacean Ecology Research Group, School of Natural Sciences, Massey University, Auckland, New Zealand
[4]Killer Whales Australia, Mornington, Melbourne, Victoria, Australia
[5]Eden Killer Whale Museum, New South Wales, Sydney, Australia
[6]Cetacean Ecology, Behaviour and Evolution Laboratory, College of Science and Engineering, Flinders University, Bedford Park, Adelaide, South Australia, Australia
[7]Molecular Ecology Laboratory, College of Science and Engineering, Flinders University, Bedford Park, Adelaide, South Australia, Australia
[8]Project ORCA, Perth, Western Australia, Australia
[9]Department of Natural History, NTNU University Museum, Norwegian University of Science and Technology (NTNU), Trondheim, Norway
[10]Department of Biosciences, Centre for Ecological and Evolutionary Synthesis, University of Oslo, Oslo, Norway

[*]Corresponding author: College of Science and Engineering, Flinders University, Bedford Park, Adelaide, South Australia 5042, Australia. Email: isabella.reeves@flinders.edu.au

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## Abstract

Cooperative hunting between humans and killer whales (*Orcinus orca*) targeting baleen whales was reported in Eden, New South Wales, Australia, for almost a century. By 1928, whaling operations had ceased, and local killer whale sightings became scarce. A killer whale from the group, known as "Old Tom," washed up dead in 1930 and his skeleton was preserved. How these killer whales from Eden relate to other populations globally and whether their genetic descendants persist today remains unknown. We extracted and sequenced DNA from Old Tom using ancient DNA techniques. Genomic sequences were then compared with a global dataset of mitochondrial and nuclear genomes. Old Tom shared a most recent common ancestor with killer whales from Australasia, the North Atlantic, and the North Pacific, having the highest genetic similarity with contemporary New Zealand killer whales. However, much of the variation found in Old Tom's genome was not shared with these widespread populations, suggesting ancestral rather than ongoing gene flow. Our genetic comparisons also failed to find any clear descendants of Tom, raising the possibility of local extinction of this group. We integrated Traditional Custodian knowledge to recapture the events in Eden and recognize that Indigenous Australians initiated the relationship with the killer whales before European colonization and the advent of commercial whaling locally. This study rectifies discrepancies in local records and provides new insight into the origins of the killer whales in Eden and the history of Australasian killer whales.

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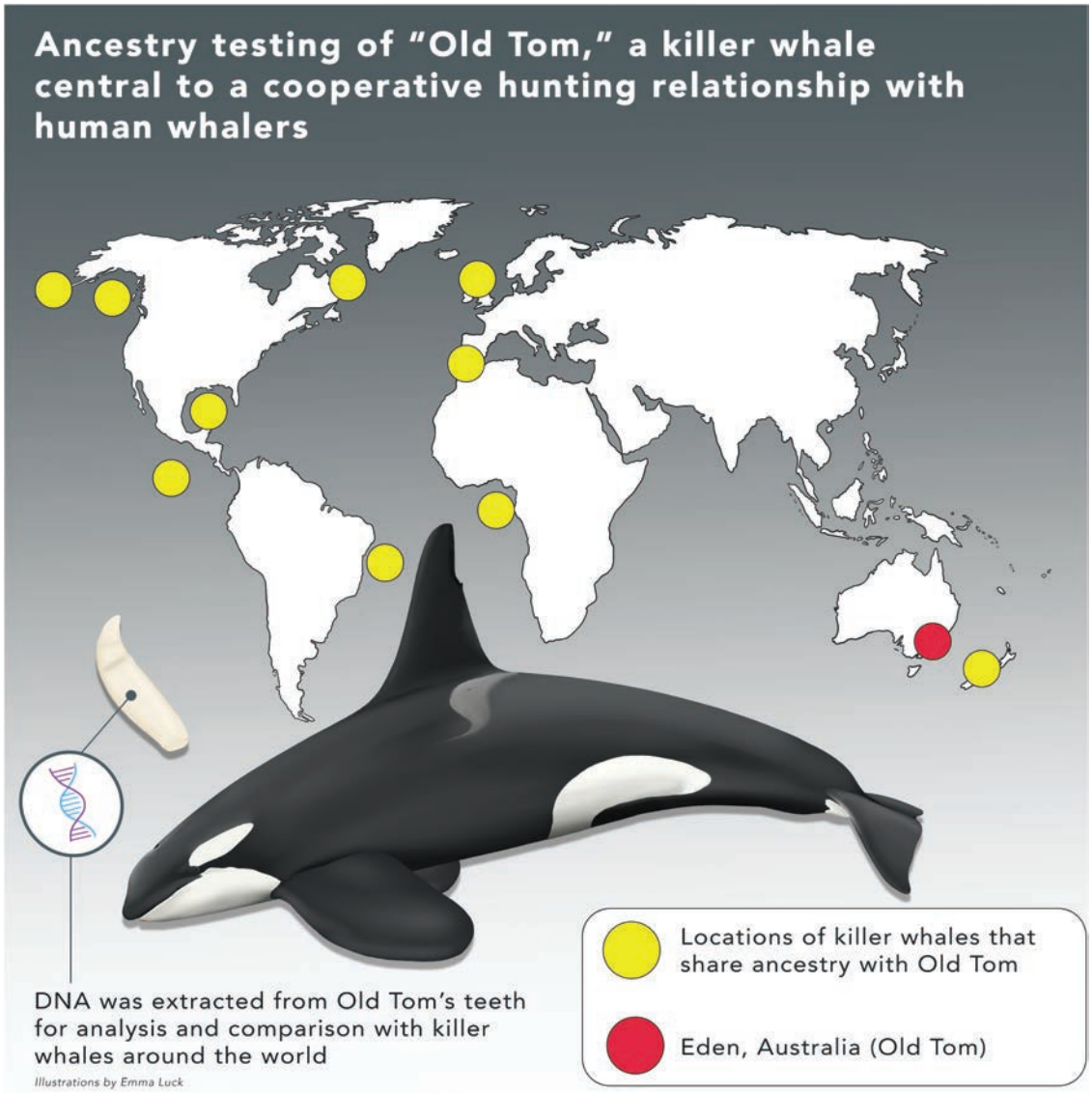
**Graphical Abstract**




**Key words:** cetaceans, human-wildlife cooperation, Indigenous knowledge, mutualism, phylogenomics, whaling

## Foreword

My name is Steven Holmes, a Thaua Traditional Custodian and this history is part of my people's legacy.

We consider beowas (killer whales) to be our brothers. Our Dreamtime stories which connect us to the beowas, is that when a Thaua member dies, they are reincarnated as a beowa. The beowas remained part of the Thaua, even after passing. The beowas would help the men by herding the other whales in the bay of Turembulerrer (Twofold Bay) for the whalers to kill. Budginbro, as his ancestors and the other Aboriginals would give the beowas the tongue of the dead whale. This was soon known as the Law of the Tongue.

My people had a long-lasting friendship with the beowa in Eden, especially Old Tom. My Nan, Catherine Holmes nee Brierly, told us about her great Grandfather, Budginbro who along with other Thaua would swim with Old Tom, holding on to his dorsal fin, my ancestors were never hurt or injured. She said that Budginbro's father, a blind man would walk along the beach (Aslings) singing to the beowas, the beowas would follow him along the beach communicating back and forth with him, it was a strong friendship between these beowas, and my people.

For the Thaua, this was a special time to be alive, a part of our history that was passed on from generation to generation. I hope one day I am able to reconnect with beowas.

## Introduction

Human-wildlife cooperation is a rare phenomenon recorded in only few known species globally, including killer whales (*Orcinus orca*) bottlenose dolphins (*Tursiops* spp.), gray wolves (*Canis lupus*), and greater honeyguides (*Indicator indicator*) (Cram et al. 2022; Spottiswoode et al. 2022; van der Wal et al. 2022). These relationships are at least partially developed by social learning and rely on tool use by humans followed by cues or signals (Cram et al. 2022; van der Wal et al. 2022). In Eden, New South Wales collaborative hunting practices with killer whales have been documented from

first- and second-hand accounts recorded in a wide range of historical sources. This includes personal journals, anthropological records, and numerous newspaper reports, which have been summarized in Brierly (1844–1851), Clode (2011), Davidson (2010), Mackenzie (1991), Mitchell (undated), and Mumbulla et al. (1995), yielding the following description of the cooperative hunting behavior.

On the eastern coast of Australia, until the early 20th century, the town of Eden was known for its mutualistic interactions between mankind and whale. Killer whales cooperatively hunted baleen whales with whalers in the region; a relationship first recorded in 1844 and that lasted for almost a century (see Fig. 1). Every June, records suggest that killer whales arrived in Turembulerrer (Twofold Bay) and would herd baleen whales into the bay or would alert whalers of the baleen whales by tail slapping and splashing in front of the whaling station. Whether it was day or night, the whalers would row out with their 9-m open boats following the killer whales to the prized baleen whale. The species of baleen whales hunted included, but were not limited to, humpback (*Megaptera novaeangliae*) and southern right whales (*Eubalaena australis*). Records suggest that part of the killer whale group would help herd the adult baleen whale, some would tear along the fins and flukes, and swim beneath the whale biting along its lip area. Others would breach on top, near the blowhole to drown the whale. The whalers would finally harpoon the exhausted whale, following it with the killing lance to pierce its vital organs. Local accounts suggest that Old Tom (henceforth referred to as "Tom") appeared to assist in the whaling efforts by directly intervening. This included pulling the tow rope along at the beginning of the chase, and seizing and pulling the harpoon line in his mouth after it was shot into the baleen whale, which was described to slow down the prey. This behavior is possible, based on the wear patterns on Tom's teeth, however, this tooth wear may have been the result of other feeding stressors (Marx et al. 2023).

After a baleen whale was killed, whalers would attach a marker buoy and anchor to prevent the drifting of the carcass for 24 to 48 h. The killer whales would then eat the lips and tongue of the baleen whale, and only after this would the whalers collect the carcass for processing. Based on the benefits for the killer whales and whalers from this interaction it appears that this was a mutualistic relationship, and one which resulted in a booming local whaling industry. It was viewed at the time that the killer whales would only eat the tongue out of respect for a relationship locally known as the "Law of the Tongue." However, killer whales, including those in Australian waters, have been recorded to favor the tongue in predations of baleen whales (e.g. Scammon 1874; Hoyt 2013; Totterdell et al. 2022). It is possible, that the tongue holds high nutritional value and is therefore favored for consumption by killer whales. The whalers may have disliked or thought the tongue held little value. Whatever the reason, there was no clear competition between human and killer whales in this interaction, and this combined with their complementary hunting skills, resulted in cooperative hunting.

To date, this relationship between mankind and whale has been more-or-less acknowledged and recorded as colonist driven. Indigenous Australians are descendants of the first people found in Australia (Rasmussen et al. 2011; Malaspinas et al. 2016), and one of the oldest living civilizations globally. They have a connection with their land which has been deeply rooted in their culture, including the Dreamtime. These Dreamtime stories and knowledge are passed through generations, intersecting with Australia's history, yet they remain largely overlooked in most ecological studies. We worked alongside Thaua Traditional Custodian, Steven Holmes, a direct bloodline descendant of Budginbro and his wife Char Ree Larra. Budginbro was the Indigenous guide and good friend of one of the Eden whaling station manager Oswald Brierly. We incorporated Steven's people's oral history in an attempt to help decolonize the recorded history.

In Twofold Bay, the coastal Thaua people, part of the Yuin nation, had a connection with the killer whales through the Dreaming, a relationship that was highly respected and relied upon due to the Thaua's dependency on the ocean for resources. Before colonization, the Thaua had an ongoing mutualistic interaction with the killer whales in the area, working in unison onshore and in the water until a carcass was ready to be shared, with the killer whales receiving the tongue. The Thaua even described a hunting practice that included singing that encouraged the killer whales to herd baleen whales close inshore for them to kill (Roberts 1904). This relationship is thought to predate European colonization by millennia and was the true start to the notion of the "Law of the Tongue." This oral history from the Thaua not only allows us to best recapture the events in Twofold Bay but also adds new insight into this long-standing relationship between humans and killer whales in Eden. Commercial whaling, which started in 1828, capitalized upon the Indigenous Australians' long-standing partnership with the killer whales, making it profitable rather than subsistent. Indigenous whalers continued to represent a considerable portion of the crews due to their sought-after skills (Brierly 1844–1851; Mumbulla et al. 1995).

It was suggested there were 25 to 30 killer whales, comprised of three groups (also called "pods"), known to visit Eden around the end of the 19th century. Recognizable individuals included Hooky, Cooper, and Tom, who when hunting joined to form a single pod. The killer whales in Eden began to disappear toward 1920, and there have been several hypotheses as to why. This includes the depletion of baleen whale stocks; a breach of the Law of the Tongue in which Tom fought for a whale with the whalers, resulting in him losing teeth; the killing of a killer whale that stranded while hunting a minke whale (*Balaenoptera acutorostrata*); or the movement of the Thaua people out of Twofold Bay. By 1928, no more baleen whales were processed in Eden and most of the killer whales had left. Tom was the last remaining whale observed by locals in Eden and was found beach cast in 1930. To this day, there have been only a few confirmed sightings of killer whales in Eden since the end of Eden's whaling era (Donnelly, unpublished). *We would like to note that we have been vigilant in highlighting factual evidence here, however, we acknowledge contention around Tom's pod structure and age* (see Supplementary Material).

Advances in genomic methods provide the potential to sequence DNA from historical skeletal remains (Lan and Lindqvist 2019). Here, we sequence DNA from Tom and compare the variation in his genome to the mitochondrial and nuclear genomes of a global dataset of killer whales. We assess evidence for the killer whales of Eden's origin, and whether their disappearance from Eden reflected the local extinction of this group.

## Methods

### DNA extraction and sequencing

The Eden Killer Whale Museum is a space dedicated to local history with a specific focus on Australia's whaling era and

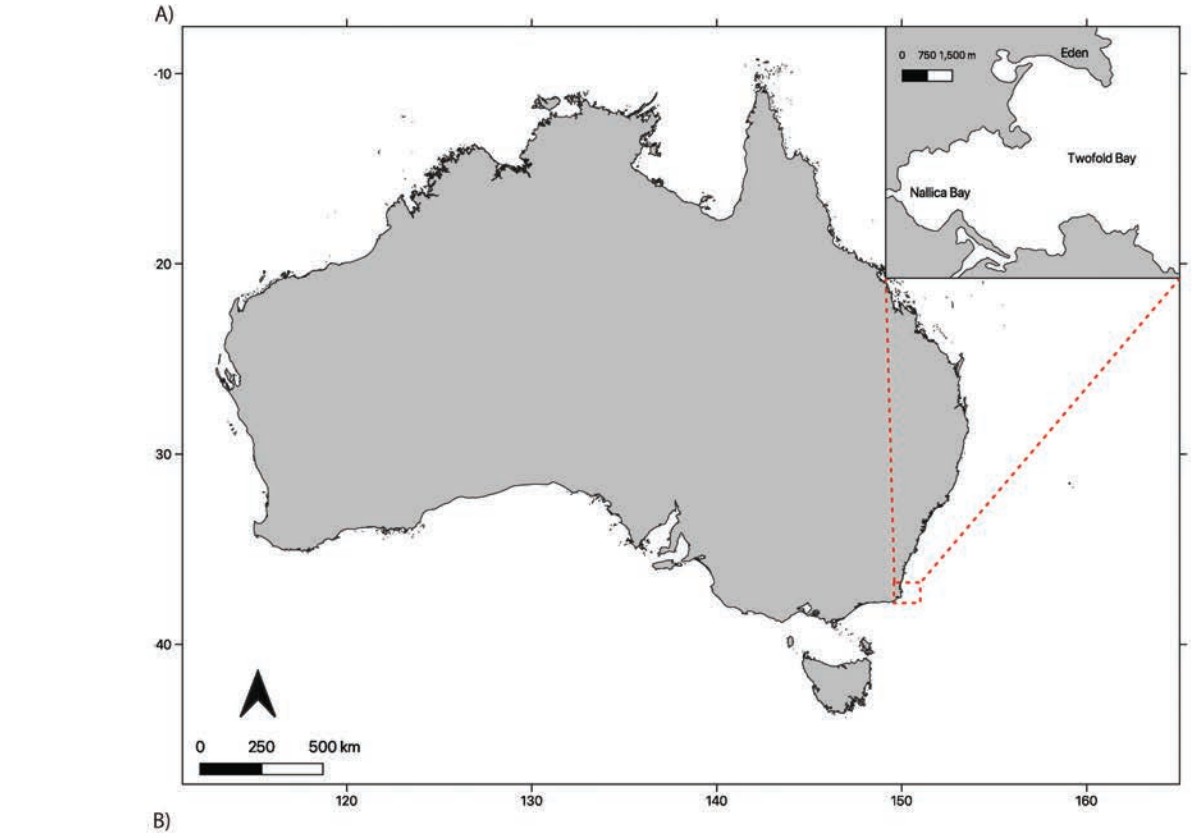



**Fig. 1.** A) Map of Eden relative to Twofold Bay (see square) in the context of Australia, detailing the location that the whalers and killer whales (*Orcinus orca*) used to cooperatively hunt baleen whales together. B) Whalers and killer whales of Eden on the chase of a humpback whale (*Megaptera novaeangliae*) captured between 1910 and 1920. Image by Charles E. Welling provided by Eden Killer Whale Museum.

the killer whales of Eden story (see Fig. 1). Tom's skeleton is now mounted on display at the museum; however, he was stored in a shed from the year of his death in 1930 until 1939, which potentially increased DNA contamination and degradation. We used a hand-held Dremel Stylo+ to drill ~0.5 g of powder both from a tooth on the upper jaw and from inside the lower mandible. We sprayed 10% bleach on the area to be sampled and left it for 30 s to remove any contaminating DNA, and then cleaned the surface with ethanol before drilling. As a positive control, we included powdered tooth drilled from a museum sample from the Natural History Museum of Denmark (museum ID: 1×), which was originally found in the Kattegat, north of Denmark in 1862 (Eschricht 1866). The Kattegat specimen was previously sampled for DNA analysis (Foote et al. 2009; Morin et al. 2015) and approximately 0.5 g of powdered tooth collected for that study was used for DNA extraction in this study. The Kattegat specimen is a similar age, but from a different climate to Tom, thus providing a comparison into the relative preservation of DNA in each climate. Samples were stored at ≤–20 °C until used for laboratory analyses.

All pre-PCR DNA lab work was conducted in a dedicated ancient DNA clean lab with positive pressure and nightly UV irradiation to maintain a sterile environment. Lab work was conducted in full-body suits and workflow was strictly controlled to prevent the introduction of any exogenous DNA into the sample. Lastly, a negative control extraction, library build, and PCR on molecular-grade water were used to screen for laboratory contamination during the protocols outlined below.

DNA extraction and purification from powdered bone and tooth broadly followed the protocol detailed by Rohland et al. (2018). DNA was extracted from approximately 0.5 g of powdered tooth and 0.5 g of powdered jawbone from Tom. This was subsequently digested in 1 mL of lysis buffer from a 25 mL stock solution composed of 22.5 mL of 0.5 M ethylenediaminetetraacetate (EDTA) at pH 8.0, for decalcification of the bone and tooth matrix, 625 µL of 10 mg/mL proteinase K, for the digestion of bone and tooth collagen, 1.863 mL of water, and 12.5 µL of Tween 20. Each tube was then sealed with parafilm and gently mixed by continuous rotation at 18 rpm while incubating at 37 °C. Optimization experiments on DNA extraction from ancient human bone samples from temperate and tropical regions have found that EDTA-based predigestion of powdered bone increases the proportion of endogenous to exogenous DNA several-fold (Damgaard et al. 2015). Therefore, after an initial digest overnight (approximately 16 h), the 15 mL centrifuge tubes containing the lysate were then centrifuged at 4,000 rpm for 5 min to separate the lysate from the pelleted bone or tooth powder. The supernatant containing DNA was removed by pipette, carefully avoiding disturbance and resuspension of the pelleted bone or tooth powder. New lysis buffer as above was then added to the pelleted bone or tooth powder, and a second digestion step of 48 h was used to maximize the extraction of endogenous DNA (see Damgaard et al. 2015).

Short DNA fragments are common in ancient and historical samples and thus, to optimize the binding to the silica membrane of the spin column, we first modified stock 500 mL Qiagen PB binding buffer by adding 2.5 mL 5 M NaCl and 15 mL of 3 M NaOAc (Rohland et al. 2018). The modified binding buffer was then added to 100 µL aliquots of lysate at a ratio of 5× the volume of lysate and mixed by pipetting and gentle vortexing. A total volume of 600 µL lysate plus binding buffer mix from each sample was added to individual Qiagen minelute spin columns and spun for 1 min at 10,000 rpm. This was repeated until all aliquots of lysate and 5× binding buffer had been passed through the column. The silica membrane in the spin column was then subject to two wash steps each using 500 µL of Qiagen PE buffer and centrifuged for 1 min at 10,000 rpm, with an additional drying centrifuge step of 1 min at 13,300 rpm. DNA was then eluted by adding 60 µL per spin column of Qiagen EB buffer, which was then incubated at 37 °C for 15 min before being spun down in a centrifuge for 1 min at 8,000 rpm.

The fragmented DNA extracts from the first and second extraction digests for the jaw and tooth samples and the negative control were converted to Illumina sequencing libraries using a New England Biolabs NEBNext Ultra II library kit. This library build protocol includes a USER treatment step to reduce sequencing errors from post-mortem damage (Orlando et al. 2021). USER treatment reduces post-mortem DNA damage as it shortens the length of DNA molecules by removing uracil sites and cleaves the abasic sites, allowing the damage to be cut out effectively. This treatment reduces sequencing errors and characteristic damage patterns. However, some post-damage pattern remain when studying CpG dinucleotides, and this is used to verify the authenticity of DNA with ancient or historical origin (Orlando et al. 2021).

## Reference DNA datasets

Libraries were subsequently dual indexed using NEBNext dual-indexing primer pairs and amplified for either 10 cycles (second extraction performed after predigest and longer digestion) or 15 cycles (first extraction performed after initial digest and negative control), then purified using SPRI beads (NEBNext). The DNA concentration and fragment length distribution of the libraries were measured using an Agilent TapeStation. These libraries were then sequenced across a partial lane of an Illumina Novoseq 6000 S4 (150 bp PE) sequencing platform using the commercial service provided by Novogene (Oxford). Patterns of post-mortem DNA damage in the genome data generated from Tom and the Kattegat museum specimen were estimated and visualized using PMDtools (Skoglund et al. 2014).

Whole genome sequences from a global dataset of 26 killer whales (Foote et al. 2019, 2021) and mitogenomes from a global dataset of 452 killer whales (Morin et al. 2015) were accessed from GenBank. Additionally, we shotgun-sequenced DNA from 37 killer whales from Australia and New Zealand to generate whole genome sequences (see Supplementary Table S1 for sample details). For the New Zealand samples, Indigenous consultation with mana whenua occurred prior to sample collection, tissue transfer, and data analysis. Further kōrero (discussions) regarding data repository and feedback of results occurred within each rohe from which Aotearoa New Zealand samples were used. To preserve data sovereignty, data repository for samples originating from Aotearoa New Zealand remains with the Aotearoa Genomic Data Repository.

To extract DNA from these tissue samples we used a salting-out protocol (Sunnucks and Hales 1996). DNA quality, integrity, and quantity were assessed using a NanoDrop 1000 spectrophotometer (Thermo Scientific), gel electrophoresis (2% agarose gels), and a fluorometer (Qubit 2.0, Life

Technologies), respectively. DNA extracts were built into genomic libraries by Novogene (Singapore) using a NEBNext Ultra II DNA library prep kit and sequenced on an Illumina NovaSeq 6000 S2 platform (150 bp PE).

### Bioinformatics of the genomic datasets

All bioinformatics and relevant analysis were undertaken using Flinders University's server Deepthought (Flinders University 2021). Reads were trimmed with AdapterRemoval2 v2.3.2 (Schubert et al. 2016) to remove adapter sequences and low-quality reads ($Q < 30$). Filtered reads >30 bp were then mapped using BWA-MEM v2.2.1 (Li 2013) to two high-quality, chromosomal reference assemblies requiring a mapping quality score >30. The two reference genomes were from genetically diverged populations (Foote et al. 2019): the first was from a Norwegian killer whale (mOrcOrc1.1; accession: GCA_937001465.1; Foote et al. 2022) the second from a North Pacific "Biggs" or "transient" (mammal-eating ecotype) killer whale (CNA0050865; CNGBdb accession: CNP0002439; Kardos et al. 2023). This was to understand the impact of reference bias on downstream population assignment analyses (see Günther and Nettelblad 2019). Clonal reads were collapsed with the markup function of the SAMtools v1.13 (Li et al. 2009). Repeat regions identified by RepeatMasker v4.1 (Smit et al. 2004) and the Cetartiodactyl repeat library (Kohany et al. 2006) were masked using BEDtools 3v2.26 (Quinlan 2014). Separate files were generated for autosomes and X-chromosomes using SAMtools view for all genomes. The FASTA file for the mOrcOrc1.1 reference assembly was concatenated with the mitochondrial genome sequence, allowing Australasian killer whale mitogenomes to be extracted using SAMtools view as above. This competitive mapping strategy is also expected to remove nuclear mitochondrial DNA from the mitogenome sequences. Both museum samples were examined for possible contamination (e.g. human sequences), resulting in the removal of a single read from the mitogenome assembly of Tom, which was replaced as Ns. This sequencing read contained multiple mismatches to all other killer whale sequences, and a BLAST+ (Camacho et al. 2009) search identified this read as likely human contamination. There were a further three ambiguous bases which were changed to "N" in the mitogenome sequence of Tom, as there was insufficient data to call the bases accurately.

### Data analyses

The newly generated mitogenome sequences were aligned with the global mitogenome dataset from Morin et al. (2015), resulting in a total sample size of 490 individuals. As per previous studies (Morin et al. 2010, 2015), we conservatively fixed polynucleotide repeats at positions 1,130 to 1,144 to a set of nine Cs, and at positions 5,210 to 5,217 to a set of seven As to reduce the likelihood of introducing erroneous variation into the phylogenetic analysis. We included an outgroup mitogenome sequence from a long-finned pilot whale (*Globicephala macrorhynchus*) (Accession number: NC_019578), to root the killer whale phylogeny in downstream mitogenome analyses.

We used PhyML (Guindon et al. 2010) to generate a maximum likelihood phylogenetic tree of the mitogenome sequences. The tree was generated assuming an HKY nucleotide substitution model (as used in Morin et al. 2010), estimating branch support using an SH-like approximate likelihood ratio test, optimizing nucleotide equilibrium frequencies and the proportion of invariable sites, considering eight rate categories, using the best of NNI (Nearest Neighbor Interchange) and SPR (Subtree Pruning and Regrafting) tree searches, with a BioNJ starting tree and five random starts. The tree was rooted with the pilot whale outgroup.

After establishing the genetic relationship of Tom to our global reference mitogenome dataset, we next compared Tom to the global reference dataset of nuclear genomes. We used a random sampling approach implemented in ANGSDv0.935 (Korneliussen et al. 2014) to generate pseudo-haploid genotypes, which reduces bias from coverage differences between the museum and modern samples. Transitions were excluded to avoid bias due to DNA deamination of cytosine in the museum samples, which leads to the misincorporation of C→T changes at read ends (Lindahl 1996; Hofreiter et al. 2001). We applied the following filters: minimum mapping quality of 20, minimum base quality of 30, minimum allele frequency that accounted for including only alleles found in at least two individuals, and probability of being a variable site of $P < 0.000002$. A minimum of 10 individuals containing a site was considered for a genotype to be called, however, for the Australasian and Tom nuclear genome analysis several variations of this filter were completed to examine potential issues from reference bias. These filtering steps resulted in pseudo-haploid genotype calls for each output. Pairwise distance between all individuals was averaged across all included sites, scoring 1 when a different base was called, and 0 if the same base was called in both individuals. Covariance was estimated as specified in Korneliussen et al. (2014). Additionally, we downsampled the mapped Kattegat sequence data using SAMtools to a comparable coverage of Tom to assess possible coverage bias affecting results.

Based on the local accounts of the killer whales in Eden, Tom was presumed to be male (Mackenzie 1991; Clode 2011). This is supported by photographs of Tom which show sexually dimorphic characteristics, such as dorsal fin height and shape (Davidson 2010). The Kattegat killer whale was also identified as a male in a necropsy (Eschricht 1866), therefore acting as a positive control. Killer whales have an XY sex-determination system. Males are the heterogametic sex, and therefore, are haploid for the X-chromosome, but diploid for autosomes, while females are diploid for X-chromosome and autosomes. The sex of the museum samples was confirmed by comparing the read counts of the X-chromosome to the mean read count of autosomes (as per Kirch et al. 2021) among the two museum samples. The Kattegat genome was downsampled five times with different seeds, and the mean read count for the autosomes and X-chromosome was used to infer the sex of the Kattegat genome.

## Results

### DNA preservation

As expected, the two museum samples differed in DNA preservation. Tom's skeleton has been in the Eden Killer Whale Museum for almost a century in southern New South Wales Australia, while the Kattegat sample is approximately 70 years older than Tom and has been kept

in the collection facility of the Natural History Museum of Denmark. The quality of DNA preservation between these museum samples differs in endogenous DNA content and therefore sequence coverage per sequencing effort. It is most likely that Australia's hotter climate and UV exposure have resulted in the degradation of Tom's DNA (e.g. Rasmussen et al. 2010; Morris et al. 2014), while the cooler climate and darkened storage facility have likely reduced the rate of enzymatic and UV degradation of DNA preserved in the Kattegat specimen.

Despite this DNA degradation, we successfully sequenced 11,464 bp of the expected 16,386 to 16,392 bp (Morin et al. 2010) mitochondrial genome of Tom. In addition, we generated 37 new mitogenome sequences (with 10 distinct haplotypes) from contemporary Australasian killer whales (Figs. 2 and 3). Tom's mitogenome sequence was a novel haplotype in the dataset. The mitogenome sequence of the Kattegat sample was identical to that previously generated for this individual (GenBank accession: KF418373; Foote et al. 2013). The effective depth of coverage (i.e. post-filtering and removal of duplicate reads) of the mitogenome assembled from Tom's DNA was 1.5×, compared with 115× for the mitogenome sequence of the Kattegat sample. The effective coverage of the contemporary Australasian mitochondrial genome dataset ranged between 193 and 3,691× (see Supplementary Table S1). This resulted in a dataset comprising 166 unique haplotypes from 490 killer whales. There are 169 variable sites, while the mean transition/transversion ratio of all sequence pairs was 7.13 within this global killer whale mitogenome dataset.

We were also able to generate low-coverage nuclear genome sequences for the two museum specimens. Effective depth of coverage (i.e. post-filtering) was <1× for both samples (see Table S1 and S2 for genome summary statistics in Supplementary Material). Post-filtering, 1,351,099 reads were generated from Tom's DNA mapped to the CNA0050865 assembly, compared with 13,729,355 reads generated from the Kattegat sample. The number of mapped reads increased to 2,062,946 (Tom) and 13,680,197 (Kattegat) when mapping to the mOrcOrc1.1 assembly. By incorporating USER treatment to the library builds, postmodern damage was reduced by removing the uracil and cleaving the abasic sites. Figure S1 (see Supplementary Material) shows the small excess of C→T changes and that the treatment removed most of the damage pattern caused by deamination. However, we observe an increase of C→T changes at the CpG sites in the termini at the 5′ end of reads, authenticating the historical origin of the DNA.

High-latitude killer whale populations generally form monophyletic clades and reflect clustering by geography such as clade 1 (Fig. 2A, Morin et al. 2015). In contrast, haplotypes sampled at lower latitudes do not necessarily cluster based on geographic sampling locality (Fig. 2, Supplementary tree; Morin et al. 2015). The haplotypes sequenced for Tom and the Kattegat sample were both found in clade 5. This clade also included haplotypes sampled from killer whales in different ocean basins such as the Atlantic and Indo-Pacific regions (Fig. 2). The mitochondrial haplotype reconstructed from Tom's DNA shares a most recent common ancestor with several Australian and New Zealand killer whales. However, the resolution of these results is reduced due to the missing data and quality of the mitogenome generated from Tom's DNA.

Using a simple measure of genetic distance, based on 1,093,893 transversions, we find relatively concordant results for the global nuclear genome dataset to those obtained from the mitochondrial genome dataset: Tom's genome has the lowest genetic distance to the New Zealand genome (Fig. 3B). However, the long branch from the shared ancestral node with the New Zealand genome to Tom indicates private genetic variation in Tom's genome and suggests Tom is not from the same contemporary population as the New Zealand sample. Tom's genome additionally clusters with genomes sampled in waters around Scotland, Gabon, Gibraltar, and the North Pacific (Clipperton Island, and the resident and offshore ecotypes). The genetic distance from Tom to the other Australian genomes, sampled from southwestern Australia and northwestern Australia, is as great as between Tom and all other included killer whale genomes (except Antarctic types B1, B2, and C). Based on mapping to the two available reference assemblies and comparing their genetic distance estimates, we see a marginally higher affinity to the reference genome's origin in both distance estimates. Overall, the relative estimates for the genetic distance between the global genomes appear to be similar despite the reference bias (see Supplementary Fig. S3).

Genetic distance and covariance based on 1,087,094 transversions from the Australasian nuclear dataset, indicates Tom's genome has the highest genetic similarity to the New Zealand genomes, and a single Tasmanian genome (Fig. 4), while predominantly comprised of private genetic variation relative to the samples included in this study. This suggests Tom is not from the contemporary Australasian populations represented by these samples. Tom's position near the middle of the axes of the PCAs (Principal Component Analysis) (Fig. 4) is likely a consequence of the high level of missing data. Samples with high missing data used in a PCA can have their variance underestimated (Li et al. 2020) which when compared with higher-quality samples, results in such samples gravitating toward the center of the PCA. The high degree of missing data and the small amount of ancestry informative SNPs (Single Nucleotide Polymorphism) sequenced for Tom likely reduce our resolution to assert the genetic relationship of Tom to other Australasian killer whales. The remaining Australasian killer whales clustered into three genetic groups, consistent with published analyses of a ddRADseq dataset by Reeves et al. (2022). Both reference assemblies yielded broadly similar results, but reference bias is apparent when the data are filtered to only include sites covered in Tom's genome (see Figs. 4S and 5S in the Supplementary Material). Under this filtering regime, far fewer sites are retained when the data was mapped to the CNA0050865 reference assembly, and the clustering pattern collapsed. In contrast, when the data were mapped to the mOrcOrc1.1 assembly, the clustering pattern was relatively consistent between the filtering changes.

## Sexing

Comparing the depth of sequencing coverage of the autosomes and X-chromosome can be used to sex individuals. Here, we use this sexing approach on our two museum specimens, both of which are well-documented males, to assess reference bias. Our genetic sexing approach confirmed the Kattegat sample

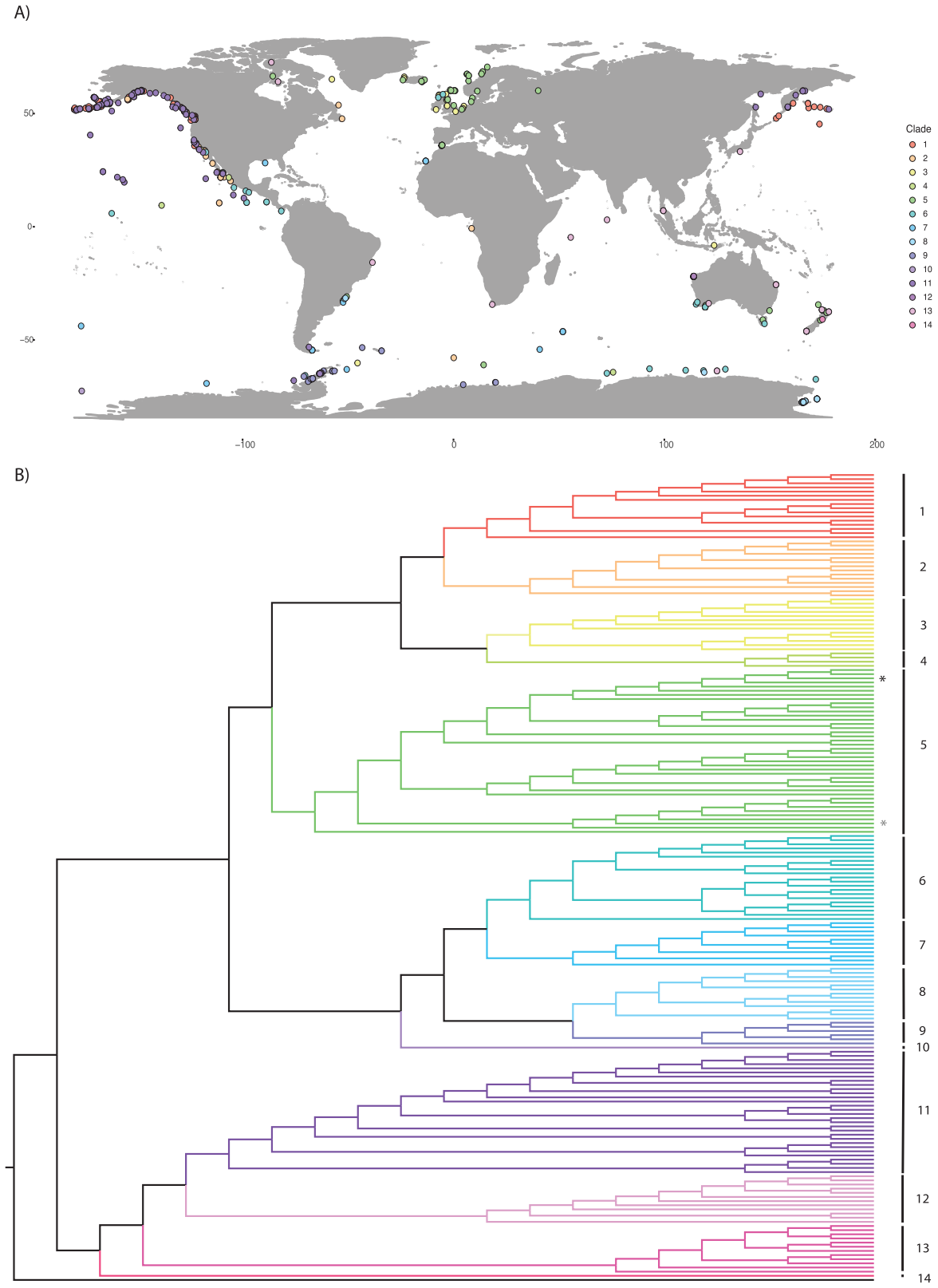



**Fig. 2.** Killer whale (*Orcinus orca*) mitogenome analysis based on 490 mitogenome sequences (166 unique haplotypes). A) World Map of 490 samples used in the study. B) Maximum likelihood phylogenetic tree of 166 haplotypes, based on an HKY nucleotide substitution model, colored by clade. The two museum samples sequenced for this study are labeled with asterisks. The tip representing the mitogenome sequence of Tom is marked with a light asterisk; the tip representing the Kattegat sample by the dark asterisk. A tree with all tips labeled can be found in Supplementary Material (Fig. S8).

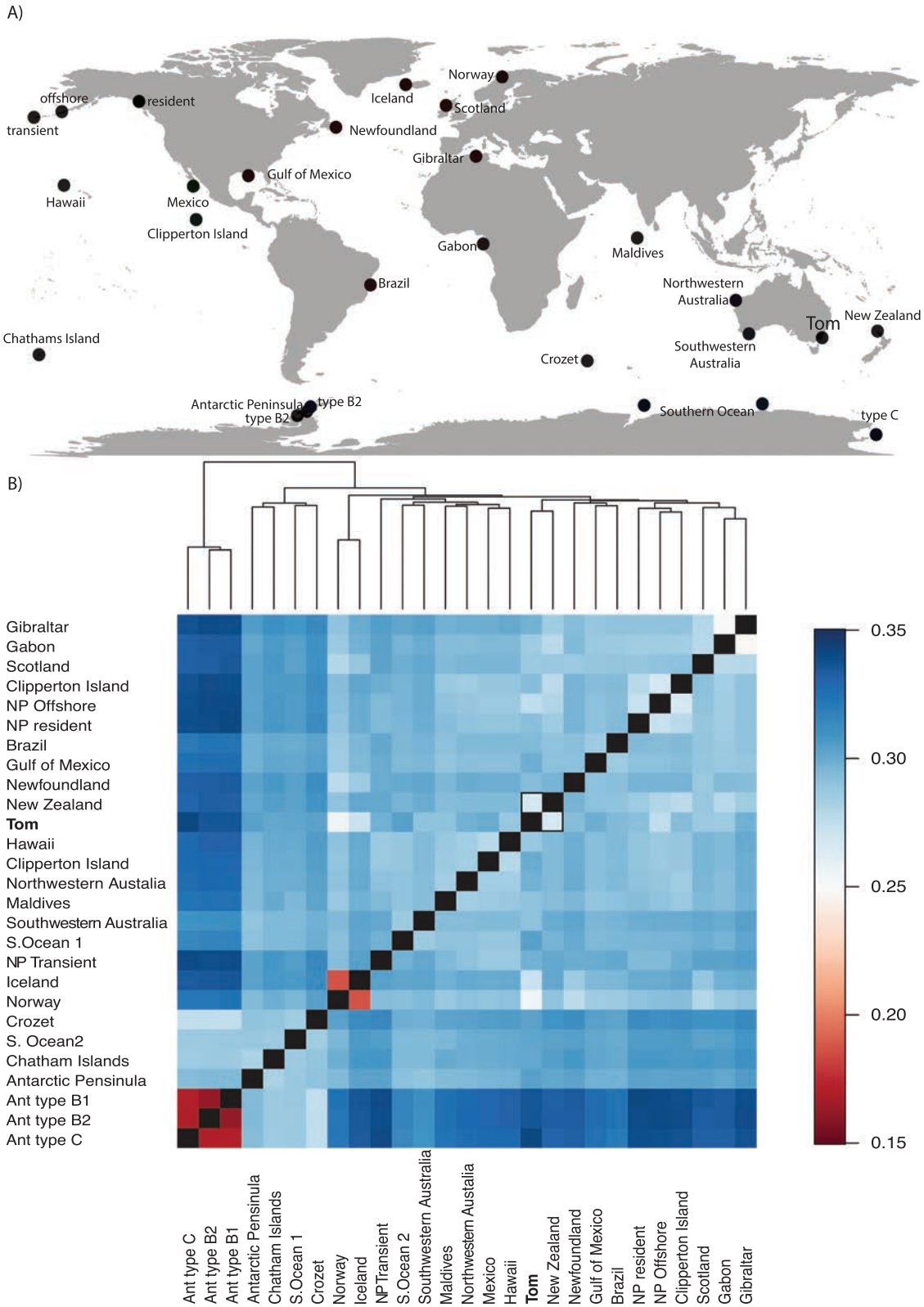



**Fig. 3.** Global dataset of Killer whale (*Orcinus orca*) nuclear genomes based on 1,093,893 transversions, A) displays a map of the sampling localities of the global dataset including Tom, with B) a genetic distance matrix in which increasing genetic distance is indicated by shading from red to blue. Self-comparisons are blacked out. The data displayed here was based on mapping to the mOrcOrc1.1 assembly. Black borders represent Tom and the New Zealand genome's relationship. Ant, Antarctica; NP, North Pacific.

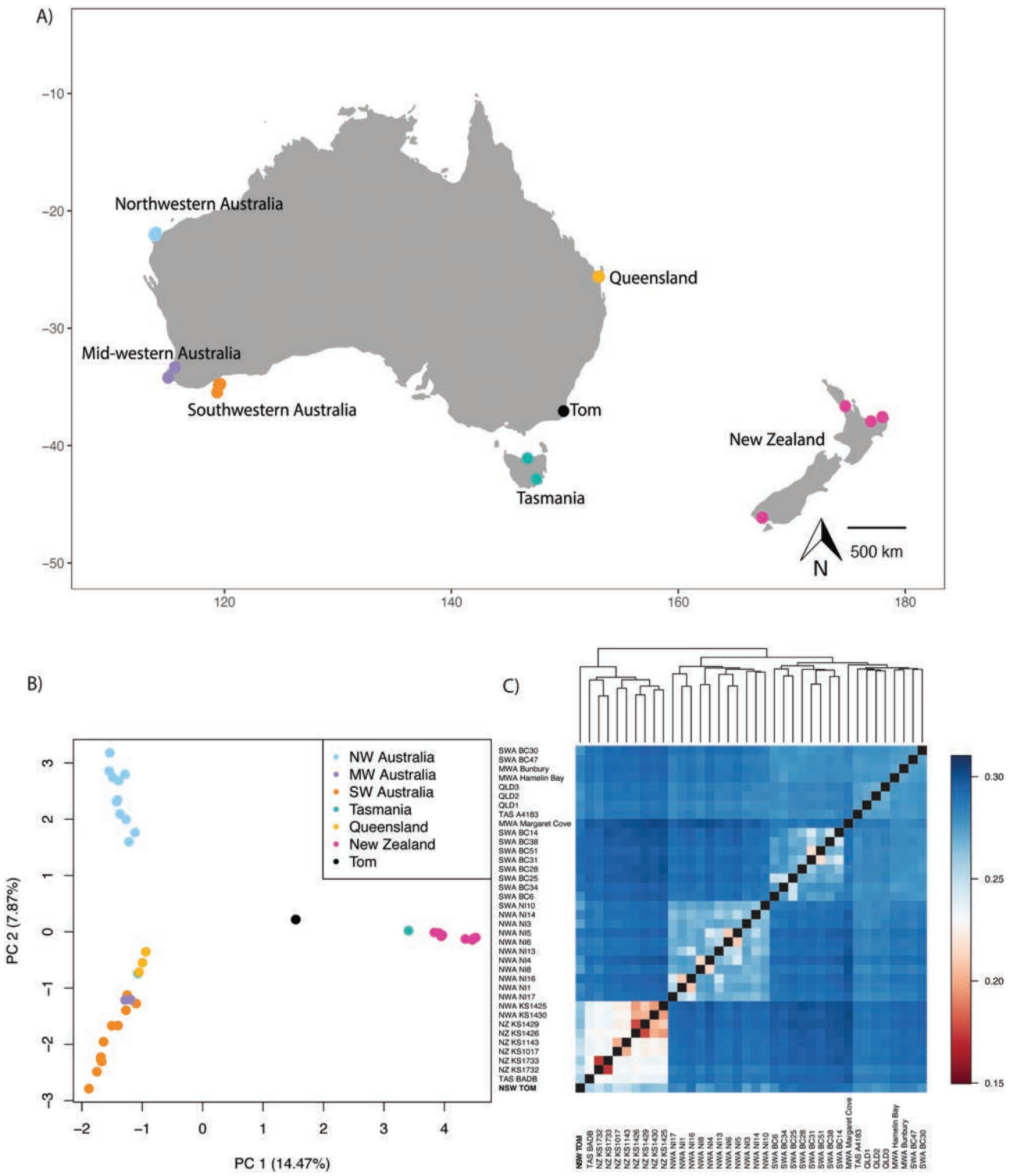



**Fig. 4.** Australasian whole genome dataset of killer whales (*Orcinus orca*) with A) sample localities displayed of 38 animals, including Tom. A total of 1,087,094 transversions were used to B) assess the genetic clustering of Tom on a regional scale using a PCA based on covariance estimates, and C) estimate pairwise genetic distance between genomes. Increasing genetic distance is indicated by shading from red to blue, with self-comparisons blacked out. The data displayed here were based on mapping to the mOrcOrc1.1 assembly. In front of each sample is the locality of origin, MWA, midwestern Australia; NWA, northwestern Australia; NZ, New Zealand; QLD, Queensland; SWA, southwestern Australia; TAS, Tasmania.

was a male, independent of the mapping reference used. Counts of sequence reads mapped to the X-chromosome were approximately half of those mapping to each an equivalent length of the autosomes (see Figs. S6 and S7 in Supplementary Material). In contrast, the low-coverage genome of Tom was impacted by reference bias. Read counts mapped to the X-chromosome and autosome reflected a 1:2 ratio (Figs. S6 and S7 in Supplementary Material) when mapped to the mOrcOrc1.1 assembly, but not when mapped to the CNA0050865 assembly. The mean read count per autosome was 64,338 for data mapped to the closely

related mOrcOrc1.1 assembly, but 18,614 for data mapped to the more distantly related CNA0050865 reference assembly. We, therefore, interpreted this difference in sexing results because of mapping bias, and cautiously interpret results when mapping data to the mOrcOrc1.1 assembly as confirmation that Tom was a male. Given this evidence for mapping bias, we expect that the geographic comparison of Tom's genome and the global dataset will be most accurate for data mapped to the mOrcOrc1.1 assembly.

## Discussion

The killer whales of Eden represent a rare partnership between mankind and whale. Tracing the genetic ancestry of Tom, arguably the best-known killer whale of Eden, provides the first insight into the demographic and evolutionary history of this group's deep past. After accounting for reference bias, there was relative concordance of both nuclear and mitochondrial genomes regarding Tom's ancestry. The ancestral matrilineal lineage of Tom, inferred from the mitochondrial sequence, was also the common matrilineal ancestor to contemporary killer whales sampled as far apart as North Atlantic, North Pacific, and New Zealand. A comparison of nuclear genome sequences also found the lowest genetic distance between Tom and the representative New Zealand killer whale genome.

Tom shared the most genetic ancestry with populations that have been previously grouped together by PCA (Foote et al. 2019) and due to sharing similar demographic histories (see Fig. 2A of Foote et al. 2021). These included genomes sampled in the North Pacific (Clipperton Island, resident, and offshore ecotypes), the North Atlantic (Gibraltar, Scotland, Gabon), and New Zealand. Coalescent-based estimates of changes in effective population size ($N_e$) through time (Li and Durbin 2011) have previously found that all these genomes had relatively stable $N_e$ of ~5,000 before 100 KYA BP, which was then followed by a small increase in $N_e$ up to ~6,000 prior at approximately 40 KYA BP, and a subsequent steep decline to a $N_e$ of ~1,000 between 40 and 10 KYA BP (Foote et al. 2021). Patterns of cross-coalescence suggest this decline in $N_e$ reflects the timing of genetic separation from a shared ancestral population (Foote et al. 2019). Note that the actual timing is dependent upon assumed mutation rates and generation time. Nevertheless, the pattern of past cross-coalescence between these now widely geographically dispersed populations provides the basis for understanding why Tom shares ancestry with such a geographically disparate subset of genomes.

The number of genealogical ancestors doubles each generation back. Tom could therefore theoretically have had over 1,000 genealogical ancestors in the generation 250 years (10 generations) before he was born. However, genealogies collapse and become tangled back in time, as ancestors from one generation may share common ancestors from prior generations (Derrida et al. 2000; Coop 2023). The further back in time, the greater the stochasticity of the genetic contribution to Tom from those genealogical ancestors due to the randomness of meiotic recombination (Coop 2020, 2023). Given this stochastic inheritance and that Tom likely descended from a common ancestor with the New Zealand and other "related" killer whale populations hundreds to over a thousand generations ago, just a subset of ancestry remains shared by Tom and the closest-related genomes included here (Supplementary Fig. S9). Under a simple model that assumes random mating, a common genealogical ancestor is expected to be shared by all individuals $\log 2n$ generations ago, where $n$ is the population size (Chang 1999). The idealized model assumption of random mating is not realistic in killer whale populations. However, even under models incorporating geographic separation of substructured populations, and preferential mating within populations, the time to a most recent common genealogical ancestor shared within a single species can be in an evolutionary short amount of time (i.e. thousands of years). For instance, it is been estimated that all humans share a common ancestor within the last 10,000 (Rohde et al. 2004), which may be possible for killer whales too. The extent of shared genetic ancestry between individuals from different populations will reflect the number of shared genealogies and be dependent upon the rate of gene flow (cross-coalescence) through time. Genetic ancestry that has survived since the time of the most recent common ancestor of Tom and populations we identified having the lowest genetic distance with Tom, suggests a pattern of coalescence that we represent in a schematic figure (Supplementary Fig. S9).

In line with expectations of the high level of structuring among insular populations typical in killer whales, ancestry shared by Tom and a subset of other killer whales in our dataset represents a small proportion of variation in Tom's genome. As in human populations (Gravel et al. 2011; The 1000 Genomes Project Consortium 2015), such genetic variation in killer whales appears to be highly localized. Killer whale populations appear to form from the expansion of kin-based social groups, i.e., families (Olesiuk et al. 1990; Baird and Whitehead 2000; Hoelzel et al. 2007; Esteban et al. 2016). Genetic variation in a founder family group can potentially rise to high frequency within the population but remain at low frequency globally, i.e., these are so-called rare genetic variants. If Tom was part of a small insular population, as is common among killer whales, then his recent genetic ancestors would come from a small pool of genealogical ancestors (see schematic Fig. S9 in Supplementary Material).

In summary, this study provides novel insight into the history of the killer whales of Eden and their relationship with Indigenous Australians. The use of ancient DNA techniques and the incorporation of Traditional Custodian knowledge allowed for a deeper understanding of the genetic origins of these whales and their historical interactions with mankind. The lack of contemporary Australasian genomes closely related to Tom suggests that the killer whales of Eden may have undergone a local extinction. The disappearance of the group may have resulted from losing either the suitable environment, the motivation from the animal or human partner, and/or their compatible interspecies knowledge of how to cooperate (van der Wal et al. 2022). However, the lack of genetic affinity of Tom's genome to the sampled modern killer whale genomes does not exclude the possibility of descendants in unsampled populations. The behavioral ecology of the killers of Eden was a natural phenomenon between humans and whales, and we hope these new insights provide some spotlight into this part of Australia's history.

The local Traditional Custodian knowledge provided valuable insight into the ancient origins of this relationship,

one that started with the Thaua people of New South Wales thousands of years ago. We encourage scientists to collaborate with first nation communities and acknowledge their role in shaping local ecosystems. Recognition of Indigenous knowledge serves not only as part of Indigenous peoples' cultural identity, but also, directly intersects with the progression of conservation and coexistence with biodiversity, both now and in the future (Marsh et al. 2022). While much of existing research highlights nature's contributions to humans, people's work with nature (referred to as "reciprocal contributions") are rarely considered (Ojeda et al. 2022). To develop meaningful research, scientists must genuinely connect with the landscapes, cultures, and individuals involved (Ban et al. 2018).

## Supplementary material

Supplementary material is available at *Journal of Heredity* online.

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Conflict of interest statement. None declared.

## Data availability

All raw sequencing data are archived at the National Centre for Biotechnology Information (www.ncbi.nlm.nih.gov), under the Orcanomics BioProject (NCBI accession: PRJNA531206). The New Zealand samples have been uploaded to the Aotearoa Genomic Data Repository (accession: https://doi.org/10.57748/4P5Q-R756).

## Author contributions

IMR conceived the study. IMR, CW, and AG sampled material from Tom. JAT, RW, DD, KAS, and ELB provided samples from contemporary Australasian populations. IMR and ADF conducted ancient DNA lab work. IMR and ADF conducted genomic analyses. SH provided historical insights. IMR wrote the manuscript with input from all coauthors. LM provided a substantial contribution to the concept of the study, and critical revision of the manuscript for important intellectual content.

## Positionality statement

The authors here originated mostly from the Global North, those who undertook academic careers largely studied in formerly colonized nations in first-class countries. We believe collaboration with Traditional Custodians is needed to decolonize research and avoid western-biased perspectives. Here, we collaborated with Thaua Traditional Custodian, Steven Holmes, a direct descendant of Thaua royalty. We ensured that cultural sensitivities were respected in the sharing process with the information provided from Steven. This collaboration, underscores a commitment to inclusivity, honoring Indigenous knowledge, and shifting from research solely viewed through a western lens.

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