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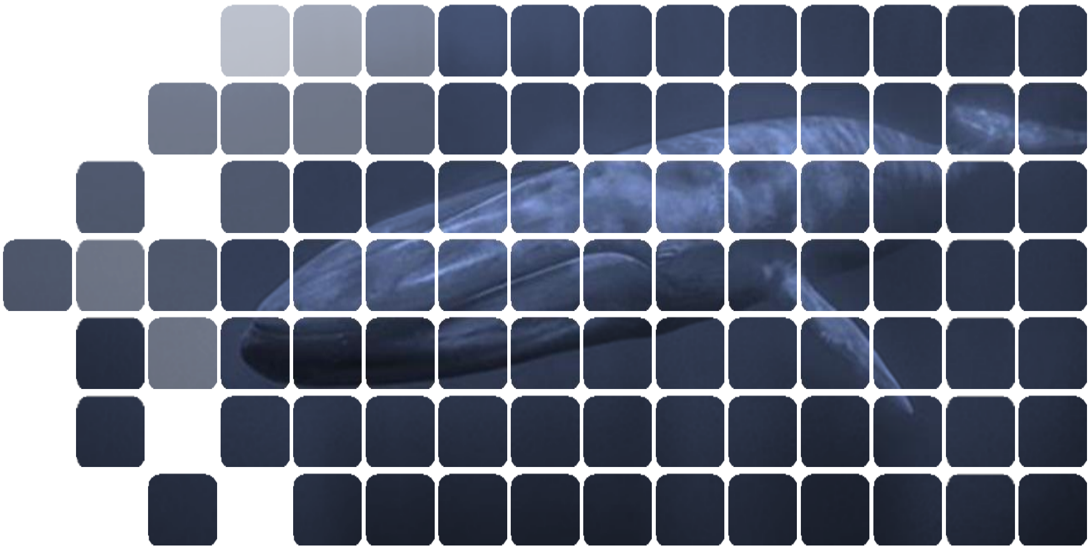
Evolutionary genomics of Balaenopterids

with a focus on the blue whale (*Balaenoptera musculus*)



BY

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Abstract

Marine ecosystems, with their minimal physical barriers and limited opportunities for allopatry, provide a unique perspective to study evolutionary adaptations. Cetaceans—whales, dolphins, and porpoises—represent a diverse group of mammals that transitioned from terrestrial to fully aquatic life approximately 50 million years ago. While the general adaptive processes underlying this transition have been well studied, the diversification of modern cetaceans, particularly within the baleen whale family (Balaenopteridae), remains a complex and poorly understood aspect of their evolutionary history. Understanding the genomic basis of species diversification is crucial in evolutionary biology as it uncovers how genetic variations drive species formation and adaptation while also informing on species resilience or vulnerability. This thesis aims to clarify the genomic basis of diversification and evolutionary history of baleen whales, with a focus on blue whales (*Balaenoptera musculus*). By analysing whole-genomes from 15 cetacean species, the study detected various genomic regions associated with the diversification and adaptation of baleen whales (Chapter 2). A robust molecular phylogeny constructed with over 10,000 orthologous genes demonstrated substantial genomic changes in balaenopterids related to aging, survival, and homeostasis. Notably, positive selection was identified in immune system genes of the largest species, blue and fin whales, suggesting a link between increased body size and enhanced immune function. The thesis further explored the impact of historical environmental changes on the connectivity, demography, and diversification of blue whales (Chapter 3). An analysis of 16,661 single nucleotide polymorphisms (SNPs) from 275 individuals, representing three of the four recognised subspecies, showed that past climatic and oceanographic events have shaped blue whale subspecies. Coalescent models indicated that divergence among blue whale lineages occurred around 300,000 years ago during the Pleistocene, a period marked by major environmental shifts. Fluctuations in climate and primary productivity likely influenced the connectivity and demographic history of blue whales, shaping their current genetic diversity and distribution. There was no evidence of recent genetic bottlenecks, possibly due to limitations in detecting such signals with a reduced representation genomic dataset and the analysis used. However, in a further examination of blue whale subspecies this time using whole-genome population resequencing data from most subspecies, including three lineages and five populations, and first-generation hybrids between Antarctic and pygmy blue whales, this signal was revealed (Chapter 4). Phylogenomic analysis based on 13,041 orthologous genes, combined with population genetic analysis based on 1,520,428 SNPs, demonstrated genetic differentiation among Atlantic, Pacific, Antarctic, and pygmy blue whales, suggesting a complex interrelationship among these groups. The whole-genome analyses provided insights into recent demographic trends, highlighting a significant population decline in Antarctic blue whales during the whaling era, with no subsequent recovery. On the other hand, Atlantic and pygmy blue whales showed a similar decline but with evidence of recovery over time. Runs of homozygosity (ROH) analyses revealed generally low inbreeding levels and larger past population sizes, except in pygmy blue whales, which exhibited a greater number and longer ROHs indicating smaller historical population sizes. Despite recent overexploitation on all lineages, heterozygosity levels and genetic load analysis suggest that blue whale populations retained considerable diversity to support their recovery. The low genetic load and minimal inbreeding in the Antarctic lineage offer a promising outlook for the long-term viability of this subspecies; however, contrasting findings from the recent demographic history analysis suggest that its recovery may be slower than anticipated. This comprehensive study underscores the importance of integrating genomic and historical data to understand the complex evolutionary processes that shape baleen whales, and in particular, blue whales. The findings contribute to a deeper understanding of cetacean evolution and have major implications for conservation strategies aimed at protecting and managing blue whale lineages in their diverse habitats.

Resumen

Los ecosistemas marinos, con sus barreras físicas mínimas y oportunidades limitadas para la alopatría, brindan una perspectiva única para estudiar las adaptaciones evolutivas. Los cetáceos —ballenas, delfines y marsopas— representan un grupo diverso de mamíferos que hicieron la transición de la vida terrestre a una vida completamente acuática hace aproximadamente 50 millones de años. Aunque los procesos adaptativos generales que sustentan esta transición han sido bien estudiados, la diversificación de los cetáceos modernos, en particular dentro de la familia de las ballenas barbadas (Balaenopteridae), sigue siendo un aspecto complejo y poco comprendido de su historia evolutiva. Comprender la base genómica de la diversificación de especies es crucial en biología evolutiva, ya que permite descubrir cómo las variaciones genéticas impulsan la formación y adaptación de especies, además de ofrecer información sobre su resiliencia o vulnerabilidad. Esta tesis tiene como objetivo clarificar la base genómica de la diversificación e historia evolutiva de las ballenas barbadas, con un enfoque en las ballenas azules (*Balaenoptera musculus*). A través del análisis de genomas completos de 15 especies de cetáceos, el estudio detectó numerosas regiones genómicas asociadas con la diversificación y adaptación de las ballenas barbadas (Capítulo 2). Una filogenia molecular robusta construida con más de 10,000 genes ortólogos demostró cambios genómicos sustanciales en los balaenoptéridos relacionados con el envejecimiento, la supervivencia y la homeostasis. De manera notable, se identificó selección positiva en genes del sistema inmunológico de las especies de mayor tamaño, como la ballena azul y el rorcual común, lo cual sugiere un vínculo entre el aumento del tamaño corporal y una mayor función inmunológica. La tesis exploró además el impacto de los cambios ambientales históricos en la conectividad, demografía y diversificación de las ballenas azules (Capítulo 3). Un análisis de 16,661 polimorfismos de un solo nucleótido (SNPs) en 275 individuos, que representan tres de las cuatro subespecies reconocidas, mostró que los eventos climáticos y oceanográficos del pasado han forjado las subespecies de ballena azul. Los modelos coalescentes indicaron que la divergencia entre linajes de ballena azul ocurrió hace unos 300,000 años, durante el Pleistoceno, un periodo marcado por importantes cambios ambientales. Las fluctuaciones en el clima y la productividad primaria probablemente influyeron en la conectividad y la historia demográfica de las ballenas azules, moldeando su diversidad genética y distribución actuales. No se hallaron evidencias de cuellos de botella genéticos recientes, posiblemente debido a las limitaciones en la detección de estas señales con los datos disponibles y el análisis empleado. Sin embargo, en un estudio adicional de las subespecies de

ballena azul, en esta ocasión analizando datos de re-secuenciación del genoma completo de la mayoría de las subespecies, incluyendo tres linajes y cinco poblaciones, e híbridos de primera generación entre ballenas azules antárticas y enanas, la señal fue detectada (Capítulo 4). El análisis filogenómico basado en 13,041 genes ortólogos, junto con el análisis genético poblacional basado en 1,520,428 SNPs, demonstrando una diferenciación genética entre las ballenas azules del Atlántico, Pacífico, Antártico y enanas, sugiriendo una compleja interrelación entre estos grupos. Los análisis de genoma completo proporcionan información sobre tendencias demográficas recientes, destacando una disminución significativa en las poblaciones de ballenas azules antárticas durante la era ballenera, sin recuperación posterior. Por otro lado, las ballenas azules del Atlántico y enanas mostraron una disminución similar, pero con evidencia de recuperación a lo largo del tiempo. Los análisis de las series de homocigocidad (ROH) revelaron niveles de endogamia generalmente bajos y grandes tamaños poblacionales históricos, excepto en las ballenas azules enanas, que exhibieron un mayor número y más largos ROHs, indicando tamaños poblacionales históricos más pequeños. A pesar de la sobreexplotación reciente en todos los linajes, los niveles de heterocigosidad y el análisis de carga genética sugieren que las poblaciones de ballena azul retienen considerable diversidad que respalda su potencial de recuperación. La baja carga genética y el mínimo nivel de endogamia en el linaje antártico ofrecen una perspectiva prometedora de esta subespecie viabilidad a largo plazo; sin embargo, los hallazgos contrastantes del análisis de historia demográfica reciente sugieren que su recuperación podría ser más lenta de lo anticipado. Este estudio exhaustivo subraya la importancia de integrar datos genómicos e históricos para comprender los complejos procesos evolutivos que moldean a las ballenas barbadas, y en particular, a las ballenas azules. Los hallazgos contribuyen a una comprensión más profunda de la evolución de los cetáceos y tienen importantes implicaciones para las estrategias de conservación dirigidas a proteger y gestionar los linajes de ballena azul en sus diversos hábitats.

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Declaration

I certify that this thesis:

1. does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university
2. and the research within will not be submitted for any other future degree or diploma without the permission of Flinders University; and
3. to the best of my knowledge and belief, does not contain any material previously published or written by another person except where due reference is made in the text.

Gabrielle Genty

6 November 2024

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I would like to acknowledge the Kaurna people as the Traditional Owners of the land where Flinders University now stands and I pay my respects to Elders past, present, and emerging.

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Dedication

This thesis is dedicated to my father, who was my biggest inspiration. He is the reason I see nature with deep admiration, respect and find comfort in it. And to my mother, Martha for being my unfaltering support.

Publications

Published

Genty, G., Sandoval-Castillo, J., Beheregaray, L. B., & Möller, L. M. (2024). Into the Blue: Exploring genetic mechanisms behind the evolution of baleen whales. Gene, 929, 148822. https://doi.org/10.1016/j.gene.2024.148822

Manuscript objectives: This study explored the molecular mechanisms underlying balaenopterid evolution using whole-genome data. The specific objectives were to: (1) clarify patterns of genomic diversification within balaenopterids as compared to other cetacean lineages, (2) characterise how rates of genomic evolution and functional genomic features correlate with broad phenotypic diversity, and (3) identify candidate adaptive genomic regions that contributed to the diversification of baleen whales.

Under Review

Genty, G., Buckey, S. J., Sandoval-Castillo, J., Attard, C. R. M., Brauer, C. J., Lang, A. R., Jenner, K. C. S., Gill, P. C., Galletti Vernazzani, B., Torres, L. G., Burton, C. L. K., Jenner, M-N. M., Sironi, M., Beheregaray, L., Möller, L. M. (in review) Out of the blue: Phylogeographic history of the largest extant animal, the blue whale (*Balaenoptera musculus*). Submitted to Journal of Heredity and currently on second round of revisions, 2024.

Manuscript objectives: This chapter leverages on previously available reduced representation genomic data to test the effects of historical events on the demography and diversification of blue whales. The objectives were to: (1) investigate how phylogenetic patterns in blue whale populations align with major historical events, such as the Last Glacial Maximum (LGM), Pleistocene glaciations, and the late Miocene-early Pliocene transition; (2) examine the influence of the Pliocene-LGM transition on population diversification; and (3) evaluate the potential genetic bottlenecks caused by human exploitation during the whaling era.

To Be Submitted

Genty, G., Sandoval-Castillo, J., Beheregaray, L. B., & Möller, L. M. (in prep.). Deep into the Blue: Unravelling the Genetic Diversity and Demographic History of Blue Whales (*Balaenoptera musculus*). To be submitted to Current Biology, 2024.

Manuscript objectives: This chapter aims to analyse genomic data to: (1) evaluate levels of genetic diversity and heterozygosity within and among blue whale lineages; (2) identify specific genomic regions associated with health and resilience against environmental stressors; and (3) correlate genomic health with the potential of population recovery after the known whaling-induced bottlenecks.

Statement of authorship

I, Gabrielle Genty, am the primary contributor to each chapter of this thesis. A/Prof. Luciana Möller, my main supervisor, and Prof. Luciano Beheregaray, my associate supervisor, are co-authors of the derived manuscripts from this thesis due to their significant guidance in study design conception, interpretation and manuscript writing and editing. Additionally, my other associate supervisor, Dr. Jonathan Sandoval-Castillo, is also co-author of the manuscripts due to his essential role in data processing, bioinformatics, and contributions to manuscript writing and editing.

Chapter 1

THE BIG BLUE: General Introduction

Geographe Marine Research

by Rodney Peterson

1.1 Evolutionary Genomics

Evolutionary genomics investigate the mechanisms driving structural and sequence changes in the genomes of organisms over time (Tiffin & Ross-Ibarra, 2014). Life on Earth exhibits extraordinary diversity, with each organism possessing a unique genome composed of numerous genes. Variations within these genes contribute to genetic diversity within species, a phenomenon known as polymorphism. These genetic systems have evolved since the emergence of life, shaping the biological diversity observed today (Ellegren & Galtier, 2016).

This genetic diversity is evident across various organisms, from plants and fungi to animals. In plants, evolutionary genomics can unravel the genetic adaptations that enable certain species to thrive in specific ecological niches or endure environmental stresses, such as drought or extreme temperatures (Browne et al., 2019; Holliday et al., 2010; Nagy & Rice, 1997; Zarzyczny et al., 2023). Similarly, in animals, evolutionary genomics has revealed the genetic foundations of traits, behaviours, and adaptations that allow species to survive and succeed within their environments (Barrett & Schluter, 2008; Song & Wangs, 2013). Even in organisms as seemingly simple as single-celled eukaryotes, such as bacteria, genomic complexity plays a crucial role in their biological functions and responses to changing conditions (Coolon et al., 2009; Csonka, 1989). By understanding genomic composition and variations, researchers can gain insights into the molecular mechanisms leading to the evolution and diversity of current species. Therefore, the study of evolutionary genomics serves to uncover the genetic foundations of traits, the origins of genetic variation, and the forces shaping the evolutionary trajectories of diverse organisms across the tree of life.

1.1.1 Past, Present and Future of Evolutionary Genomics

In 1976, the first sequenced genome of the RNA bacteriophage MS2 became available (Fiers et al., 1976), followed by the bacteria *Haemophilus influenzae*, the first organism to have its entire genome fully assembled in 1995 (Figure 1.1a), an achievement largely attributed to their small genome size of 1.83 Mb (Fleischmann et al., 1995). Since then, the use of comparative genomics to examine differences and similarities among genomes has been crucial in deepening the understanding of the genetic composition of organisms.

Evolutionary genomics integrates molecular biology, population genetics, and bioinformatics (Figure 1.1) to unveil the complexity of genomic evolution (Saitou, 2018). Advances in genome sequencing have been significant, with the mapping of the first human genome, albeit incomplete, in 2001 as part of the Human Genome Project (Lander et al., 2001). Following the technological and bioinformatics advancements, the human genome was finally completed in April 2022 (Figure 1.1a) (Nurk et al., 2022), together with a thorough analysis of its genetic variation (Aganezov et al., 2022). This holistic approach has helped researchers better understand the molecular mechanisms leading to the adaptation and diversification of species, providing valuable insights into the origins of genetic variation and the forces that shape the evolutionary paths of organisms.

Presently, thousands of species have their entire genome sequences available, most available in two comprehensive genome databases: National Centre for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/datasets/genome/) and Ensembl Genome (http://ensemblgenomes.org/). Numerous databases are dedicated to specific groups of organisms, and a significant portion of these databases offer access to their genomic sequences. In alignment with these efforts, various initiatives focusing on eukaryotes, including DNA Zoo, Darwin's Tree of Life, and the Vertebrate Genome Project, aim to sequence all known eukaryotic species within the next decades. Several other databases contribute extensively to the domain of evolutionary genomics, providing comprehensive resources that allow researchers to advance the understanding of genetic intricacies.

There are several ongoing projects, particularly for humans and mice, that aim to collect extensive data to understand the relationship between phenotypic traits and genetic information. For example, one of these projects is the 'Mouse Genome Informatics' (MGI; https://www.informatics.jax.org/), which serves as an international database resource for

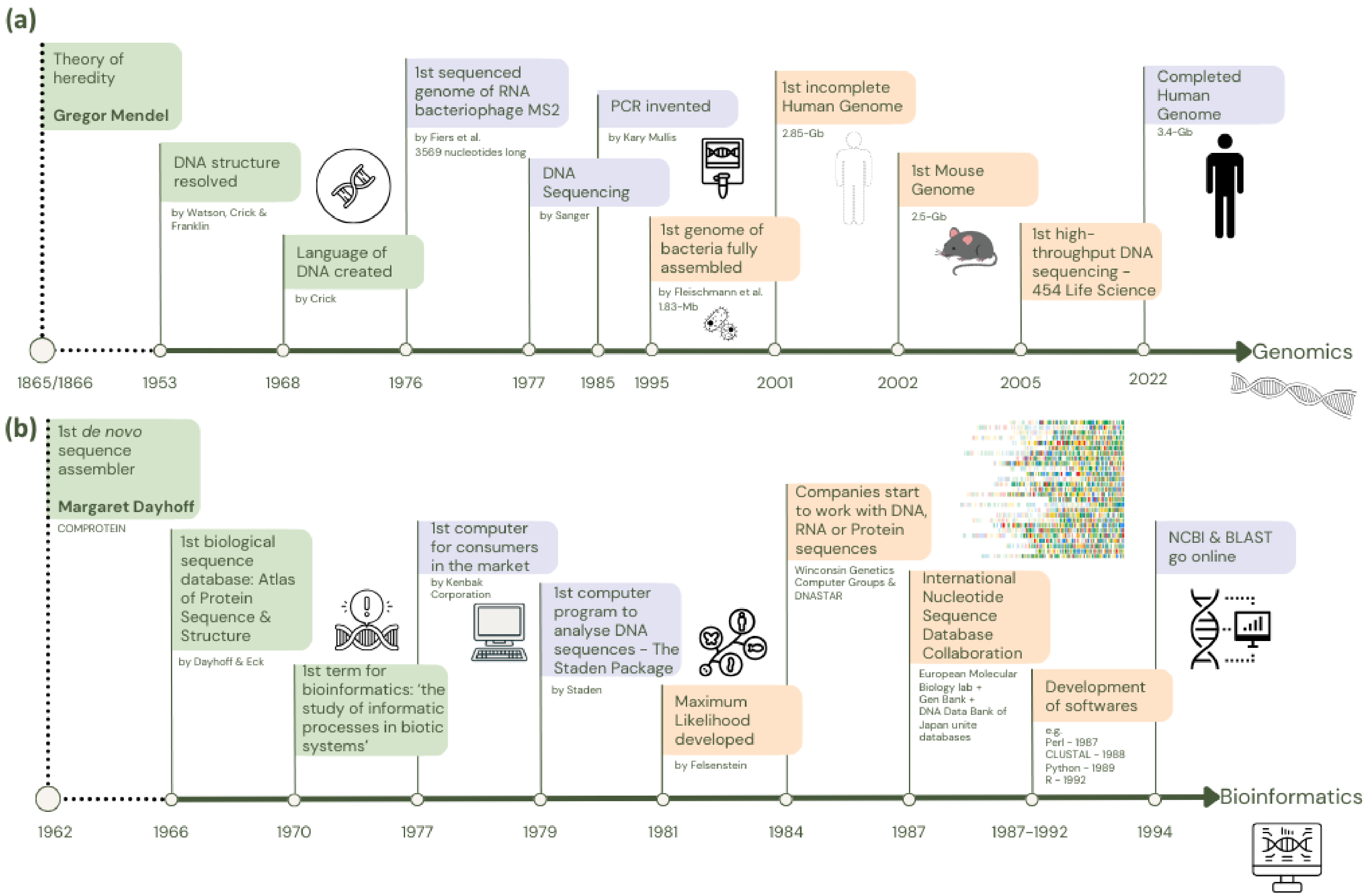


Figure 1.1. Chronology of key milestones in the fields of **(a)** genomics and **(b)** bioinformatics. (Adapted from: Aganezov et al., 2022; Fiers et al., 1976; Fleischmann et al., 1995; Gauthier, Metcalfe, & Sears, 1997; Giani, Gallo, Gianfranceschi, & Formenti, 2020; Lander et al., 2001; Mishra et al., 2023; Nurk et al., 2022).

laboratory mice, integrating genetic, genomic, and biological data to enable research on mammalian health and disease, with a focus on applications for humans. Other complementary databases are, for example, BUSCO (https://busco.ezlab.org/), which employs evolutionarily guided criteria for gene content using single copy orthologs genes to assess the completeness of genome assemblies, gene sets, and transcriptomes (Manni et al., 2021). BUSCO's scores, expressed as the percentage of marker genes within the analysed assembly or gene set, offer a valuable measure to evaluate the completeness of genomes being assessed. Another example is the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (http://www.genome.jp/kegg/pathway.html) (Kanehisa, 2019; Kanehisa et al., 2023; Kanehisa & Goto, 2000). It integrates genomic, chemical, and functional data to map out the interactions between genes, proteins, and metabolic reactions in various biological systems. By organising this information into pathway maps, KEGG allows scientists to analyse how genes and proteins contribute to complex biological processes and to better understand the functional implications of genomic data. Additionally, for a systematic exploration of gene functions, researchers have available the Gene Ontology (GO) database (http://www.geneontology.org) (Ashburner et al., 2000; The Gene Ontology Consortium et al., 2023). GO terms are categorised into three domains: biological processes, cellular components, and molecular functions. This hierarchical system enables researchers to annotate genes and proteins based on their roles in specific cellular processes, their location within the cell, and their biochemical activities. By standardising gene function descriptions, GO allows for the comparison of gene functions across different organisms and enhances the interpretation of genomic data in different biological contexts. These resources offer invaluable and complementary tools for dissecting gene composition, biological pathways and understanding gene functions in various organisms.

This increase in information is likely to offer a new perspective on the amount, distribution, and functional importance of genetic variation within natural populations. Exploring genetic diversity within populations provides a retrospective view of the evolutionary history of taxa (Ellegren & Galtier, 2016) and a current glance of genetic structure within and among populations (Whitlock, 2014). It also provides predictions on the evolutionary potential of populations and species (e.g.

Bay et al., 2018; Hoffmann, Weeks, & Sgrò, 2021; Turbek et al., 2023; Wood et al., 2021) to adapt to current and future environments.

1.1.2 Species Classification and Lineage Diversification

Species or subspecies are usually classified as genetically and geographically distinct populations, exhibiting phenotypic differences and commonly reproductive isolated (Mayr, 1996). Understanding the ecology and evolution of organisms requires species and subspecies to be correctly defined to ensure their effective conservation and management (De Queiroz, 2007). In recent years, the field of evolutionary genomics has developed the ability to analyse genetic information for the understanding of lineage diversification (Allendorf, Hohenlohe, & Luikart, 2010; Luikart, England, Tallmon, Jordan, & Taberlet, 2003; Thomas, 2004), and assist with proper management of different populations. For example, genomic analysis of polar bear (*Ursus maritimus*) and brown bear (*U. arctos*) lineages revealed ancient hybridisation events, which highlighted the need to reassess their distinct species classifications and management strategies (Cahill et al., 2013). Similarly, genomic studies of the African elephant species (*Loxodonta africana* and *L. cyclotis*) demonstrated clear genetic divergence despite physical similarities, providing evidence to prioritise the conservation of both as distinct species (Rohland et al., 2010).

Some organisms exhibit higher capacity for diversification into new species than others. The processes influencing species diversity are multifaceted and may potentially interact, as geographic modes of speciation, divergent adaptation, adaptive radiations, mass extinctions, species selection, and introgression from distant lineages (Seehausen et al., 2014). Genomics offers valuable insights into unravelling the evolution of genes and traits, providing a deeper understanding of the genetic mechanisms underlying lineage diversification. Certain genes involved in divergent adaptation exhibit limited exchange between populations (Nosil et al., 2009). However, neutral genes that are not linked to divergent selection can be shared between species through gene flow resulting from migration (Pinho & Hey, 2010). Over time, genetic differences accumulate in regions with low recombination rates ultimately leading to complete reproductive isolation (Wu, 2001). Divergent accumulation influences compatibility within populations leading to the eventual emergence of distinct species. For instance, the colonisation of the Lake Victoria basin in East Africa by freshwater fish around 150,000 years ago led to the diversification of haplochromine cichlids into several hundred species within the lake (Seehausen, 2002). This well-recognised example underscores the role of evolutionary genomics in elucidating the processes driving species diversification and adaptation.

Evolutionary genomics facilitates the investigation of evolutionary relationships among species, aiding in the resolution of complex phylogenetic patterns and providing insights into the evolutionary history of lineages (Miles & Dunham, 1993; Yang & Rannala, 2012). Although comparing gene expression among multiple species has many challenges (Dornburg et al., 2022; Naylor & Gerstein, 2000), multiple studies have revealed, for example, that uncommon cases of directional selection are caused by mutations in coding sequences, changes in gene regulatory regions, and that gene duplications are responsible for large effects on phenotype and function among divergent species (Labbé et al., 2014; Lemos et al., 2005; Wittkopp & Kalay, 2012). By understanding species-specific adaptations, scientists can have a grasp of the complex interactions between functional divergence, gene expression evolution, and gene regulatory networks. This knowledge is essential for unravelling the mechanisms driving phenotypic and functional differences among species, shedding light on the intricacies of evolution and biological diversity.

1.1.3 Detecting Positive Selection: Synonymous and Nonsynonymous Substitutions

In navigating the landscape of evolutionary genomics, the exploration of positive selection through synonymous and nonsynonymous substitutions enhances the understanding of molecular dynamics and establishes a crucial link to the broader context of lineage diversification and genetic mechanisms shaping species evolution. This approach examines nucleotide substitutions within amino acid-coding sequences to reveal the dynamics of evolution (Saitou, 2018) and links adaptive genetic regions to broader evolutionary processes (Song & Wangs, 2013). Synonymous substitutions, which do not alter amino acids, are considered neutral, while nonsynonymous changes can be positively selected, neutral, or under purifying selection (Kafatos et al., 1977; Saitou, 2018). The ratio of nonsynonymous to synonymous differences (dN/dS) helps infer natural selection, with values close to one suggesting neutral evolution, values below one indicating constrained changes and values above one denoting rapid evolution favoured by natural selection (Kafatos et al., 1977; Miyata & Yasunaga, 1980; Nei & Gojobori, 1986; Yang & Nielsen, 1998). The dN/dS value offers an average of the evolutionary process, providing insights into constraint, rapid evolution, or uncertainty, making it a conservative test. Although the dN/dS ratio provides only an average view of evolution, advancements in tools like PAML (Yang, 2007), have significantly improved the ability to assess genetic variation and its role in adaptation.

1.1.4 Genetic Load

While methodologies such as the dN/dS ratio offer valuable insights into adaptive evolution and the role of selection in shaping genetic variation, not all genetic changes are beneficial. Alongside positive selection, the accumulation of deleterious mutations—also referred to as genetic load—can shape population dynamics and evolutionary trajectories (Bertorelle et al., 2022).

Genetic load refers to the accumulation of deleterious mutations within a population, which can reduce overall fitness and influence evolutionary trajectories (Bertorelle et al., 2022; Muller, 1950). It is a critical concept in conservation genetics, as it affects the long-term viability of populations, particularly those experiencing reduced genetic diversity due to bottlenecks, habitat fragmentation, or small population sizes (Dussex et al., 2023; Mathur & DeWoody, 2021). Two key processes can influence the genetic load in populations: purging and inbreeding depression. Purging occurs when selection removes deleterious alleles from a population, potentially mitigating the negative effects of inbreeding (Hedrick & Garcia-Dorado, 2016). However, inbreeding depression, which is the reduction in fitness due to increased homozygosity of deleterious alleles, can lead to reduced survival and reproductive success, especially in small or isolated populations (Charlesworth & Charlesworth, 1987).

In conservation, understanding genetic load of a population is essential to maintaining population health. For instance, in vertebrate species such as the Florida panther (*Puma concolor coryi*), genetic rescue efforts have introduced genetic variation from other populations to reduce inbreeding depression, successfully increasing survival and reproductive rates (Johnson et al., 2010). Similarly, a study on the vaquita (*Phocoena sinus*) has shown how genetic load influences the survival of small, isolated populations (Robinson et al., 2022). Despite their critically low numbers due to by-catch, genomic analyses suggest that the vaquita harbors a relatively low burden of deleterious variation, reducing the risk of inbreeding depression. These findings underscore the importance of how the understanding of genetic load is crucial for assessing the evolutionary potential of populations and informing conservation management strategies, ensuring that species maintain adaptive capacity in changing environments.

1.2 Paleoceanography and Evolution of Whales

Paleoceanography is used to reconstruct and understand the changes in the oceans through time. The study of paleoceanography involves analysing sedimentary records, tectonic plate motions, glacial changes, and the relationship between present sediment patterns and environmental factors (Thiede, 2015). The extensive climatic changes from the most recent epochs have played a fundamental role in shaping contemporary biodiversity patterns in both terrestrial and marine habitats. The dispersal and genetic structure of populations are products of current and historical processes. Past environmental changes can be retained in the genetic information of individuals. Understanding palaeoceanographic changes that have occurred through time can help inform how a globally distributed species may be moulded by such changes. Hence, when evaluating a split, contraction, expansion, and/or migration of populations, changes in genetic variation can be used to investigate signals that might be associated with major historical events (Hahn, 2018).

Paleoceanography, through its examination of past oceanic conditions and environmental factors, helps in understanding how organisms evolved and adapted to changing marine ecosystems. One group of organisms that are closely intertwined with the history of the world's oceans and have shown remarkable adaptability to a wide range of oceanic conditions are the Balaenopteridae, a family within the suborder Mysticeti (baleen whales) of the Cetacea order, which include the largest animal on earth, the blue whale (*Balaenoptera musculus*). The integration of palaeoceanographic data provides valuable insights into the dynamic interplay between environmental shifts and the evolutionary trajectory of balaenopterids, shedding light on the selective pressures that have shaped their genomic adaptations over time.

1.2.1 Historical Demographic Analyses

Historical demographic analyses help reconstruct past population size (Ne) changes, migration patterns, and selection pressures, offering insights into how species respond to environmental shifts (Lowe et al., 2017). Given that past environmental changes are often retained in the genetic information of individuals, integrating paleoceanographic insights with genomic analyses can provide a more comprehensive understanding of how populations have responded to historical climatic shifts. Two main genomic approaches are used: coalescent-based methods, which model genealogical histories (Excoffier et al., 2013; Nielsen, 2000), and linkage disequilibrium (LD)-based methods, which infer Ne changes from genetic variation patterns (Ramstetter et al., 2017).

Coalescent methods like PSMC and MSMC analyse heterozygosity across genomes to infer long-term Ne (Li & Durbin, 2011; Schiffels & Durbin, 2014). While PSMC and MSMC are well-suited for detecting ancient demographic events (10,000–1,000,000 years ago), they are less effective at resolving recent changes due to limited recombination signals and assumptions of panmixia, making them less suitable for species with complex population structures or high gene flow, such as cetaceans (Chikhi et al., 2018; Mazet et al., 2016).

Site frequency spectrum (SFS)-based methods, such as fastsimcoal2, improve resolution by incorporating allele frequency data from multiple individuals, allowing for detailed modelling of demographic events (Excoffier et al., 2013; Excoffier & Foll, 2011). Unlike PSMC and MSMC, which rely on a single or a few diploid genomes, SFS-based approaches use allele frequency data from multiple individuals, allowing for the explicit modelling of demographic events such as bottlenecks, expansions, and migration. This method is particularly useful for species with known historical population perturbations and varying population structures, as it enables the testing of complex demographic scenarios. By simulating genetic data under different historical models and comparing them to observed SFS patterns, tools such as fastsimcoal2, offer a statistically robust approach for inferring past population changes.

Complementary to coalescent-based methods, LD-based approaches such as GONE, provide high-resolution estimates of recent Ne changes, making them useful for structured populations and conservation applications (Coombs et al., 2012). LD-based methods explore the decay of nonrandom associations between alleles at different loci, which occurs over time due to recombination and genetic drift, allowing for detailed reconstructions of more contemporary demographic events (Santiago et al., 2020).

By integrating coalescent and LD-based approaches, a more comprehensive reconstruction of demographic history can be achieved, capturing both ancient and recent timescales. The combination of fastsimcoal2 and GONE, for example, allows for a nuanced understanding of how past climatic changes, habitat shifts, and anthropogenic pressures have shaped population dynamics. These integrative approaches are particularly valuable for studying species with complex life histories, migration patterns, and population structures, such as cetaceans, as they help refine conservation strategies and improve the understanding of evolutionary processes in natural populations.

1.2.2 Evolution of Balaenopteridae

Cetaceans are marine mammals that are thought to have originated approximately 52.5 Mya during the early Eocene (McGowen, Tsagkogeorga, Álvarez-Carretero, et al., 2020) with the hippopotamus (*Hippopotamus amphibius*) being the closest living terrestrial relative (Geisler & Theodor, 2009). The evolution of extant cetacean species may be attributed to various patterns of ocean restructuring during climatic oscillations in the Oligocene and Miocene (Steeman et al., 2009). The diversification of cetaceans coincides with the late Eocene and early in the Oligocene when widespread sea-ice and glaciations first appeared. During the Oligocene, a significant drop in the sea floor temperatures promoted Antarctica to become physically and oceanographically isolated at high latitudes (Barrett, 1996). The radiation of the Balaenopteridae family began around 15.74 to 4.75 Mya in the late Miocene (Árnason et al., 2018; McGowen, Tsagkogeorga, Álvarez-Carretero, et al., 2020), with the development of long-distance migrations and increase in body sizes, including gigantism (Marx & Fordyce, 2015).

The glaciations during the Pleistocene, shaped the distribution of primary productivity, prey resources and suitable habitats, and dictated the capacities and dispersal strategies of species, ultimately affecting the demographic history of marine species (Grant & Dickens, 2001; Ratnarajah et al., 2016). For example, Cetacean diversification, as evidenced by the early radiation of baleen whales in Antarctica (Fordyce & Marx, 2018), is thought to have occurred during the development of the Southern Ocean and the origin of diatoms as the base of the food chain (Marx & Uhen, 2010). However, since the fossil record is sparse and biased towards the northern hemisphere, it is difficult to confirm whether the lineage of baleen whales originated in Antarctica (Pyenson, 2017).

Furthermore, during extended glaciations, the availability of prey played an important role in shaping the genetic structure and dispersal patterns of cetaceans (Jackson et al., 2014; Liu et al., 2011) and contributed to the isolation of some populations, reducing gene flow and promoting genetic differentiation. The glacial oscillations of the Pleistocene (2.5 Mya to 11.7 kya) are known to have had a significant impact on the genetic structure, distribution, and connectivity of different cetacean species (e.g., Harlin-Cognato et al., 2007; Jackson et al., 2016). For this reason, it is important to have a historical context (e.g., the reconstruction of past demographic histories) for predicting future biological impacts of environmental changes, such as species range shifts and/or genes expected to face selection pressures.

By exploring historical demography, genomic approaches enable the reconstruction of past population dynamics and demographic events (Avise, 2009). This sheds light on the factors that have shaped the evolutionary trajectories of species, by relating genetic disjunctions to histories of climate or physical barriers(e.g. Booth et al., 2022; Buckley et al., 2021; Jensen et al., 2019). If the genetic structure of related but evolutionarily independent populations is similar, it may suggest a common process driving genetic divergence (Bermingham & Moritz, 1998), and this divergence can be further shaped by the historical size of the population, which influences the rate of genetic differentiation (Cabrera et al., 2021). Understanding these genetic patterns is crucial, especially as climate change is already substantially impacting marine ecosystems (A. K. et al., 2023; Brierley & Kingsford, 2009; Williamson & Guinder, 2021).

Climate change, in the form of increasing sea surface temperatures, changing currents, shifting ocean salinity, and increasing weather variability is predicted to have a substantial impact worldwide, resulting in shifts and alterations of many biotas (IPCC, 2019). Marine species, such as baleen whales, experience few physical barriers to migration and gene flow, therefore contact between populations is likely to occur. Previous studies on marine mammals have shown that climate changes can significantly reduce prey availability (Kaschner et al., 2011), cause a shift and reduction of distributions (Schumann et al., 2013), increase pollutants and pathogen loads (Sanderson & Alexander, 2020), increase stress levels (Atkinson & Dierauf, 2018) and decrease survival rates (Wild et al., 2019). Globally, monitoring sentinel species is key to unravelling the health state of marine ecosystems and identifying hotspots at high risk of population declines (Avila et al., 2018). Therefore, it is crucial to investigate and understand the interactions between marine species and past environmental changes for predicting the potential impacts of contemporary climate change on different species.

1.3 Balaenopteridae family

The Balaenopteridae family or rorquals are within the cetacean suborder of baleen whales. These organisms are distinguished by the adaptation of baleen plates that enable them to filter feed (Pivorunas, 1979). They are also characterised by a symmetrical skull, paired blowholes, and the lack of ribs that attach to the sternum (Bannister, 2018).

Balaenopterids range in size from the smallest, the common minke whale (*Balaenoptera acutorostrata*), reaching about 7 to 8 meters in length, to the largest, the blue whale (*B. musculus*), reaching up to more than 30 meters (Table 1.1). In this family, the females are commonly slightly larger than the males (McNeill Alexander, 1998), and most species are known to undertake long migrations (Stern, 2009).

1.3.1 Distribution and General Biology

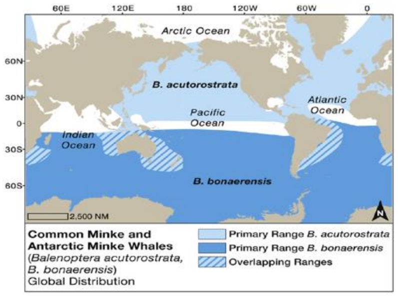
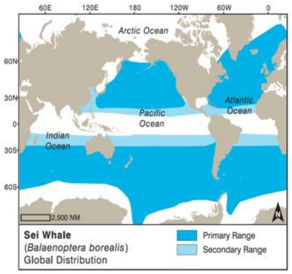
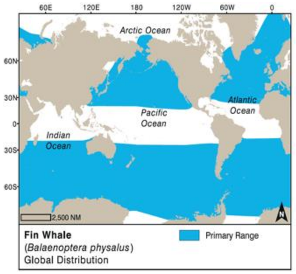
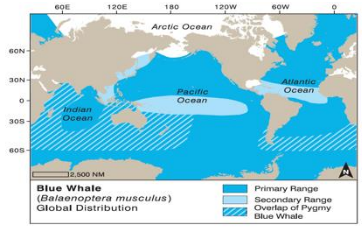
The rorquals include the genus *Balaenoptera* (common minke, *B. acutorostrata*, Antarctic minke, *B. bonaerensis;* sei, *B. borealis*; fin, *B. physalus*; **blue, *B. musculus***; Bryde’s, *B. edeni*; Omura’s, *B. omurai*; rice’s, *B. ricei*), and the genus *Megaptera*, represented by the humpback whale (*Megaptera novaeangliae*) as its only member (Bannister, 2018). Their heads are relatively short, less than a quarter of the length of their bodies. Baleen plates are smaller and wider than those of right whales (*Eubalaena spp.*). The dorsal fin is sometimes quite reduced, and they have numerous ventral grooves. On the inside of the mandible, they have a long upper jaw, bowed mandibles, coronoid process, and free cervical vertebrae (Bannister, 2020). In terms of genetics, all rorquals are diploid and have 44 chromosomes. In multiple molecular analyses, it has been found that the morphologically distinct gray whale (*Eschrichtius robustus,* a benthic suction feeder and the only representative of the Eschrichtiidae family today) belongs to the engulfment feeding rorquals of Balaenopteridae, although the relationships between balaenopterids at the base vary (Árnason et al., 2018; Hassanin et al., 2012; McGowen, Tsagkogeorga, Álvarez-Carretero, et al., 2020; Sasaki et al., 2006; Steeman et al., 2009).

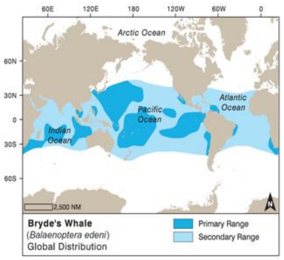
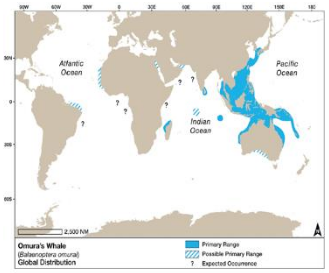
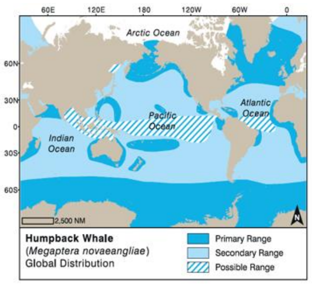
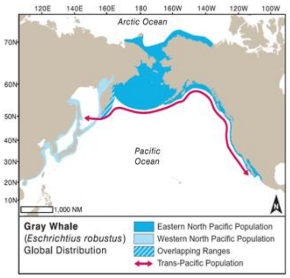
Distributed worldwide, baleen whales can be found in a wide range of habitats (Table 1.1). Within this family, the fin and sei whales are the most oceanic species (Aguilar & García-Vernet, 2018; Horwood, 2018). Minke whales are also wide-ranging and together with blue whales can be found closest to the ice edge during summer (Branch, 2007; Perrin et al., 2018). Both species seem to migrate, but these migrations are less defined and predictable, and for some populations in certain regions, they may be present year-round (Stern & Friedlaender, 2018). Blue whales are often found in deep coastal canyons but can be located closer inshore due to the upwellings they feed on (Sears & Perrin, 2018). Humpback whales and gray whales are coastal baleen whales, with long migrations from tropical breeding aggregation sites to cold feeding grounds (Clapham, 2018; Swartz, 2018) and are the most morphologically dissimilar within the family. Bryde's whales are the most geographically localised baleen whale. They are exclusively found in tropical waters and possibly do not undertake long migrations (Kato & Perrin, 2018). However, some populations may be connected through gene flow despite limited migration (Constantine et al., 2018). Among the mysticetes, gray whales are the most coastal and migrate the longest (Swartz, 2018).

For larger animals, filter-feeding is the most efficient feeding strategy (Friedman et al., 2010). Therefore, these giants are primarily feeding on very small organisms like zooplankton, mainly euphausiids, copepods, or small fish (Christensen et al., 1992). During the summer season in the Southern Hemisphere, the distribution of baleen whales highly depends on the presence of Antarctic krill (*Euphausia superba*) (Amaral et al., 2016; Buchan et al., 2021; Valenzuela, Sironi, Rowntree, & Seger, 2009). In the Northern Hemisphere the species have a more variable diet including small fish (Christensen et al., 1992; Oliver & Slattery, 1985; Rita et al., 2023). For example, fin whales feed on multiple species of schooling fish in the North, while feeding exclusively on krill in the South (Bannister, 2018). However, recent evidence suggests that humpback whales in the Southern Hemisphere also consume a variety of prey species, similar to their Northern Hemisphere counterparts (Bury et al., 2024). Baleen whales feed mainly in colder waters but may feed opportunistically elsewhere (Christensen et al., 1992). It is reported that most baleen whales, except for gray whales, typically feed within 100 meters of the surface (Swartz, 2018), although this pattern may reflect a lack of comprehensive data on deeper or alternative feeding behaviours across different habitats. Gray whales feed mainly on bottom-living organisms, almost exclusively on benthic crustaceans found in shallow waters (Bannister, 2018; Christensen et al., 1992).

Across species, the life history of baleen whales is quite similar. The gestation period is about ten to eleven months (Christensen et al., 1992). Calves depend on their mothers' milk for approximately 7 months and grow at an accelerated rate (Bannister, 2018). Generally, males reach sexual maturity during their fifth to tenth years of life, while females reach maturity at their fourth to fifteenth years, and reproduce successfully every two to three years (Bannister, 2018). However, some species, such as humpback and minke whales, have a higher reproductive rate as they have been observed reproducing annually (Pallin et al., 2018; Perrin et al., 2018). The similarities and varying aspects in the life history of baleen whales across species provide a foundational understanding for applying genomic methods to explore the intricacies of their evolutionary processes and adaptations, offering valuable insights into the genetic mechanisms that underlie their shared and contrasting biological traits.

Table 1.1. Summary of dive depth (Panigada et al., 1999), body length, diet (Bannister, 2018; Rosel et al., 2021) and distribution of the Balaenopteridae and Eschrichtiidae families. Maps of distribution for all species, except *B. ricei*, taken from Jefferson, Webber, and Pitman (2015). Map distribution for *B. ricei* based on Rosel, Wilcox, Yamada, & Mullin (2021).

	Balaenoptera acutorostrata **Common minke whale**	***Balaenoptera bonaerensis*** **Antarctic minke whale**	***Balaenoptera borealis*** **Sei whale**	***Balaenoptera physalus*** **Fin whale**	***Balaenoptera musculus*** **Blue whale**
Dive depth	Average 19m Max 106m	Average 18 (+/-0.5)m Max 105m	Rarely >300m	>200m	100m no more than 500m
Body length	Males 7.8 to 8.2m Females 8.5 to 8.8 m Dwarf Male 6.5m Female 7m	Males 8.5m Females 9m	Northern Hemisphere Males 17.1m Females 18.6m Southern Hemisphere Males 18.6m Females 19.5m	Northern Hemisphere Males 21m Females 22.5m Southern Hemisphere Males 25m Females 26m	Largest recorded Northern Hemisphere Female 28.1m Southern Hemisphere 31.7-32.6m
Diet	North Atlantic: Schooling fish; euphausiids North Pacific: Euphausiids; copepods; schooling fish Dwarf: Euphausiids, schooling fish	*Euphausia superba, E. crystallorophias*	Northern Hem: Schooling fish Southern Hem: Copepods incl. *Calanus* ; *Euphausia superba*	Northern Hem: Schooling fish; squid; euphausiids; copepods Southern Hem: *Euphausia superba* (Antarctic); other euphausiids (ex Antarctic)	Northern Hem: Euphausiids; calanoid copepods; occasional amphipods, squid Southern Hem: -Antarctic: *Euphausia superba* (Antarctic); other euphausiids (ex Antarctic) -Pygmy: Euphausiids, incl. *E. vallentini, E. recurve, Nyctiphanes austra*
Distribution	 Wide ranging from polar to tropical waters in both hemispheres **Dwarf** - lower latitudes in Southern Hemisphere	Southern Hemisphere	 Cosmopolitan - most oceanic together with the Fin whale	 Cosmopolitan - most oceanic together with the Sei whale	 Cosmopolitan The **Antarctic** - *B. m. intermedia*; The **North Atlantic** and **North Pacific** blue whale - *B. m. musculus*; And the Southern Hemisphere, mainly **Indian Ocean** - *B. m. brevicauda* & *B. m. indica*. The last two being more restricted to temperate waters.

	Balaenoptera edeni **Bryde's whale**	*Balaenoptera omurai* **Omura's whale**	*Megaptera novaeangliae* **Humpback whale**	*Eschrichtius robustus* **Gray whale**	*Balaenoptera ricei* **Rice's whale**
Dive depth	Average 15.24m Max 305m	Unknown maybe similar to Bryde's whale	~200m	155m	Average 15m Max 271m
Body length	Average of 13m Female usually 0.5-0.6m bigger than Males Reach 15.5m Southern Hemisphere are larger than Northern Hemisphere	Average 10-12m Males 9.6 to 10.0 m Females 10.1 to 11.5 m	Average 14-15m Females are typically 1-1.5m longer than Males Reach 16-17m	Average 15.3m	Male 7 to 10m. Largest known 11.26m Female 12.65m
Diet	Northern Hem: Pelagic crustaceans including euphausiids Southern Hem: Schooling fish; euphausiids	Both crustacean and piscine remains were found in the stomach of a 7 m female from Japan. Specimens from Solomon fed only on *Euphausia diomedeae*	Northern Hem: Schooling fish; euphausiids Southern Hem: *Euphausia superba* (Antarctic); euphausiids, post-larval *Munida gregaria*, occasional fish (ex-Antarctic)	Gammarid amphipods; occasional polychaetes, small fish	*Not observed*
Distribution	 ***B.brydei*** more oceanic & ***B. edeni*** more coastal. Most localised rorqual restricted to tropical/warm temperate waters	 Current knowledge of the distribution of Omura's whale is rapidly expanding with new discoveries, but it still remains incomplete	 Coastal with long migrations between temperate breeding grounds to cold water feeding grounds.	 Coastal with long migrations (Mexico to Alaska)	 Exclusive from the Gulf of Mexico

1.3.2 Genomics in Balaenopterids

By using genomic data, insights into the evolution of genes and traits (Lopes-Marques et al., 2019; Seim et al., 2014; Themudo et al., 2020; Tollis et al., 2019), historical demography (Brüniche-Olsen et al., 2018; Foote et al., 2019; Morin, Archer, Avila, Balacco, Bukhman, Chow, Fedrigo, Formenti, Fronczek, Fungtammasan, Gulland, Haase, Heide-Jorgensen, et al., 2020; Westbury, Petersen, Garde, et al., 2019; Zhang et al., 2020) and evolutionary relationships (Árnason et al., 2018; Sun et al., 2019; Zhou et al., 2018) of marine mammals species can be tackled.

Balaenopterids offer an excellent opportunity to investigate the genomic challenges that have driven their evolutionary trajectories. The differentiation of species within baleen whales involves a complex interplay of genetic, ecological, and environmental factors (Bannister, 2018), making them a suitable subject for genomic exploration. In this context, exploring the unique genomic features that distinguish different baleen whale species has presented as a valuable tool to understand the mechanisms that have shaped their evolutionary history (Árnason, 1993; Bisconti & Carnevale, 2022; Randall et al., 2022). This focused examination promises valuable insights into the adaptive strategies, historical demography, and evolutionary relationships within this group of marine mammals and is essential for conservation management.

Phylogenomics, which integrates genome-wide data into phylogenetic analyses, has refined the understanding of cetacean evolution by revealing intricate relationships between species and populations (McGowen, Tsagkogeorga, Álvarez-Carretero, et al., 2020). Genome-wide studies have provided evidence of historical hybridisation and introgression, highlighting the role of gene flow in shaping diversity (Árnason et al., 2018; Westbury et al., 2023). However, factors such as incomplete lineage sorting and interspecies hybridisation can sometimes mask true evolutionary relationships, requiring advanced analytical approaches to resolve these complexities (Westbury, Petersen, & Lorenzen, 2019).

1.3.2.1 Species differentiation

The current era of technological advancements has revolutionised the field of molecular biology (Butlin, 2010; Sousa & Hey, 2013; Wolf & Ellegren, 2017). Previously overlooked species and subspecies, undetectable through morphology alone, are now unveiled through the analysis of genetic information (Arias et al., 2016; Seehausen et al., 2014). New species identification has also occurred in Balaenopterids. For instance, the northern and southern fin whale populations do not appear to encounter one another due to their seasonal migrations, which follow alternate schedules in each hemisphere (Edwards et al., 2015). Three subspecies are currently recognised: *Balaenoptera physalus physalus* in the Northern Hemisphere; *B. p. quoyi* in the Southern Hemisphere; and *B. p. patachonica*, the pygmy fin whale, in the Southern Hemisphere and tropics (Archer et al., 2019). However, Archer and collaborators (2019) concluded that the North Pacific fin whale should be considered a different subspecies from the North Atlantic fin whale due to low gene flow and their phylogenetically distinct mitogenomes.

Advancements in molecular biology have significantly influenced the refinement of taxonomic classifications within balaenopterids, highlighting the transformative impact of genomic methods on our comprehension of marine biodiversity. Beyond reconstructing evolutionary histories, genomic data are also essential for defining conservation management units (MUs). These MUs are identified based on genetic, demographic, ecological, and cultural differences, ensuring that distinct populations receive appropriate protection. Genetic differentiation, assessed through mitochondrial DNA and genome-wide markers, helps determine historical and contemporary gene flow (Taylor et al., 2010), while demographic independence, inferred from movement patterns and population dynamics, highlights populations requiring separate management (Sveegaard et al., 2015). Ecological specialisation, including habitat use and foraging strategies, can differentiate populations even without genetic divergence, with tools such as stable isotope and acoustic analyses aiding classification (Hoyt, 2012; Katona & Whitehead, 1988). Additionally, cultural traits, such as vocal dialects and socially learned behaviours, contribute to population structure and should also be considered in conservation planning (Whitehead & Rendell, 2014).

A deeper understanding of these species' genomic adaptations can help predict how each population may respond to environmental changes. Genetic analyses provide the exploration of the evolutionary processes within marine environments, offering valuable perspectives on their resilience and adaptive strategies. Integrating these factors with ecological and cultural traits provides a more comprehensive framework for defining conservation units, ensuring that management strategies reflect both genetic resilience and ecological adaptation in balaenopterids and other cetaceans.

1.3.2.2 Aquatic genomic adaptations

The family of baleen whales, including balaenopterids, has undergone significant adaptive evolution to thrive in their aquatic habitat (McGowen, Tsagkogeorga, Williamson, Morin, & Rossiter, 2020; Steeman et al., 2009; Sun et al., 2013). Positive selected genes associated with immune system function, lipid metabolism, thermoregulation, metabolism, sensory perception, and growth/size have been identified in baleen whales (Keane et al., 2015; Sun et al., 2019; Tollis et al., 2019; Tsagkogeorga et al., 2015; Yim et al., 2014). For example, blood lactate concentration in whales increases after prolonged diving, and hypoxia is known to control lactate concentration by activating hypoxia-inducible factors (Firth et al., 1995). Several whale-specific genes have been strongly associated with stress resistance (Yim et al., 2014), which can be associated with their resistance to hypoxia and diving capabilities. Each species of balaenopterid has different diving capabilities, some whales like minke, fin, humpback and gray whales have been estimated to dive between 100-200 meters, while others like the Bryde’s, rice’s and sei whales can reach depths exceeding 300 meters (Bannister, 2018). Blue whales, in particular, have been recorded diving as deep as 500 meters (Calderan et al., 2023). Also, previous studies have revealed positive selected genes (PSGs) associated with cancer resistance pathways and tumour suppression in baleen whales, contributing to their ability to evolve larger body sizes without an increased risk of cancer (Tejada-Martinez et al., 2021; Tollis et al., 2019; Yim et al., 2014). Baleen whales have also developed adaptations for deep diving, including enhanced oxygen storage capacity, cellular stress responses, and regulation of homeostasis (Tian et al., 2016). These are some of the genomic adaptations which contribute to their unique adaptations for filter feeding, diving capabilities, and sensory environments.

These adaptations are crucial for their survival in the face of environmental challenges, including prolonged diving and exposure to hypoxic conditions. Understanding these genomic adaptations is crucial for the conservation and management of baleen whale populations, particularly with ongoing anthropogenic threats across the oceans. Continued research on the evolution of gene expression and functional divergence can provide further insights into the mechanisms underlying rapid phenotypic diversification in balaenopterids.

1.4 Focus species: Blue Whales (*Balaenoptera musculus*)

1.4.1 Taxonomy – Classification and Description

Blue whales *Balaenoptera musculus* are the largest of the balaenopterids and the largest extant vertebrate on earth. *B. musculus* was first described by Carl Linnaeus in 1758 in his book Systema Naturae (Linnaeus, 1758). The Latin origin of *Balaenoptera* means 'winged whale', referring to its dorsal fin, and the species name, *musculus,* can mean either muscle or a diminutive of a mouse (Davis et al., 2007).

The record of the largest blue whale in the Southern Ocean was at 32.6 meters long and weighed around 200,000 kilograms (Lockyer, 1976). The body of a blue whale is long and tapered, their head is relatively flat and broad, with a distinctive ridge running from the blowhole crest to the tip of the rostrum. An adult blue whale can shoot a spout that reaches a height of 10-12 meters. Their giant mandibles carry black one-meter-long baleen plates on each side of the upper jaw. In addition, they have between 60 to 88 ventral pleats on the lower jaw, which enable the ventral pouch to expand when feeding (Sears & Perrin, 2018). These throat pleats give the blue whale and other rorquals a unique feeding strategy. The dorsal fin of blue whales is at the very rear of the body, and it is smaller than other balaenopterids. The round-end flippers can measure up to 15% of the total body length of the animal (Tinker, 1988; Walker, 1999).

The colouring of blue whales is generally lighter than other large whales, with a characteristic mottled pigmentation of black and white colours scattered with patches of varying sizes. Pigmentation varies among individuals in two ways: one is predominantly pale with sparser dark patches, while the other one has a darker background with more pale patches. The mottled patterns are unique for each whale and are used to identify them through photos. Other features can also be used for identifying individuals, including distinct chevrons curving back from behind the blowhole, or ventral pigmentation patches (Pastene et al., 2020; Sears et al., 1990). In addition to visual identification, genetic analyses have also been used to identify individual blue whales (Barlow et al., 2018). Four blue whale subspecies are presently recognised by the Society of Marine Mammalogy's Committee on Taxonomy: The Northern blue whale (*B. m. musculus*), the Antarctic blue whale (*B. m. intermedia*), the Northern Indian Ocean blue whale (*B. m. indica)*, and the pygmy blue whale (*B. m. brevicauda*), with several populations present (Committee on Taxonomy, 2022).

Unravelling the population structure of blue whales poses a persistent challenge because genetic analyses consistently classify them as the same species, warranting a uniform conservation status. However, bioacoustics has been instrumental in identifying and differentiating populations, as distinct song types are linked to specific regional groups (Mcdonald et al., 2006). Despite this, conservation measures are often implemented at the population level, recognising that some groups may be more vulnerable than others due to various factors such as distinct migration patterns, ecological preferences, or exposure to specific threats (Geijer et al., 2016). This has led to the identification of culturally, ecologically, and demographically distinct units, which provide a more nuanced framework for conservation efforts. Addressing these complexities requires a multidisciplinary approach incorporating genetics, bioacoustics, and ecological data. Such an approach is essential for conservation management, as it considers both the genetic unity of blue whales and the potential vulnerabilities of specific populations along their migratory routes. While the Society for Marine Mammalogy recognises four subspecies, these have been further subdivided into nine units for management purposes (National Marine Fisheries Service, 2018). Additionally, the International Whaling Commission has designated six stock areas in the Southern Hemisphere, based on evidence of discrete feeding grounds. For example, Antarctic blue whales are the subspecies with the least discrepancies, with bioacoustics, mark-recapture, and genetics supporting their recognition as one biological unit (Branch et al., 2007; Sirovic et al., 2009; Thomisch et al., 2016; Warren et al., 2021).

Despite the similarities, clear differences are found within the different subspecies. These differences may be allocated to some ecological barriers, cultural barrier or some other factor that are yet not understood. Recent population genomic studies reveal that divergence can occur over relatively small geographic and temporal scales in cetaceans (Barceló et al., 2021; Pratt et al., 2022; Reeves et al., 2022). This can be attributed to either behaviour or local oceanographic features that restrict dispersal or to ecological variables rather than physical obstructions that limit gene flow and drive adaptation. To recognise the factors promoting the diversification of the subspecies, further understanding on their adaptability is needed and how this was inherited.

1.4.2 Life History and Ecology

Given the enormous size of the blue whale, their daily food intake is immense. Therefore, the distribution of blue whales seems to be largely determined by the availability of their prey. Most of their diet consists of krill (euphausiids), in areas of cold current upwellings (Sears & Perrin, 2018). While feeding, they gulp large amounts of water and filter it through their mouth's fringed baleen plates until only their prey remains (Bannister, 2018). Blue whales usually dive to at least 100 meters during daylight and rise to feed near the surface at dusk, following their prey's diel vertical migration. Diving time for this species is usually around 20 to 30 minutes. The longest recorded dive time was 36 minutes (Sears & Perrin, 2018).

Blue whales are usually solitary species and have no defined social structure other than the mother-calf relationship, and in some cases, males follow females for mating purposes (Sears & Perrin, 2018). A high productivity area may have fifty or more individuals spread out. Vocalisation occurs frequently during all seasons, with peaks in summer and winter. They make one of the loudest and most powerful sounds of any animal ranging between 8 to 25 Hz at 188 decibels (corresponding to 1uPa at 1m). The low frequency is ideal for communicating across large distances (Sears & Perrin, 2018).

Females in the northern hemisphere can reach a length of 28 meters on average, while the ones in the southern hemisphere up to 32 meters. Mating behaviour in blue whales is poorly understood. Only a few observations have been made in St. Lawrence of female-male pairings lasting up to five weeks. Breeding grounds have not been identified, however, some areas like the Costa Rica Dome seem to be frequented by mothers and calves (LeDuc et al., 2017). The mating season for blue whales typically extends from late autumn to mid-winter, with sexual maturity reached between 8 and 10 years of age. Females, having a gestation period of 10 to 12 months, can give birth every 2 to 3 years, with the newborn calf measuring as much as 7 meters

(Atkinson et al., 2020; Ichihara, 1966). Calves stay with their mothers until they are around 16 meters long, at about 6-8 months old. Lifespan is estimated to be between 80 and 90 years (Sears & Perrin, 2018).

Additionally, migratory baleen species can transport nutrients across great distances from cold to warm masses of water. According to estimates, blue whales carry around 88 tons of nitrogen to their calving grounds each year (Roman et al., 2014). Whales are therefore capable of transporting nutrients both vertically, between depths and surfaces, and horizontally, across oceans, which promotes primary production and fixation of carbon dioxide in the atmosphere (Jefferies, 2018; Roman et al., 2014). It is, therefore, crucial to understand the ecological context in which human activities occur when developing strategies to manage increasing anthropogenic pressures on these animals and other baleen whales.

1.4.3 Distribution and Abundance

Blue whale subspecies are wide-ranging and inhabit different geographic regions around the world (Table 1.1) (Fiedler et al., 1998; Paarman et al., 2021; Pérez-Jorge et al., 2020; Schoenherr, 1991). Like other baleen whales, they are capital breeders that provision their offspring using previously-stored energy (Houston et al., 2007). As a result of this characteristic, they are able to separate feeding and breeding areas and have migration patterns (Hoelzel, 1998; Kasamatsu, 1988), although some flexibility is apparent for feeding throughout their migratory range (Geijer et al., 2016).

According to photo-identification studies, all blue whales in the western North Atlantic seem to be part of the same population (Lesage, Gavrilchuk, Andrews, & Sears, 2017; Sears et al., 1990). In contrast, blue whales from the Azores, Northwest Africa, Spitsbergen, and Iceland appear to belong to different populations. There is, however, an imbalance in the studies associated with the eastern North Atlantic, since the majority of photo identification research has been conducted in the Azores and Iceland (Lesage et al., 2017). Blue whales can be found in the North Atlantic, reaching as far as Baffin Bay in the west and Spitzbergen in the east during the summer of the northern hemisphere (Sears & Calambokidis, 2002). However, the distribution of the populations during winter remains elusive and the southernmost observations from the North

Atlantic basin are around 30 degrees north (Reeves, Smith, Josephson, Clapham, & Woolmer, 2004; Sears & Larsen, 2002; Silva, Prieto, Jonsen, Baumgartner, & Santos, 2013).

In the North Pacific, acoustic analyses have identified two populations (Stafford, 2003). These two populations can be found as far north as the Gulf of Alaska during the northern hemisphere summer and reach as far south as the Costa Rica Dome during winter (Rankin et al., 2006; Schall et al., 2019).

Chilean blue whales, are currently placed as part of the eastern Pacific population (LeDuc et al., 2017). Based on their genetic information and the body length, Chilean blue whales are a separate group from the Antarctic and the pygmy blue whales (Attard et al., 2024; Leslie et al., 2020). They present high site fidelity in the Corcovado Gulf (Buchan et al., 2021). However, during the austral winter, part of the population migrates to the tropics, near the Galapagos Islands (Hucke-Gaete et al., 2018). A seasonal cycle of primary production and the water temperature near the Costa Rica Dome has been suggested as the reason for this migration (Block et al., 2011), but no empirical evidence supports this hypothesis.

The pygmy blue whales spend half of the year feeding in highly productive upwelling areas, south of Australia and in the subtropical zone of the Indian Ocean (Garcia-Rojas et al., 2018; Gill et al., 2011). In Australia, pygmy blue whales feed in the Great Southern Australian Coastal Upwelling System (GSACUS) during the summer and migrate to the West Timor and Indonesia during the winter (Attard et al., 2018; Möller et al., 2020; Thums et al., 2022). Additionally, a distinct population of pygmy blue whales resides in New Zealand waters, where they are observed year-round, primarily feeding in the South Taranaki Bight (Barlow et al., 2018). Although, the range of pygmy blue whales is known to cross the southern Indian Ocean (Branch et al., 2007), most studies done have been based on bioacoustics and photo-ID data (e.g. Cerchio et al., 2020; Ilangakoon & Sathasivam, 2012; Vos et al., 2014). For this reason, additional research is needed in these areas to understand the movements and behaviour of the populations within the Northern and Western Indian Oceans.

The Antarctic blue whales are known to be in sympatry with the Chilean and the pygmy blue whales. When they migrate during the southern hemisphere autumn and winter they overlap in the feeding grounds (Balcazar et al., 2017; Samaran et al., 2019). However, a proportion of the Antarctic population seems to prevail year-round in the Southern Ocean (Branch et al., 2007; Paarman et al., 2021; Sirovic et al., 2009). However, the lack of data on this population makes it unclear whether they migrate or present high site fidelity.

Australian pygmy blue whales have a roughly estimated abundance of 662-1,559 passing along the west coast of Australia (McCauley & Jenner, 2010). Additionally, the pygmy blue whales on the Madagascar Plateau, have an estimated abundance of 424-472 individuals (Best et al., 2003), and a recent photo identification catalogued created from Sri Lanka contains around 100 more individuals (Vernazzani et al., 2018). These studies suggest a total of at least around 2,000 individuals of pygmy blue whales. Nevertheless, in the subantarctic, the number of pygmy blue whales was estimated to have decreased from 7598 to 3996 (Zemsky & Sazhinov, 1982).

For the North Pacific, the current abundance is around 1,000 individuals in the West and 2,000 in the Central and East (Calambokidis & Barlow, 2004). In the North Atlantic, there is an estimated abundance of 400-600 blue whales in the Northwest (Ramp et al., 2006; Sears et al., 2005) and 1,500-2,500 blue whales in the Central and Northeast (Pike et al., 2009). Altogether, there seem to be at least 10,000 individuals of blue whales worldwide. The most depleted subspecies of blue whales are the Antarctic group, with an estimated abundance of 1,700 individuals (Branch et al., 2007).

Continued research is imperative to gain a comprehensive understanding of the distribution and abundance of blue whales, including the distinctions of subspecies and population dynamics. This ongoing exploration is essential for accurate population assessments and also for devising effective mitigation strategies against the current threats they are facing.

1.4.4 Threats

The preservation of endangered species, like blue whales, is driven by anthropogenic disturbances in an environment already undergoing significant changes. Despite being less numerous than other marine organisms, blue whales play an important role in marine ecosystems. Given their high mobility across ocean basins and role as secondary consumers in the marine food web, feeding primarily on zooplankton, they are excellent indicators of the overall ecosystem health and, consequently, habitat changes (Roman et al., 2014; Schumann et al., 2013; Simmonds & Isaac, 2007). Global monitoring of such sentinel species, therefore, plays a key role in assessing the health of the marine ecosystem (Reddy et al., 2001). Together, the whaling era, climate change and other anthropogenic factors are an immense threat to the survival of this species.

1.4.4.1 Whaling

During the whaling era, blue whales experienced extensive hunting, leading to severely diminished numbers, with certain subspecies and populations teetering on the brink of extinction (Thomas et al., 2016). The development of steam-powered vessels and explosive harpoons during the Industrial Revolution led to an increase in whale hunting in the 20th century. Around 400,000 individuals were killed during this period worldwide (Sears & Perrin, 2018). They are presently listed by the IUCN as an endangered species (Cooke, 2018). They were historically more abundant, especially in the Southern Ocean (Allen, 1980). Currently, only around nine of the acoustically different populations are left (Buchan et al., 2018; Frank & Ferris, 2011; McCauley & Jenner, 2010; Miller et al., 2014; Samaran et al., 2010). Since the whaling ceased at the end of the 1970s, populations have only recently started to recover.

Not many studies have focused on the estimated population size pre-whaling for the derived populations. However, the few available studies present an exponential decline from before and after the intense whaling period. For example, in the Antarctic population, an estimated abundance of 239,000 individuals was calculated, from which only around 360 individuals were left by 1973 (Branch et al., 2004). Similarly, on the coast of Chile around 6,200 individuals were estimated to exist pre-whaling, and around 4,300 were hunted in this area (Torres-Florez et al., 2014). In the North Atlantic, the number of individuals remains unknown but it is estimated to be around the low hundreds and more than 11,000 individuals are estimated to have been taken between the 1800 and 1960s (Jonsgård, 1955).

For species with long generation times, such as blue whales, whaling may have been too recent to leave a substantial impact on effective population size. The impacts may not be apparent for several generations, and detailed assessments will require additional population-scale studies. Blue whales, however, have a relatively high degree of genome-wide heterozygosity (Árnason et al., 2018). This suggests a substantial genetic diversity is still present within the species, which is crucial for adaptability and resilience to environmental changes. High heterozygosity often indicates a greater potential for the species to evolve and respond to selective pressures, contributing to its overall health and survival in dynamic marine ecosystems. Because they have been under the protection of the International Whaling Commission (IWC), there is evidence that they are in a slow process of recovery (Branch et al., 2004). Unfortunately, whaling is not the only risk that this species is facing. Major changes in their environment due to climate change and other anthropogenic factors, such as depletion of their food resources such as krill and small pelagic fish, are also currently threatening their survival.

1.4.4.2 Climate Change

Global climate change is a pressing concern for the conservation of the endangered blue whale. With temperatures predicted to increase rapidly, it is expected this will have unavoidable shifting and alterations of food webs worldwide and potentially niche separation of species (IPCC, 2019). Whales are now recognised by the IWC in the resolution 2016-23 to play a major role in mitigating climate change (IWC, 2016). Hence, it is crucial to investigate and understand the interactions between blue whales and environmental stressors.

Blue whales have few physical barriers to migration and gene flow, therefore, contact between populations is likely to occur. For example, blue whales are starting to present shifts in their distribution (Peters et al., 2022). Across the Indian, Pacific, and Atlantic Oceans, blue whales seem to avoid the oligotrophic central gyres (Branch, Stafford, et al., 2007). The abundance of blue whales is more prevalent in places that have high phytoplankton densities and dynamic oceanographic processes, such as upwelling and frontal meanderings (Busquets-Vass et al., 2017; Gill et al., 2011; Guilpin et al., 2019; Möller et al., 2020). However, climate shifts are already being observed. While blue whales have historically been sighted throughout the North Pacific Ocean

(Mizroch et al., 1984) and were once widely hunted across the Pacific Rim from Mexico to Japan, past shipboard and aerial surveys found no evidence of their presence in the Aleutian Islands (Rice & Wolman, 1982; Stewart et al., 1987). Yet, recent reports have indicated that blue whales can now be found in the western North Pacific, including the Aleutian Islands, Kamchatka, Kurils and Japan (Rankin et al., 2006). Whether this represents a true shift in distribution due to environmental changes or a reoccupation of historical ranges following population recovery remains unknown.

Furthermore, their habitat is predicted to decrease significantly due to climate change (Hazen et al., 2013). Warmer oceans could affect their main source of food, krill species, by deepening the thermocline, increasing the stratification of the water column, and shifting the distribution poleward due to changes in coastal upwelling patterns and destruction of favourable habitats (Simmonds & Isaac, 2007). Changes in prey distribution and abundance due to climate change will negatively impact marine mammals' nutrition and, therefore, their reproductive success and survival (Simmonds & Isaac, 2007). It is crucial for the fitness of species to reproduce successfully because it has far-reaching implications for the viability of a population (Scheiner, 1992). Also, whales release nutrients through their defecation, which helps sustain fish stocks and promote marine productivity (Herr et al., 2016). In the Southern Ocean, researchers found that blue whales fertilise their feeding grounds with nutrients necessary to sustain the growth of krill (Lavery et al., 2014; Savoca et al., 2021). Therefore, drastic changes in species composition at any extent of the food chain can cascade and adversely affect the recruitment and abundance of the entire ecosystem (e.g., Lavery et al., 2014; Pirotta et al., 2019; Roman et al., 2014; Tulloch et al., 2019).

1.4.4.3 Other Anthropogenic Stressors

Apart from the loss of hundreds of thousands of individuals hunted during the whaling era and an increased pressure from a warming climate, there are a variety of other threats blue whales must contend with. This includes other anthropogenic stressors such as pollutants, underwater anthropogenic noise, ship strikes, diminishing food resources and entanglements in fishing gear.

High levels of pollutants have been linked to adverse health effects on marine mammals (Hütt et al., 2023; Moore et al., 2021). Over the long term, persistent contaminants are not only toxic, but they bioaccumulate, potentially damaging the immune and endocrine systems as well as reproduction, offspring survival rates, and population growth (Bossart, 2011; Desforges et al., 2016; Hall et al., 2018; Sharp et al., 2019). Due to reproduction, breeding, and migration, baleen whales often spend extended periods fasting, depending mostly on their lipid storage. As a result of using these lipid resources, previously secluded lipophilic pollutants will also be absorbed, increasing the chemical exposure for these species (Bengtson Nash et al., 2013; Desforges et al., 2016). The accumulation of pollutants appears to differ between sexes, with lower loads in females perhaps due to pollutants passing to calves (Muñoz-Arnanz et al., 2019). In the Northern Hemisphere, blue whales exhibit elevated concentrations of pollutants, potentially restricting both their reproduction and the recovery of populations (Fossi et al., 2014; Gauthier et al., 1997; Metcalfe et al., 2004; Trumble et al., 2013).

However, pollutants are not the only anthropogenic threat. Whales rely on sound for communication, foraging and perhaps other vital functions. Therefore anthropogenic noise has an incredibly detrimental impact on not just whales but all cetaceans (Richardson et al., 2013). Noise can result in altered behaviour, disorientation, hearing thresholds changing temporarily or permanently, obliviousness to important sounds, and even death (Clark et al., 2009; Merchant et al., 2014; Nowacek et al., 2007; Rolland et al., 2012). Chilean blue whales seem to change communication behaviour and reduce their communication range due to shipping noise (Colpaert et al., 2016). Also, it has been noted that military sonar and other naval activities have caused significant behavioural changes in some species, possibly leading to mass strandings and deaths (Nowacek et al., 2007; Wade et al., 2010; Wang et al., 2021). In blue whales, studies so far have shown that sonar has led to changes in their foraging behaviour, likely affecting their individual fitness (Goldbogen et al., 2013).

Blue whale injuries and deaths from ship collisions are also on the rise, and they can hinder a species' recovery. In the Gulf of St. Lawrence, 25% of the blue whales carry scars from collisions with ships (Redfern et al., 2013). Northeast Pacific blue whales have been noted to suffer similar scarring, which can lead to a negative impact on the populations (Laggner, 2009). Likewise, expanding commercial fisheries can lead to the entanglement of blue whales. Blue whales in the Northwest Atlantic already carry marks from contact with fishing gear (Guilpin et al., 2020). While few lethal entanglements have been reported, larger whales are often capable of pulling fishing gear with them. In turn, the animal will be unable to feed, resulting in a protracted death from starvation (Clapham et al., 1999) as observed for humpback whales. As a result of a lack of data, it is unclear to what extent this problem threatens populations.

Overall, several factors continue to threaten the recovery of blue whales, including pollution, ship noise, seismic surveys for offshore oil exploration, military sonar, ship collisions in busy shipping lanes, and entanglement in fishing gear. These human activities have not only diminished blue whale populations but also jeopardised the survival of this already endangered species. As a result, it is crucial to closely monitor and manage the regions where blue whales are found to mitigate these threats and support their recovery.

1.4.5 Previous Genetics/Genomics studies

Genetic and genomic research on blue whales (*Balaenoptera musculus*) has significantly advanced understanding about their population structure, evolutionary history, and conservation status. Early studies focused on mitochondrial DNA (mtDNA) and microsatellite markers to assess genetic diversity and population differentiation, while more recent genomic approaches have used whole-genome sequencing to unravel deeper evolutionary patterns and adaptative responses in blue whale populations.

Initial genetic work on blue whales relied heavily on mtDNA, a maternally inherited marker, to investigate population structure and assist with conservation management programs. These studies revealed low levels of genetic differentiation between blue whale populations across the globe, indicating limited genetic divergence (Attard et al., 2010; LeDuc et al., 2007, 2017; Sremba et al., 2012; Torres-Florez et al., 2014), and high gene flow. After mtDNA, the use of polymorphic microsatellite loci was developed and characterised from blue whale genomic DNA, leading to greater information about their genetic diversity, which was applied to aid disclose their population boundaries and to further inform conservation management (Torres-Florez et al., 2012). For example, Barlow et al. (2018) identified a genetically distinct pygmy blue whale population in New Zealand waters using a multidisciplinary approach, including genetic samples. Their findings highlighted a high degree of isolation in this population, reinforcing the importance of genetic studies in uncovering cryptic populations and informing conservation strategies. However, mtDNA and microsatellite markers alone provided limited resolution worldwide in detecting more subtle patterns of population structure, such as differences between more cryptic populations as the Northern and Southern Indian Ocean, and between Pacific and Atlantic populations.

Other studies enhanced resolution by analysing nuclear genes and mtDNA. These analyses confirmed blue whales' genetic distinction from fin whales, aiding in identifying potential hybrids in commercial markets (Palumbi & Cipriano, 1998). Notably, Chilean blue whales demonstrated high mitochondrial and nuclear diversity despite their low population size, likely reflecting resilience from long generation times and recent recovery post-whaling (Torres-Florez et al., 2014). Similarly, Sremba et al. (2023) investigated the impact of 20th-century commercial whaling on genetic diversity in Southern Hemisphere great whales, including blue whales, using mtDNA. Their findings revealed that, although surviving populations retain relatively high genetic diversity, there is evidence of a probable loss of mtDNA lineages compared to pre-whaling levels. In contrast, Australian pygmy blue whales exhibited lower genetic diversity, potentially due to a founder event associated with past climatic glaciations (Attard et al., 2015). This underscores the lasting effects of exploitation on blue whale populations and the impact of past climatic changes on the species and highlights the significance of historical genetic data for understanding recovery dynamics.

The advent of next-generation sequencing has greatly expanded blue whale genetic research. Whole-genome sequencing revealed that blue whales, among all rorquals, maintain relatively high levels of genome-wide heterozygosity, based on 100,000 single nucleotide polymorphisms (SNPs) (Árnason et al., 2018). The study suggested that speciation among baleen whales likely occurred with ongoing gene flow, as different evolutionary histories are equally probable across their genomes. Notably, hybridisation events—particularly between blue and fin whales—have left to significant genomic signals of ancestral gene flow (Westbury, Petersen, & Lorenzen, 2019), underscoring the importance of hybridisation in whale evolution.

Genomic studies have also deepened insights into blue whale conservation. In 2022, a high-quality blue whale genome was assembled, uncovering gene duplications, amplifications, and links between the IGF1 gene and body size, as well as population heterozygosity and historical demography (Bukhman et al., 2022, 2024). This genome now serves as a reference for ongoing genomic studies. For instance, specific gene studies have revealed high immunogenetic variability, identifying 22 distinct DQB alleles and evidence of positive selection at the MHC Class II DQB locus (Moreno-Santillán et al., 2016). Additionally, positive selection in the ASPM gene suggested a role in brain size evolution, although specific blue whale analyses remain limited (Xu et al., 2017). Other studies have also explored genomic diversity. Recent research using the largest DNA dataset for blue whales to date—16,661 SNPs and mtDNA from 276 individuals—found significant genetic divergence among populations in the eastern Pacific, Indo-western Pacific, and Antarctic regions (Attard et al., 2024). Further differentiation was found within these regions, particularly between populations in the eastern North and South Pacific, and across the Indian Ocean. However, no genetic divergence was detected within Antarctic populations. These findings confirm the distinct subspecies status of Antarctic and Indo-western Pacific blue whales but challenge the current subspecies classification of eastern Pacific blue whales. Additionally, despite opposite breeding seasons across the equator, connectivity remains, highlighting the need for a refined understanding of population structure to conserve blue whale diversity (Attard et al., 2024). Genomic studies have also identified potential subpopulations, such as those in the North Atlantic, showing low population structure but high genetic diversity and gene flow from fin whales (Jossey et al., 2024). These findings underscore the complexity of blue whale genomes, its importance in population dynamics and the need for the refinement of conservation strategies.

1.5 Aims and Research Objectives

This thesis aims to explore the molecular signatures of selection associated with the diversification of the Balaenopteridae family, with a focus on the blue whale. By investigating the adaptive divergence of the blue whale subspecies and their responses to various environmental and anthropogenic threats, this work examines how historical climate changes, oceanographic shifts, and human activities have influenced demographic trends in these animals. The general objective is to provide a comparative analysis of the species' past and present adaptive capacities. The specific objectives of this thesis, corresponding to the three data chapters, are outlined below.

Objective 1 (Chapter 2): Baleen whales serve as a model to explore the genomic drivers of cetacean diversification, highlighting how evolutionary processes shaped their genomes and phenotypes during their adaptive radiation. To further this understanding, this chapter investigates the genes and pathways undergoing accelerated evolution that have driven diversification within the balaenopterid family using a comparative genomic framework. (This chapter has been published in *Gene,* doi: 10.1016/j.gene.2024.148822).

Objective 2 (Chapter 3): Historical climatic events and oceanographic conditions have significantly influenced the evolutionary trajectories of blue whale subspecies. This chapter aims to investigate how these factors influenced the timing of diversification and demographic shifts of blue whale subspecies and assess the potential genetic impacts of human exploitation (e.g., bottlenecks caused by whaling) using ddRAD-seq data. (This chapter is currently in its second round of review with the *Journal of Heredity*).

Objective 3 (Chapter 4): Understanding the genomic health of blue whale lineages is crucial for evaluating their resilience and recovery potential after the recent human-induced population declines. Using whole-genome data, this chapter aims to assess genomic diversity and population structure within and among lineages, identifying genomic features that may contribute to their recovery post-whaling. (This chapter is intended for submission to *Current Biology*).

1.6 Thesis Structure

The thesis comprises five chapters, including the General Introduction (1), three data chapters (2-4), which were written as stand-alone manuscripts suitable for publication, and the General Discussion (5). Each chapter is structured to offer the essential background information necessary for comprehending and interpreting the presented findings. As a result, some overlap between chapters is inevitable. Every chapter commences with a title page, followed by the main text along with relevant tables and figures. To facilitate readability, all references from the chapters are consolidated into a unified reference list at the conclusion of the thesis.

Chapter 2

INTO THE BLUE: Evolutionary Adaptations Associated with the Diversification of Baleen Whales

Geographe Marine Research

2.1 Contributions

Gabrielle Genty – conception of study design, bioinformatics, data analysis and interpretation, writing of manuscript.

Luciana Möller – primary supervisor – conception of study design and interpretation and drafting and revision of manuscript.

Luciano Beheregaray – associate supervisor – guidance in study design and interpretation, drafting and revision of manuscript.

Jonathan Sandoval-Castillo – associate supervisor – guidance for bioinformatics, study design, drafting and revision of manuscript.

2.2 Abstract

Marine ecosystems are ideal for studying evolutionary adaptations involved in lineage diversification due to few physical barriers and reduced opportunities for strict allopatry compared to terrestrial ecosystems. Cetaceans (whales, dolphins, and porpoises) are a diverse group of mammals that successfully adapted to various habitats within the aquatic environment around 50 million years ago. While the overall adaptive transition from terrestrial to fully aquatic species is relatively well understood, the radiation of modern whales is still unclear. Here high-quality genomes derived from previously published data were used to identify genomic regions that potentially underpinned the diversification of baleen whales (Balaenopteridae). A robust molecular phylogeny was reconstructed based on 10,159 single copy and complete genes for eight mysticetes, seven odontocetes and two cetacean outgroups. Analysis of positive selection across 3,150 genes revealed that balaenopterids have undergone numerous idiosyncratic and convergent genomic variations that may explain their diversification. Genes associated with aging, survival and homeostasis were enriched in all species. Additionally, positive selection on genes involved in the immune system were disclosed for the two largest species, blue and fin whales. Such genes can potentially be ascribed to their morphological evolution, allowing them to attain greater length and increased cell number. Further evidence is presented about gene regions that might have contributed to the extensive anatomical changes shown by cetaceans, including adaptation to distinct environments and diets. This study contributes to our understanding of the genomic basis of diversification in baleen whales and the molecular changes linked to their adaptive radiation, thereby enhancing our understanding of cetacean evolution.

2.3 Introduction

One of the greatest challenges in evolutionary biology is explaining the lineage diversification process. It is well known that a combination of intrinsic genetic and extrinsic ecological factors promotes species diversification (Grant & Grant, 2020). However, the relative contribution of these factors remains unclear, particularly when rapid successions of speciation events occur within a lineage. This is particularly problematic in an adaptive radiation, whereby several species rapidly evolve from a common ancestor to occupy different ecological niches (Givnish & Sytsma, 1997; Schluter, 2000). Although several adaptive radiations have been investigated to understand factors promoting rapid diversification (e.g. Beltrán et al., 2002; Freeland & Boag, 1999; Glor, 2010; Smith, Konings, & Kornfield, 2003), the rates and drivers of speciation events vary considerably among lineages, limiting the understanding of the process of diversification (McGee et al., 2020). The vast majority of adaptive radiation studies have focused on terrestrial or freshwater organisms, with a few notable exceptions from the marine real (Litsios & Salamin, 2014; Richards & Martin, 2017; Sandoval-Castillo & Beheregaray, 2020). Under the allopatric speciation model, the high species diversity found in the world's oceans has been regarded as a paradox (Bierne et al., 2003) due to the expected high level of connectivity between marine populations. However, the adaptive radiation process provides an alternative explanation to this paradox, emphasising the role of niche specialisation, trophic divergence, and/or ecological adaptation in driving the observed diversity.

Among marine taxa, cetaceans are of great interest for investigating the processes of diversification. Having transitioned from a terrestrial back to an aquatic habitat around 50 million years (Myr) ago, cetaceans underwent radical morphological and molecular changes to adapt to the aquatic environment (McGowen, Tsagkogeorga, Álvarez-Carretero, et al., 2020; Thewissen, Cooper, George, & Bajpai, 2009). They have shown remarkable evolutionary pathways resulting in complex morphological, ecological, and behavioural specialisations (Barrett & Schluter, 2008). The diversity of extant cetaceans has been attributed to both allopatric divergence (Steeman et al., 2009) and adaptive radiation (Marx & Uhen, 2010; McGowen, 2011), with evidence of geographic dispersal and ecological divergence (Holt et al., 2020). Some clades, however, exhibit a strong geographical signal, suggesting the possibility of allopatric speciation (Holt et al., 2020). Thus, investigations at a species level that can be extrapolated to higher taxonomic levels might offer valuable insights into the evolutionary pathways among related taxa.

Cetaceans, which encompass whales, dolphins and porpoises, diversified while facing many challenges within their new environment (Nikaido et al., 1999; Steeman et al., 2009; Sun et al., 2019). This diversification resulted in a minimum of 89 currently recognised extant species, divided into two main suborders: 74 belonging to Odontoceti (toothed whales, dolphins and porpoises) and 15 to Mysticeti (baleen whales) (See Figure 3 of McGowen, Tsagkogeorga, Álvarez-Carretero, et al., 2020; Perrin, 2023). Mysticeti, or baleen whales, possess baleen plates for filter-feeding (Goldbogen et al., 2017), while Odontoceti, or toothed whales, have teeth and are known for their echolocation abilities (Ridgeway & Harrison, 1981). The present study focuses on balaenopterids (the Balaenopteridae family within the Mysticeti), which are represented by nine currently recognised species (Committee on Taxonomy, 2022). These cetaceans are distinguished by their baleen plates and by their ability to communicate with sound signals, engage in social foraging (Tershy, 1992), and undertake migrations spanning vast parts of the globe (Edds-Walton, 1997; Janik, 2014). They are also characterised by a symmetrical skull, paired blowholes, and a lack of ribs that attach to the sternum (Bannister, 2018). Despite similar body shapes, balaenopterids exhibit wide interspecific variation in size. They range from the small common minke whale (*Balaenoptera acutorostrata*), reaching approximately 7-8 meters in length, to the largest mammal on Earth, the blue whale (*Balaenoptera musculus*), exceeding 30 meters. Recent research suggests that a massive size increase was the last step in the evolution of present-day whales, likely driven by the improved thermal isolation of larger body sizes (Gearty et al., 2018). In addition, baleen whales present a wide range of feeding mechanisms related to a range of energetic strategies and ecological niches (Goldbogen et al., 2017).

Baleen whales are highly mobile and pelagic, so studying them is challenging. Recent advances in conservation genomics have revolutionised the study of non-model species, including cetaceans. The analysis of a species' genome is particularly useful for assessing genetic diversity, population structure, demographic changes, and genetic adaptability in species with global distributions (Carroll et al., 2020; Foote et al., 2019; Jossey et al., 2024). However, little is known about the genomic adaptations that have led to the evolution of baleen whales. The partial mixing of gene pools due to both ancient and recent gene flow between some species of Mysticeti are indicative of the evolutionary complexity of this group (Gatesy & McGowen, 2021). A multi taxa approach is therefore crucial to assess the response of closely related lineages to past natural selection and to understand factors promoting their adaptive radiation.

This study investigates molecular mechanisms of balaenopterid evolution using whole-genome data. The objectives include (1) clarify patterns of genomic diversification in balaenopterids with respect to other cetacean lineages, (2) characterise how the rate of genomic evolution and functional genomic features relate to broad phenotypic diversity, and (3) identify candidate adaptive genomic regions that contributed to the evolution of baleen whales. Baleen whales provide an ideal system to study the genomic underpinnings of diversification in cetaceans and to understand how evolution has shaped genomes and phenotypes during the adaptive radiation of an iconic group of marine mammals.

2.4 Methods

2.4.1 Data acquisition

Genomic data of 17 mammals, including eight mysticetes, seven odontocetes and two terrestrial species (*Hippopotamus amphibius* and *Bos taurus*) were downloaded from NCBI (www.ncbi.nlm.nih.gov), CNGBdb (db.cngb.org) and dnazoo (www.dnazoo.org). *Hippoppotamus amphibius*, a semiaquatic species, is the closest relative of extant cetaceans, and *Bos taurus*, with its well-assembled and annotated genome, serves as terrestrial outgroup. Recent phylogenetic studies have shown that the gray whale (*Eschichtius robustus*) is part of the monophyletic group of the Balaenopteridae family (McGowen, Tsagkogeorga, Álvarez-Carretero, et al., 2020; Themudo et al., 2020; Wolf et al., 2023) and, as such, was included in this phylogenomic analysis. Of the acquired genomes, 15 were assembled at the chromosome level (Table 2.1).

2.4.2 Bioinformatics and orthologous genes

All bioinformatics analyses were conducted using the DeepThought HPC (Flinders University, 2021). The BUSCO v5 pipeline was employed for gene prediction and for comparing the mammalian genomes with the Cetartiodactyla orthologous database OrthoDB v10 (Kuznetsov et al., 2023). In BUSCO the MetaEuk (Levy Karin et al., 2020) workflow was used with default parameters and the Cetartiodactyla orthologous to obtain the gene sequences present in each genome. Completeness was assessed based on the proportion of orthologous genes found (Figure S2.1, initial test for completeness, using 34 whole-genomes available online relevant to the study). Only single-copy genes present in more than 85% of the species were included for further analyses (Table 2.1; Figure S2.2).

From the gene sets identified in over 85% of the species, multiple nucleotide alignments were created using PRANK with default settings, resulting in 10,159 single-copy genes aligned. As suggested by Jordan & Goldman (2012), PRANK can align sequences reliably even without additional filtering and trimming. Their study suggested that filtering and trimming may sometimes improve phylogenetic signal, but it can also potentially reduce the power for detecting positive selection in certain cases. Similarly, Spielman et al. (2014) noted that no filtering method can improve positive-selection inferences. Thus, PRANK's default settings were used here to maintain robustness without incorporating additional filtering steps. A partitioned file in nexus format with each gene considered a different partition was created using the 'nexusIQ3.pl' in-house script available at https://github.com/Yuma248/MELFUnction/scripts.

To infer branch-specific evolutionary acceleration, a phylogenetic tree is essential. By incorporating odontocetes, the semiaquatic and the terrestrial lineages into the alignments, it enabled the transition from conducting pairwise comparisons between balaenopterids and other mysticetes, to inferring common ancestral states and branch-specific changes. These approaches gained strength both statistically and biologically as more genomes of mysticetes were included, facilitating the identification of genes that have undergone accelerated substitution since the most recent common ancestor (Clark et al., 2003). To construct the cetacean orthologous phylogenomic tree, a maximum likelihood approach was employed using IQ-TREE (Minh et al., 2020). The program was run using the phylip file, with the model option -m TEST, to determine the best-fit model, which performs model selection (ModelFinder) and tree inference separately for each gene, using the 10,159 aligned genes. *B. taurus* and *H. amphibious* were used as outgroups, and the tree was rooted with *B. taurus* and the results were confirmed with 1,000 bootstraps using UFBoot. The final tree was visualised using iTol (Letunic & Bork, 2021)

Table 2.1. Mammal genomes with over 87% of the genes on the Cetartiodactyla database from OrthoDB. Genomes sourced from NCBI or DNAZoo, along with their respective publication or 'Owner' and year of public availability, where available. The sources include: VGP (https://vertebrategenomesproject.org/), NWPU (Northwestern Polytechnology University) and UoA (University of Arizona).

Order	Family	Common Name	Species	Assembly ID	Coverage	No. Scaffolds	Contig N50 (kb)	Publication	Assembly	Busco Coverage
Artiodactyla	Balaenopteridae	Rice's whale	*Balaenoptera ricei*	Balaenoptera_ricei_HiC	n/a	141,314	71	DNA Zoo	Chromosome-length	C:93.0%
		Bryde's whale	*Balaenoptera edeni*	Balaenoptera_bryde	349x	66,600	103,910	Yuan et al. (2021)	Scaffold	C:92.3%
		Northern blue whale	*Balaenoptera musculus*	GCF_009873245.2	51.16x	105	6,316	Bukhman et al. (2024)	Reference/Chr-length	C:95.6%
		North Atlantic fin whale	*Balaenoptera physalus*	GCA_023338255.1	30x	1,361,899	146	Wolf et al. (2022)	Chromosome-length	C:93.9%
		North Atlantic humpback whale	*Megaptera novaeangliae*	GCA_004329385.1	102x	102	12	Tollis et al. (2019)	Chromosome-length	C:94.3%
		Gray whale	*Eschrichtius robustus*	Eschrichtius_robustus_HiC	n/a	119,935	67,1	DNA Zoo	Chromosome-length	C:94.0%
		North Pacific minke whale	*Balaenoptera acutorostrata*	GCF_000493695.1	92x	10,776	22.69	Yim et al. (2014)	Scaffold	C:95.1%
	Balaenidae	Southern right whale	*Eubalaena australis*	RWref_HiC	n/a	3,234	27,7	DNA Zoo	Reference/Chr-length	C:94.8%
	Physeteridae	Sperm whale	*Physeter macrocephalus*	GCF_002837175.2	248x	14,677	42.5	Fan et al. (2019)	Chromosome-length	C:92.2%
	Ziphidae	Gervais' beaked whale	*Mesoplodon europaeus*	Mesoplodon_europaeus_HiC	n/a	569,347	44.9	DNA Zoo	Chromosome-length	C:87.8%
	Monodontidae	Beluga	*Delphinapterus leucas*	ASM228892v2_HiC	n/a	6,972	158	DNA Zoo	Chromosome-length	C:95.7%
	Phocoenidae	Vaquita	*Phocoena sinus*	GCF_008692025.1	67.26x	64	20,218	Morin et al. (2020)	Reference/Chr-length	C:95.2%

Delphinidae	Orca	*Orcinus orca*	GCF_937001465.1	34x	448	45,583	Foote et al. (2015)	Reference/Chr-length	C:95.7%
	Commerson's dolphin	*Cephalorhynchus commersonii*	Cephalorhynchus_commersonii_HiC	n/a	143,722	92	DNA Zoo	Chromosome-length	C:94.1%
	Bottlenose dolphin	*Tursiops truncatus*	GCF_011762595.1	63.7x	362	9,739	VGP 2020	Reference/Chr-length	C:95.4%
Hippopotamidae	Hippopotamus	*Hippopotamus amphibius*	GCA_023065835.1	69x	697	49,478	NWPU 2022	Chromosome-length	C:95.2%
Bovidae	Cow	*Bos taurus*	GCF_002263795.2	80x	2,210	25,896	UoA 2018	Chromosome-length	C:95.7%

2.4.3 Testing for positively selected genes (PSGs)

Evidence for positive selection on each Balaenopterid species were analysed using the codeml package PAML v4.9 (Yang 2007). This package was adapted with an in-house script 'paml.pl' available at https://github.com/Yuma248/MELFUnction/scripts. It is important to note that the true topology within the baleen whales likely remains uncertain. Phylogenomic studies showed direct disagreements (Árnason et al., 2018; McGowen, Tsagkogeorga, Álvarez-Carretero, et al., 2020; Wolf et al., 2023), with some proposing a polytomy for certain branches (Wolf et al., 2023). In particular, high bootstrap support values can be artificially elevated with large datasets, so the assumed topology may still be incorrect. Independent analyses were conducted for each individual species within the mysticetes, the odontocetes and the outgroups. The inclusion of odontocetes and outgroups in the phylogenomic framework was crucial for several reasons. Firstly, a broader context for understanding cetacean evolution was provided, enabling branch-specific evolutionary changes within mysticetes to be assessed more accurately. Secondly, adaptations unique to mysticetes, potentially linked to their aquatic lifestyle and large body size, could be discerned by contrasting PSGs across mysticetes, odontocetes, and terrestrial species.

Additionally, further analyses were performed at key bifurcations within the phylogenomic tree of mysticetes. These bifurcations included the separation between the mysticetes and odontocetes, by using the sperm whale as outgroup, as well as the family Balaenopteridae, which encompasses all balaenopterid species and the gray whale, and the family Balaenidae, represented by the southern right whale. Within the Balaenopteridae family, the bifurcations analysed were those involving the clade containing Bryde's and Rice’s whales, the clade containing blue, Bryde's, and Rice’s whales, the clade containing fin and humpback whales, and the clade containing fin, humpback, and gray whales. Additionally, the bifurcation between the monophyletic clade of blue, Bryde’s, and Rice’s whales and the monophyletic clade of fin, humpback, and gray whales was analysed. The analyses were performed at different taxonomic levels to determine if changes in genes are linked to specialised distributions at a family or species level.

The set of 10,159 genes were analysed for signs of positive selection through the application of the branch-site model, which considers the ratio of non-synonymous to synonymous substitutions (dN/dS, represented by ω) to vary across both sites and branches (Yang 2007). The four models used under the branch-site model were M0, M2a1, M2a and M2a_rel. M0 (one ratio) assumes a constant ω ratio across sites and lineages (Goldman & Yang, 1994; Yang & Nielsen, 1998) while M2a1 (nearly neutral) assumes the presence of two classes of sites, one with a fixed ω value between branches and another with a constant ω value across sites (Yang, 2007; Yang & Nielsen, 1998). M2a tests for positive selection by postulating three classes of sites, one with an ω value of 0, one with an ω value of 1, and a third with a non-fixed ω value (Yang, 2007; Yang & Nielsen, 1998). M2a_rel is similar to M2a, with the difference that it employs $\omega > 0$, whereas M2a has $\omega > 1$ (Weadick & Chang, 2012; Yang, 2020). A significance assessment of model fit differences was conducted through a likelihood-ratio test (LRT): model M2a was compared to the neutral and nearly neutral (M0 and M2a1) models, while M2a_rel was compared against the nearly neutral model (M2a1), using an in-house script 'lrt.pl' available at https://github.com/Yuma248/MELFUnction/scripts. Specifically, comparison M2a_rel vs M2a1, have demonstrated notable efficacy in detection of positive selected genes (PSGs) using codeml (Álvarez-Carretero et al., 2023). A gene was classified as a 'candidate gene' (i.e. gene undergoing positive selection) if it was significant (α=0.5) on all three model comparisons.

The functional implications of the unique genes under positive selection were then analysed using gene identifiers from diverse sources using an in-house script 'BUSCO2GKO.pm' available at https://github.com/Yuma248/MELFUnction/. With this script, each gene had the Gene ID, Gene Ontology (GO) and Kegg Orthology (KO) terms associated to them. However, due to the extent of genes found in this study these terms are only reported and will not be further explained here.

By using the Gene ID, the genes were then further explored for functional enrichment analysis on the MGI database (Law & Shaw, 2018) through MouseMine (https://www.mousemine.org/mousemine/begin.do) (Motenko et al., 2015). Each list of positively selected genes (PSGs), identified for individual species (Table S2.2) and bifurcations within mysticetes, is evaluated independently (Table S2.4), and referencing the MGI model organism, *Mus musculus* (common mouse), to identify enriched terms. MGI provides six enrichment widgets, but the Mammalian Phenotype Ontology (MPO) enrichment was chosen because it offers specialised and curated terminology that is specifically tailored to describe phenotypic traits and biological functions relevant to mammalian biology. This enrichment method employed a hypergeometric distribution with the Bonferroni test correction used, with a maximum p-value of 0.05. This approach ensures robust identification of over-represented MPO terms associated with the PSGs, providing insights into the functional implications of gene sets across different species. Enrichment terms specific to each species of balaenopterids and each bifurcation of the balaenopterids, were visualised using RStudio (R Core Team, 2021).

A literature search was used to determine the extent of studies on mysticetes that have previously investigated positively selected genes. This was done using all databases in Web of Science on 23rd February 2023. The literature search was done using: '((TS=whale$ OR TS=baleens OR TS=mysticetes OR TS=mysticity) AND TS= (gene adaptation in whales OR aquatic adaptation) AND (TS=positive adaptation OR TS=PSG OR TS=selection))', to capture genomic studies, positively selected gene tests and only baleen whales or mysticetes from any journal or article type within the databases.

2.5 Results

2.5.1 Phylogenomic tree

A robust dataset of 10,159 single copy and complete genes, focusing on genes present in at least 85% of lineages across 17 mammalian species, including eight mysticetes, seven odontocetes and two terrestrial species, was assembled. The Odontoceti phylogenomic relationships, in the current study, remains consistent with findings from previous literature (McGowen, Tsagkogeorga, Álvarez-Carretero, et al., 2020), while the Mysticeti phylogeny shows some differences from previous findings. The phylogenomic analysis indicates that most branches among the Mysticeti have strong support with bootstrap values between 95-100% confidence. The analysis shows that the balaenopterids, including the gray whale (*Eschrichtius robustus*), formed a monophyletic group that is separate from the Balaenidae family, represented here by the southern right whale (*Eubalaena australis)*. Bryde's whales (*B. ricei* and *B. edeni*) and the blue whale (*B. musculus*) formed a well-supported clade. Similarly, but with a lower confidence value of 51%, the humpback whale (*M. novaeangliae*), fin whale (*B. physalus*), and gray whale (*E. robustus*) form a clade. This lower bootstrap value may be due to several factors, such as insufficient phylogenetic signal in the dataset for this particular grouping or evolutionary rates that vary significantly among the genes sampled. Both clades exhibited a sister relationship to the minke whale (*B. acutorostrata*) (Figure 2.1c). These findings support the classification proposed by Wolf et al. (2023) based on 20 kbp fragments from an 1.3 Gbp alignment, which indicated that Rice's whale (formerly considered a subspecies of Bryde's whale) is more closely related to blue whales, and that humpback whales, fin whales, and gray whales form a monophyletic group. However, this last grouping is not strongly supported

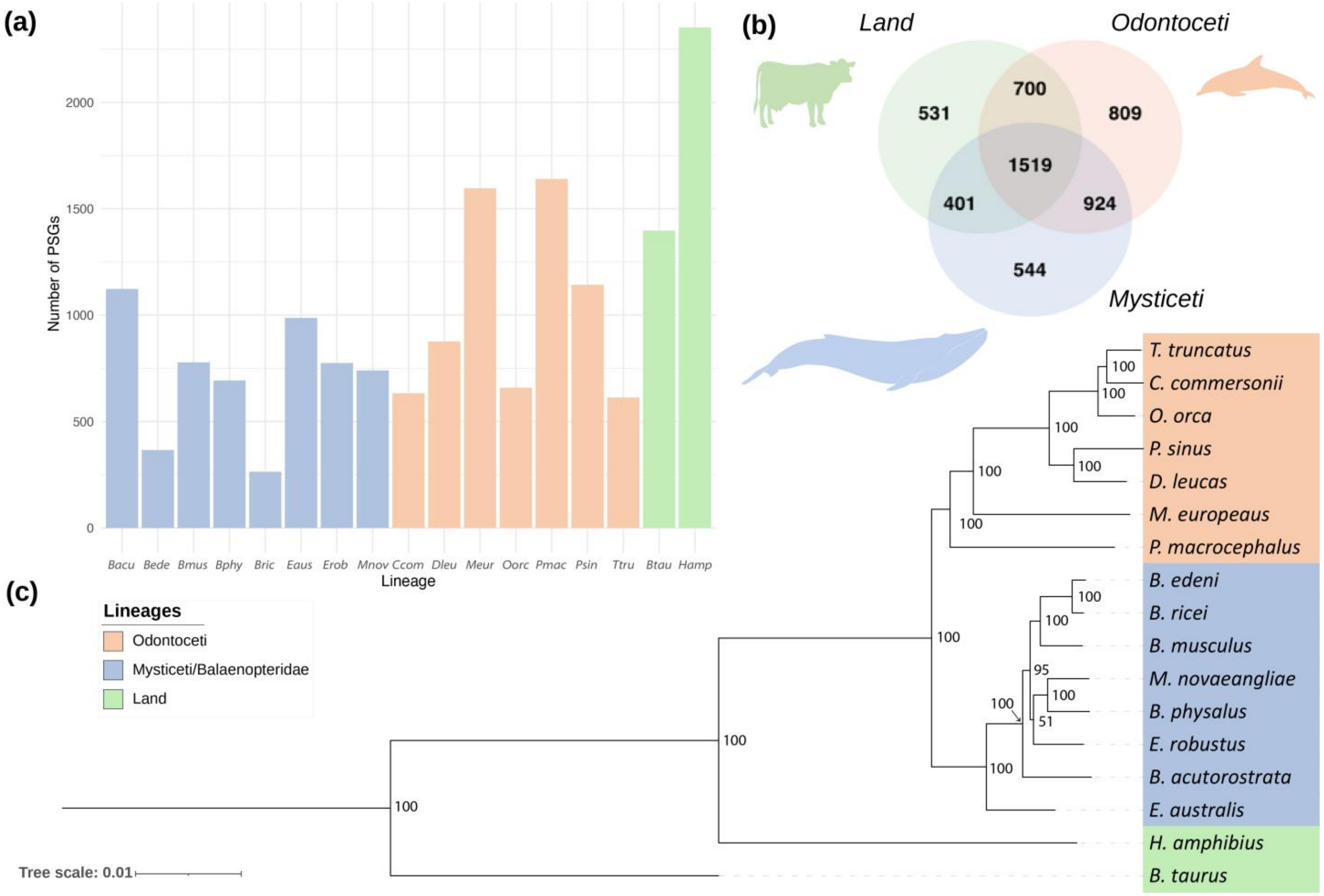


Figure 2.1. Comparative Analysis of Candidate PSGs, Lineage-Specific PSG Counts, and Phylogenomic Tree: **(a)** PSGs - Candidate genes found for each species used in this study. Each lineage is represented by a distinct colour, and the candidate PSGs associated with specific species. **(b)** Venn diagram of PSGs identified in each group of the species analysed as well as shared among groups, with a total of number of 5,428 PSGs. **(c)** A phylogenomic multi-species maximum likelihood tree is presented, inferred from 10,159 orthologous genes. Branch lengths represent the genetic divergence between species. The tree depicts the evolutionary relationships among the species under investigation and serves as a framework for interpreting the lineage-specific PSG counts. The numbers on the tree represent the bootstrap values, indicating the support level for each branch. A high-quality version the figure is available on figshare https://figshare.com/s/a4c63a537aa4563eb8c7

bootstrapping and, as suggested by Wolf et al. (2023), the placement of the gray whale remains uncertain. The authors proposed a polytomy-like situation for this lineage, where the grouping can vary with sampling of different gene subsets.

2.5.2 Evidence of Positive Selection in balaenopterids

To assess positive selection, PAML models were employed, which identified 5,428 genes across all three groups combined (Figures 2.1a & 2.1b; See Figure S2.3 for the percentage of total genes found to be under selection for each species). Among these, 809 genes were unique to Odontoceti and 531 for terrestrial representatives (see Table S2.1 for all tested genes for each group). However, given the focus on balaenopterids, the latter results will not be discussed further. Within mysticetes, 3,388 genes exhibited positive selection based on likelihood ratio tests (LRTs) (Figure 2.1a). Further analysis focused on 3,150 PSGs unique to balaenopterids, with 2,779 genes showing no duplication across species (Table S2.2).

The number of PSGs varied among balaenopterids, with notable counts found for the blue whale (*B. musculus)* with 662 genes, followed by the gray whale (*E. robustus)* with 537 genes, humpback whale (*M. novaeangliae)* with 509 genes, minke whale (*B. acutorostrata)* with 508 genes, fin whale (*B. physalus)* with 479 genes, edeni whale (*B. edeni)* with 281 genes, and Rice’s whale (*B. ricei)* with 174 genes (Figure S2.4). Overall, gene functions overlapped between species of balaenopterids, but overlapping genes were less frequent (Figure S2.5), which may suggest the effects of divergent selection and adaptation to different ecoregions (Sandoval-Castillo et al., 2020) or provide additional evidence supporting the convergent evolution of larger body size (Slater et al., 2017; Wolf et al., 2023) and diving capabilities (Nery et al., 2013; Yuan et al., 2021; Zhu et al., 2018).

Additionally, bifurcation analysis within balaenopterids identified 1,801 PSGs (Table S2.3), highlighting specific evolutionary adaptations along the different branches of the Balaenopteridae family (Figure 2.2). Distinct sets of PSGs were observed between clades containing blue, Rice’s, and edeni whales versus those containing fin, humpback, and gray whales (Table S2.4).

The literature search disclosed 86 articles, but upon manual filtering, 41 articles discussed gene assessment in cetaceans, and only 14 investigated PSGs in Mysticeti. From the 584 genes reported in these 14 studies (Table S2.5), only 120 were found in the set of 2,779 unique PSGs for the balaenopterids (Table S2.2). Therefore, this study presents 2,659 novel PSGs within the balaenopterids.

2.5.3 Over-enriched terms in the balaenopterids

To understand the functional implications of the positively selected genes, an enrichment analysis was conducted using the MGI dataset through MouseMine. The analysis identified significantly over-enriched MPOs within the set of candidate genes for balaenopterids. This approach employed a hypergeometric distribution and Bonferroni test correction, with a maximum p-value of 0.05, ensuring robust identification of enriched terms.

Based on the MPO enrichment analysis, evidence was found for 81 categories significantly over-enriched in the set of candidate genes for balaenopterids, and 36 categories for the bifurcations within balaenopterids. These mammalian phenotype (MP) categories included mortality/aging (MP:0010768), abnormal survival (MP:0010769), preweaning lethality (MP:0010770), homeostasis/metabolism phenotype (MP:0005376), abnormal homeostasis (MP:0001764), abnormal cell physiology (MP:0005621), abnormal blood homeostasis (MP:0009642), among many others (Figure 2.3; Table S2.6 and Figure S2.6). No enrichment was found for the monophyletic clade including fin, humpback, and gray whales, nor for the bifurcation separating blue, edeni, and Rice's whales, from fin, humpback and gray whales (Figure 2.2). Nonetheless, four enrichment terms (mortality/aging, abnormal survival, homeostasis/metabolism phenotype and preweaning lethality) were consistently observed across various bifurcations and were identified during the early stages of balaenopterid evolution (Figure 2.2). These findings may indicate adaptive processes across species and different evolutionary timescales of the balaenopterids.

Species-specific terms provided insights into the variations that contributed to the diversification of each species. For example, PSGs were observed in fin whales and blue whales associated with terms on the immune system and anti-cancer mechanisms. And gray whales exhibited positive selection for renal and urinary adaptations, while humpback whales showed positive selection for movement-related terms (Figure 2.3; Table S2.6 and Figure S2.6).

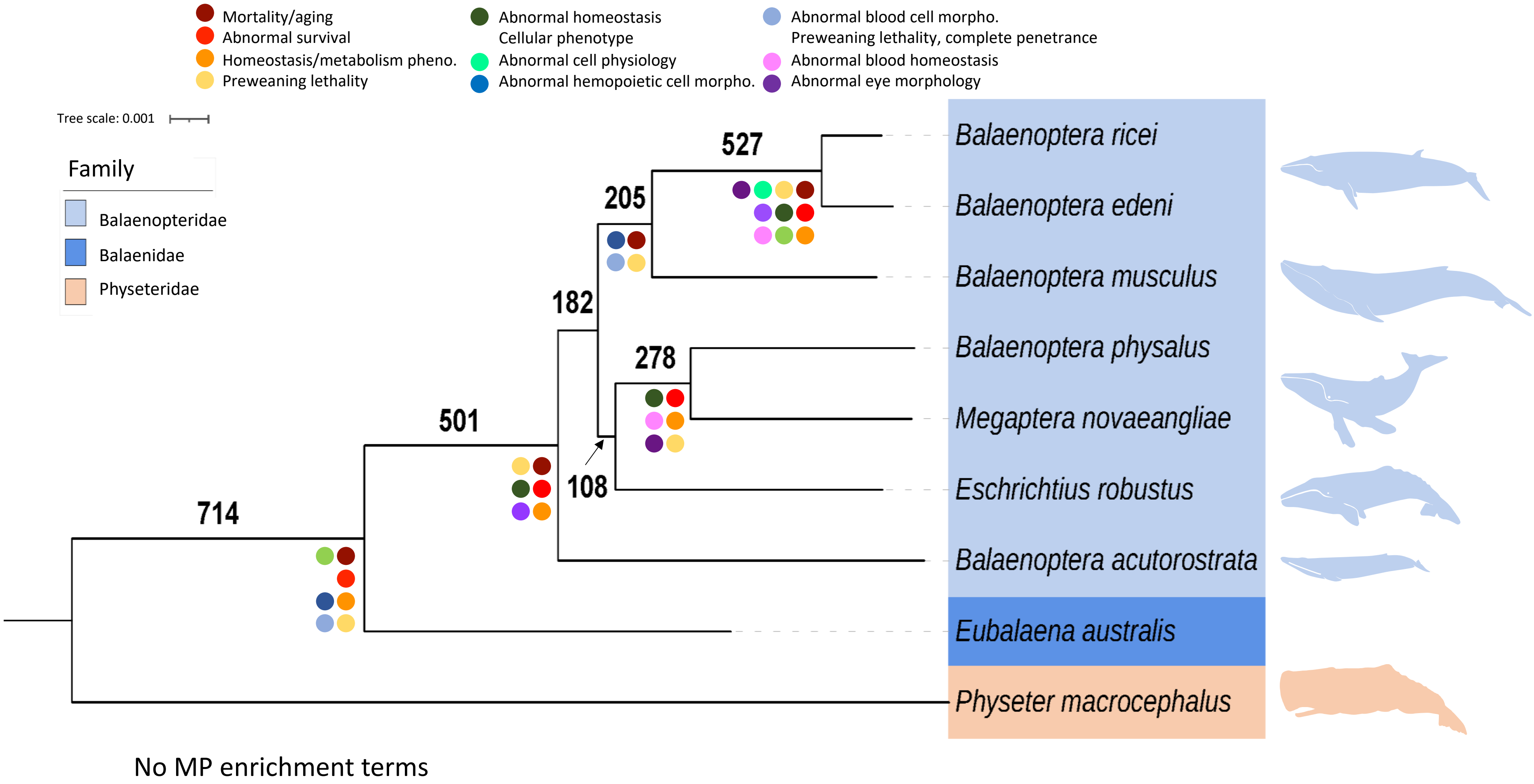


Figure 2.2. Positively selected genes during the Mysticeti evolution. Species tree relationships of eight modern cetaceans with complete genome assemblies, estimated from 10,159 orthologs. 1,810 PSGs found specifically for the balaenopterids bifurcations and 714 for the bifurcation with the sister clade balaenids. The tree was rooted using the sperm whale, *Physeter macrocephalus*. Numbers indicate the amount of positively selected genes for each bifurcation in the tree. Circles indicate the top twelve from the thirty-six total enriched MP terms detected on each bifurcation after Bonferroni correction (p-value > 0.05).

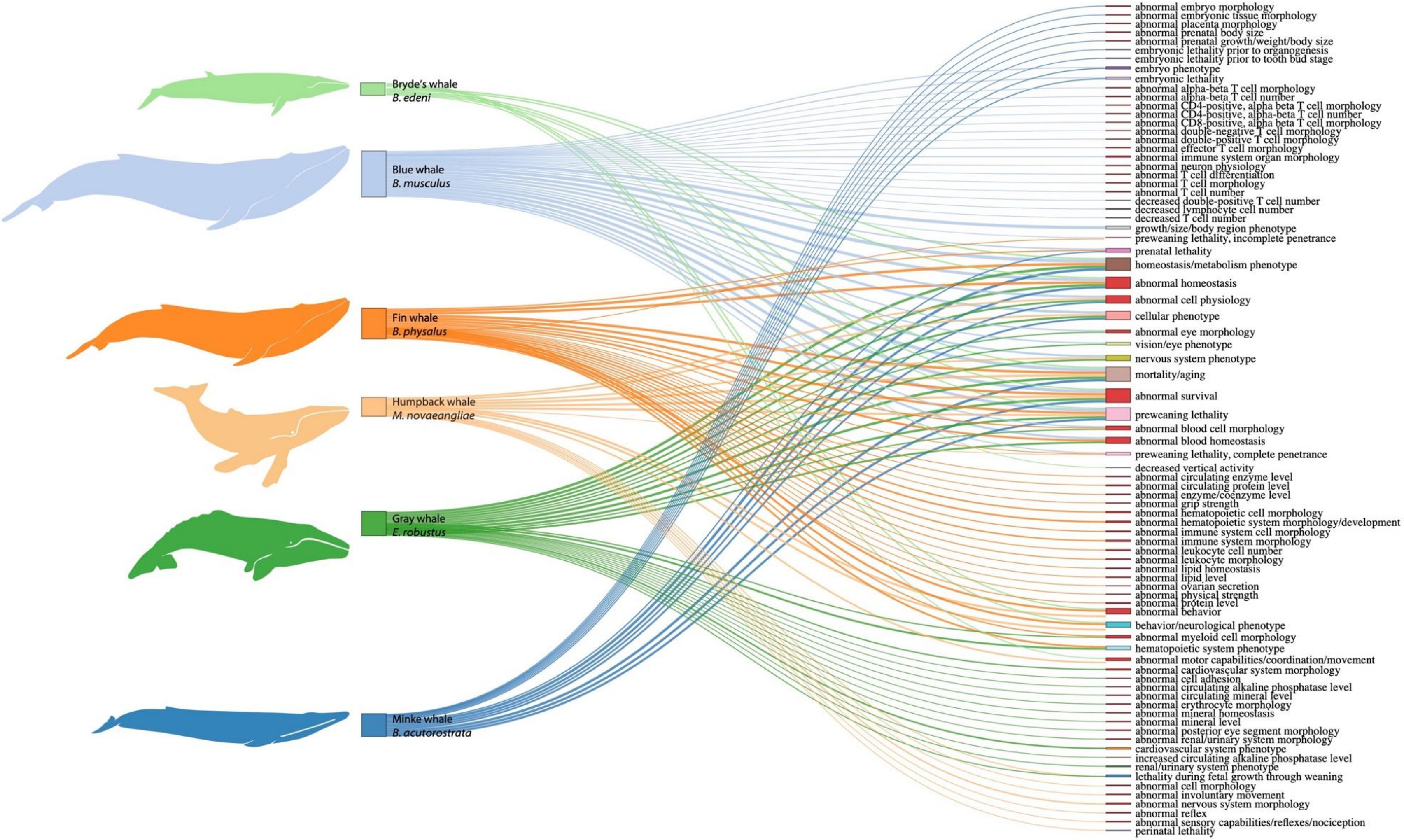


Figure 2.3. Sankey diagram illustrating the significant associations between species of balaenopterids and MP Terms, adjusted using Bonferroni correction (p-value ≤ 0.05). The diagram visually represents the flow of significant relationships, with species of balaenopterids on one side and MP Terms on the other, connected by directed flows representing the significant associations. The width of the flows corresponds to the strength of the associations based on the number of terms, highlighting the most significant connections between species and MP Terms in the dataset. A high-quality version of the figure is available on figshare https://figshare.com/s/a4c63a537aa4563eb8c7.

2.6 Discussion

The molecular mechanisms of balaenopterid evolution were explored through a comparative approach based on whole-genome data. This was achieved by characterising patterns of genomic diversification in balaenopterids, identifying genomic regions under positive selection in this group, and determining the relationship between genomic evolution and functional genomic features with broad phenotypic diversity.

The evolutionary history of cetaceans and the baleen whale suborder, Mysticeti is characterised by multiple radiation events. These include the transition to an aquatic environment by early cetaceans, the subsequent radiation within Cetacea, and the distinct radiation of baleen filtering within Mysticeti (Steeman et al., 2009; Sun et al., 2013). Recent phylogenetic analyses also suggest further radiations within specific cetacean lineages, such as the rorquals (Árnason et al., 2018; McGowen, Tsagkogeorga, Williamson, et al., 2020). These evolutionary transitions and diversifications have allowed cetaceans to occupy a variety of habitats worldwide, making them a suitable system to study the genomic mechanisms of adaptive radiations. Despite having an axiomatic ecological role, cetaceans face significant threats from anthropogenic factors, which may result in either acute or chronic impacts (Kennish et al., 1984; Kovalenko, 2019; Popper & Hawkins, 2016). As a result of current and emerging threats caused by climate change, and other anthropogenic stressors (Thomas et al., 2016), many balaenopterid populations are struggling. Understanding their evolutionary mechanisms is therefore important to improve the efficiency of management and conservation efforts. Gigantism, diving capabilities, migration, and preweaning lethality provide a focus through which key adaptive strategies enabling the evolution and persistence of balaenopterids can be uncovered. These terms highlight the genetic foundations and phenotypic evolution that enable their ability to manage environmental challenges and reproductive demands.

2.6.1 Adaptive evolution in balaenopterids

Genes that have undergone positive selection are frequently associated with adaptive phenotypic evolution (Fan et al., 2019) and positive fitness (Chase et al., 2021; Endo et al., 2018; Qiu et al., 2012). The emergence of high-quality genomes from eukaryotes spanning diverse evolutionary lineages has enabled comparative studies of adaptation at the levels of lineages, genes and molecular pathways (Themudo et al., 2020). In spite of technological advances, understanding the diversification processes of the large balaenopterid radiation is challenging (Norris & Hull, 2012).

Several studies have investigated PSGs in balaenopterids (Keane et al., 2015; Sun et al., 2019; Tollis et al., 2019; Tsagkogeorga et al., 2015; Yim et al., 2014). Some have identified PSGs involved in the immune system, lipid metabolism, and thermoregulation, that may have contributed to the adaptation of balaenopterids to their aquatic environment (Shen et al., 2012; Tejada-Martinez et al., 2021; Yim et al., 2014). Other PSGs have been related to metabolism and sensory perception (Chikina et al., 2016; Yuan et al., 2021), and likely reflect adaptations to the unique diet and sensory environment of each species. However, previous studies focused on specific genes or utilised smaller gene sets. For instance, Yim et al. (2014) employed a PAML approach similar to ours and remains the only study to date that analysed 9,848 orthologous genes (excluding singletons) for four artiodactyl genomes (minke whale, bottlenose dolphin, cow and pig). Similarly, Yuan et al. (2021) used PAML for 7,525 orthologous genes on 17 marine mammal genomes. In contrast, our study takes a more comprehensive approach, using 17 higher-quality artiodactyl genomes now accessible and analysing a larger orthologous gene set of 10,159. This significantly broadens the scope of identified PSGs and species and enhances our understanding of the potential functions in these species.

In this study, we identified several novel PSGs not previously reported in balaenopterids. For instance, genes such as ACO2, AIFM1, DENND1C and TAT are associated and were found to be enriched for terms like preweaning lethality, mortality/aging, and abnormal survival. Additionally, ABCG8 and ADORA2B are linked to abnormal homeostasis and abnormal blood homeostasis. Other examples include BZW2, associated with decreased circulating iron, cholesterol and glucose levels, which was enriched for the term abnormal homeostasis; and EPO, associated to hypoxia and pericardial edema was related to abnormal homeostasis; among many other genes identified in this study.

These novel findings confirm and expand on the genes previously identified in the literature, such as RAD52, involved in DNA repair; KIF3C, related to intracellular transport; DAGLA, involved in neurotransmitter signalling; NPC1, associated with cholesterol transport (Tsagkogeorga et al., 2015); GOLGA1, associated with Golgi apparatus structure (Yim et al., 2014); and GNPAT, linked to lipid metabolism (Wang et al., 2015). By uncovering these additional PSGs and their associated phenotypic terms, our study provides a broader understanding of the evolutionary adaptations in balaenopterids and highlights the complex interplay of genes involved in their unique adaptations. This comprehensive approach enables a more nuanced comparison and deeper insight into the evolutionary biology of these marine mammals.

2.6.2 Gigantism

Mortality/aging and abnormal survival genes were found to be enriched in all species of balaenopterids and in the branch-set analysis for all baleen whales. The strong selection over these genes could allow the increment in body size without increase of cancer incidence, an adaptation that could explain the Peto's paradox. Peto's paradox refers to the phenomenon where large-bodied, long-lived animals have not shown an increase in cancer rates proportional to their size, in contrast to what might be expected based on the risk associated with accumulating more cells over time (Peto et al., 1975).

Whales have become a subject of significant interest due to their cancer-resistant capabilities, which may be linked to their adaptations to the marine environment (Tejada-Martinez et al., 2021; Tollis et al., 2019; Yim et al., 2014). Recent studies exploring the genome of humpback whales also suggest that the evolution of body mass in cetaceans may be associated with strong selection over cancer resistance pathways (Tollis et al., 2019). As such, cetaceans may be a suitable model for investigating the genetic basis of body size, aging and cancer resistance evolution (Sun et al., 2022), as previously demonstrated for giant terrestrial species (Herrera-Álvarez et al., 2021; Quesada et al., 2019). Additionally, the identification of selection on genes associated to the terms of mortality/aging and abnormal survival may provide insights into how large-bodied whales have managed to evolve without a commensurate increase in cancer incidence, contributing to the understanding of the Peto's paradox.

The much higher rate of body mass evolution in cetaceans compared to their terrestrial relatives has been attributed to factors such as aquatic weightlessness, abundant diet, thermoregulation, predator defence, and migratory behaviour (Evans et al., 2012; Montgomery et al., 2013). Although baleen filter feeding likely evolved around 25 Myr ago (Peredo et al., 2017; Tsai & Fordyce, 2015), gigantism became ecologically advantageous during the Plio-Pleistocene epoch (around 5.3 million to 2.6 million years ago), likely due to intensified seasonal upwelling (Slater et al., 2017). For instance, the blue whale which can weight up to 180,000 kg (Jefferson et al., 2015), presented in this study 250 genes, such as ATP13A1, ATP18B1, DNAH9, DIS3L2, EHMT1 and FLVCR1, associated to the term growth/size/body region phenotype. Larger body sizes are typically associated to longer lifespans, and genes that exhibit evidence of positive selection were found to play a role in DNA repair, cell cycle regulation, resistance to ageing and cancer confirming what has been previously presented in other studies on cetaceans (Firsanov et al., 2023; Keane et al., 2015). The lack of significant enrichment in the term growth/size/body region phenotype for species other than the blue whale may imply potential constraints stemming from incomplete genome assemblies, quality of annotations, or database limitations. These factors should be considered when interpreting results and assessing the evolution of gigantism in balaenopterids. However, it could also suggest that blue whales, in achieving the largest body mass among balaenopterids, have evolved additional anti-cancer mechanisms facilitating their gigantism.

2.6.2.1 Diving capabilities and migration

Marine mammals are skilled at deep diving and spend most of their time in deep waters, except when the density of prey is higher near the ocean's surface. Diving capabilities vary among species, with minke, fin, humpback, and gray whales estimated to dive down to 200 meters, while the blue, Bryde's, Rice's, and sei whales reach depths exceeding 300 meters (Würsig et al., 2018). Their high myoglobin concentration and large muscle mass contribute to the total oxygen stored, particularly in their locomotor muscles (Arregui et al., 2021; Christiansen, Uhart, et al., 2022). However, deep diving poses a risk of cellular stress, such as hypoxia, oxidative, and osmotic stress (Fabrizius et al., 2016; Ngoh et al., 2011).

Cetaceans have adapted to the hypoxic conditions induced by deep diving, which is crucial for their survival (Tian et al., 2016). Several whale-specific genes have been strongly associated with stress resistance (Yim et al., 2014). For instance, glutathione has been demonstrated to enhance the antioxidant capacity in cetacean cells, protecting against damage caused by reactive oxygen species (ROS) under hypoxic conditions. This protective effect involves genes such as PRDX6, SOD1, and GSTP1 (Yim et al., 2014). This study identified enriched terms in balaenopterids associated with either the homeostasis/metabolism phenotype or blood homeostasis. Homeostasis phenotype refers to the set of traits, functions, and mechanisms that enable an organism to maintain stable internal conditions despite changes in the external environment (Weinstein, 2000). It involves the regulation of various physiological and metabolic processes, such as temperature, fluid balance, pH, and nutrient levels, to keep them within a narrow range suitable for optimal functioning (Weinstein, 2000).

Enriched homeostasis/metabolism terms were consistently observed in different species (blue, edeni, fin and gray whales) and persist across various bifurcations in the evolutionary lineage of balaenopterids, highlighting their adaptive strategies in challenging environments facilitated by their large body size and robust homeostatic mechanisms. Some species of balaenopterids exhibit a dynamic balance between short-term residency in cold, productive areas at low latitudes, and lipid storage (Brodie, 1975, 1977). Together with their increment in size, the migratory response to seasonal changes and cold resistance to guarantee an increment of their lipid storage may be partially attributed on how homeostasis is maintained as a response to extreme environments.

Previous studies compared deep-diving odontocetes, such as sperm whales and Blainville's beaked whales, which dive over 1,000 meters, focused on hypoxia related genes, and identified genes under selection related to myoglobin (Nery et al., 2013), in genes like for example SETX, GIF, and TMPRSS11D (Yuan et al., 2021). While baleen whales do not exhibit PSGs specifically related to hypoxia, they may have evolved alternative adaptations to compensate for reduced diving capabilities. For instance, fin and gray whales, exhibited enrichment in myeloid cell morphology and the hematopoietic system phenotype, indicating adaptations that supports the immune response under high pressure conditions during dives. For example, the genes found to be related to the term hematopoiesis (i.e. CD55, ERG and FLNB), which occurs in the bone marrow and involves stem cell differentiation into blood cell types responsible for oxygen transport, immune response, and haemostasis (Itkin et al., 2016), can further highlight the adaptations for coping with the pressures at greater depths during diving.

In the current studies, no signal of positive selection was found for previously reported genes related to cetacean diving capabilities. However, this study has a broader approach and evidence was found for PSGs enriched on the baseline of the balaenopterids branch, such as those related to abnormal blood cell morphology, abnormal apoptosis (i.e., the physiological process of cell death that occurs during an organism's growth and development) and abnormal erythrocyte morphology (i.e., the primary function involved in the transportation of oxygen and carbon dioxide to and from the body's tissues). These adaptations may be linked to the abilities of whales to dive and grow larger while protecting themselves against tumour development (Tejada-Martinez et al., 2021). Although baleen whales do not possess the extensive diving skills of odontocetes, they have developed mechanisms to cope with the pressure in the marine environment, showing additional evidence of convergent evolution between cetaceans.

2.6.2.2 Preweaning lethality

Preweaning lethality was another term consistently found within the different species of balaenopterids and prevailed in the bifurcations of the different branches. Here, baleen whales exhibited signals of positive selection over multiple genes associated with the MP term preweaning lethality. The presence of such signals suggests an evolutionary advantage associated with managing the substantial risk of mortality from fertilisation to weaning, usually occurring at 5-6 months of age for calves (Chivers, 2009). This adaptation may reflect strategies honed through natural selection, where genes associated with preweaning lethality undergo positive selection to enhance the survival prospects of offspring. By favouring traits that reduce mortality rates during the vulnerable preweaning period, balaenopterids can increase the likelihood of reproductive success and passing on advantageous genetic variations.

Baleen whale females generally outweigh males because of potential energy demands of reproduction and increased fat storage (Ralls, 1976). However, the cost of foetal growth increases rapidly during gestation, particularly in the latter stages of pregnancy, as foetal mass increases exponentially with foetal length (Christiansen, Bejder, Burnell, Ward, & Charlton, 2022; Christiansen et al., 2022). A similar phenomenon was observed in southern elephant seals, where pups experienced a remarkable average growth rate during the lactation period, with the proportion of body mass represented by fat increasing from less than 3% at birth to 12.7% at weaning (Hindell et al., 1994). These findings highlight the significance of maternal energy reserves and body condition in shaping the growth rates and overall health of offspring in different marine mammal species.

Estimating neonatal mortality in baleen whales is challenging due to limited data. Growth models revealed that baleen whale calves can nearly double their length and achieve nearly eight times their body mass during the nursing period (Agbayani et al., 2020; Fortune et al., 2021). Nevertheless, precocial development in mammals has detrimental implications on the body development of the infant (Ashmeade et al., 2007; Gaillard et al., 1997; Lezama-García et al., 2022; Magrini et al., 2023). Mothers under poor body conditions can be a significant risk for the survival of their calves. In humpback whales, the Gulf of Maine population showed a one-year birth interval rate of 6.3% (Clapham et al., 1993), while a higher rate of 14% was reported from the Hawaiian breeding range (Glockner-Ferrari & Ferrari, 1990). Clapham et al. (1993) partially attributed this to neonatal mortality before the mother's return to high-latitude feeding grounds, as any calf that dies in the West Indies or during migration north would not be recorded in the Gulf of Maine later in the year. Considering the substantial lactation cost in baleen whales (Lockyer, 1976, 1981), females with shorter interbirth intervals, notably one year, and with poor body condition (Christiansen et al., 2014), could experience higher calf mortality. It is important to consider the vulnerability of pregnant females, embryos, and calves during this period. Anthropogenic threats and disturbances to the mother and calf, such as navigation, seismic activities and other offshore developments, can have a significant impact on whale populations (Pirotta et al., 2018). Previous studies have shown that reduced survival of embryos and calves plays an important factor for the recovery of protected populations (Cramer et al., 2008; Hin et al., 2019), underscoring the necessity of considering these factors in the management and conservation of baleen whale populations, with special attention to their breeding and calving areas.

2.6.3 Species-specific adaptations

2.6.3.1 Immune system in the largest species, the blue and fin whales

Despite the common adaptations found in balaenopterids, species-specific adaptations have likely evolved independently, contributing to their success during diversification. For instance, blue and fin whales were found to exhibit unique immune system adaptations that may play a crucial role in their survival. In this study, positive selection was observed for genes associated with terms enriched with immune responses, particularly within T cells for blue whales and leukocyte cells for fin whales.

In blue whales, there is significant enrichment in genes related to the terms of morphology and number of cytotoxic T cells (CD8+) and CD4+ cells, as well as alpha-beta T cell differentiation. These T cells are vital for immune responses, including tumour recognition and fighting against cancer-infected cells and foreign antigens (Van den Eynde & van der Bruggen, 1997). CD4+ and CD8+ are responsible for identifying and fighting against cancer-infected cells and foreign antigens (Smith-Garvin et al., 2009). The presence of tumour-infiltrating lymphocytes, particularly CD8+ T cells, is a favourable prognostic marker across various solid tumours (Fridman et al., 2012). However, these cells often struggle to eliminate cancer cells due to immunosuppressive mechanisms within the tumour microenvironment (Chen & Mellman, 2013; Schreiber et al., 2011). Optimising CD4+ T cell assistance has shown promise in enhancing cancer immunotherapy outcomes in humans (Borst et al., 2018). The significant enrichment of genes related to morphology and the number of cytotoxic T cells (CD8+) and CD4+ cells, along with alpha-beta T cell differentiation in blue whales, underscores their evolutionary advantage in combating cancer as they acquire a long body size and maintaining immune resilience.

In fin whales, there is clear evidence of immune system enrichment, particularly in leukocyte cells, which suggests a robust immune system crucial for their adaptation. The emphasis on leukocyte cells suggests a specialised immune response tailored to combat environmental pathogens and maintain overall health in fin whales. These specialised immune responses, critical for tumour recognition, defence against foreign antigens, and enhancing resilience in dynamic marine ecosystems, highlight adaptations that contribute to the blue and fin whales thriving despite environmental and physiological challenges in their marine habitat.

2.6.3.2 Feeding and diving strategies of gray whales

While most of the water needed by whales is obtained through metabolising fat from their food, they may still consume seawater in certain situations (Ortiz, 2001). Gray whales use a unique benthic-suction feeding mechanism (Werth et al., 2016). Their kidneys have specialised adaptations, such as glycogen stores, vasa recta bundles, and a sporta perimedullaris musculosa, to support diving and produce concentrated urine in hyperosmotic environments (Pfeiffer, 1997; Vardy & Bryden, 1981). Despite these adaptations, their kidney efficiency is lower than expected, but they compensate with hormonal regulation (Bester, 1975; Vardy & Bryden, 1981). The analysis of gray whales revealed a high enrichment of genes associated with renal/urinary and mineral homeostasis, as well as their cardiovascular system and circulating alkaline phosphatase and minerals. These genes are thought to be critical in maintaining blood pressure and water balance, especially in environments with elevated sodium intake and exposure to sediment-laden conditions on the seafloor (Oliver & Slattery, 1985). These adaptations are probably allowing gray whales to efficiently process high-salt intake and to extract maximum nutrients from their food source, but it is also placing them at a higher risk to contaminants accumulated on the seabed (Dehn et al., 2006).

2.6.3.3 Movement and reflexes of humpback whales

Despite the common adaptations found in balaenopterids, adaptations within each species have likely occurred independently and contributed to their success during diversification. For instance, humpback whales, are renowned for their agility despite their large (12-16m) and rigid bodies (Clapham, 2018). They exhibited positive selection in genes associated with reflexes, sensory capabilities, coordination, movement, and involuntary movements. These adaptations might relate to their specialised foraging techniques and famous singing repertoire, aiding their communication and mating system (Clapham, 2000). Humpback whales exhibit unique behaviours among cetacean species, as they are the only ones known to form leks. Humpback males engage in vigorous combat for access to females (Clapham, 2000). The presence of protuberances along the leading edge of its pectoral flippers have been suggested as the reason for their agility. Fish and Battle (1995) hypothesized that these structures play a role in the species' feeding strategy. However, it could also be attributed to their significant male-male competition (Chu & Nieukirk, 1988; Tyack & Whitehead, 1982). Further studies noted that their tight turning radii during extreme manoeuvres, such as bubble netting (Baker & Herman, 1984; Tyack & Whitehead, 1982), are unique, and the protuberances may facilitate passive flow control and/or drag reduction (Fish & Lauder, 2006). Positive selection was observed in genes enriched within the humpback whale species associated with reflexes, sensory capabilities, coordination, movement, and involuntary movements. Given their unique feeding techniques and famous singing repertoire, it is possible that these whales also possess gene variations that contribute to their specialised adaptations for foraging, male competition and communication.

2.7 Conclusions

This study provides further evidence of the success of balaenopterids and they underwent adaptive evolution, enabling them to thrive in their aquatic habitat and diversify into distinct species with variations in size, morphology, mating systems, feeding strategies and diving abilities. By identifying positively selected genes and enriched mammalian phenotypic terms, this research highlights the genetic and physiological adaptations that underpin the diversification and specialisation of balaenopterids. The work advances our understanding of the genetic mechanisms behind the evolution of baleen whales, offering new insights into their evolutionary triumph.

Chapter 3

OUT OF THE BLUE: Phylogeographic History of the Largest Extant Animal, the Blue Whale (*Balaenoptera musculus*)

The Fat Whales Project

3.1 Contributions

Gabrielle Genty – conception of study design, data analysis and interpretation, writing of manuscript.

Luciana Möller – primary supervisor – conception of study design and interpretation and drafting and revision of manuscript.

Luciano Beheregaray – associate supervisor – guidance in study design and interpretation, drafting and revision of manuscript.

Jonathan Sandoval-Castillo – associate supervisor – guidance for bioinformatics, study design, drafting and revision of manuscript.

Sean James Buckley – guidance in study design, data analysis and interpretation, drafting and revision of manuscript.

Catherine R.M. Attard – data collection, preparation of samples in the laboratory, initial bioinformatics and revision of manuscript.

Chris J. Brauer – guidance in study design, data analysis and interpretation, drafting and revision of manuscript.

Aimee R. Lang – data collection and revision of manuscript.

Curt S. Jenner – data collection and revision of manuscript.

Peter C. Gill – data collection and revision of manuscript.

Bárbara Galletti Vernazzani – data collection and revision of manuscript.

Leigh G. Torres – data collection and revision of manuscript.

Christopher L. K. Burton – data collection and revision of manuscript.

Micheline-Nicole M. Jenner – data collection.

Mariano Sironi – data collection.

3.2 Abstract

Historical environmental fluctuations have influenced the distribution, connectivity, demography, and diversification of present-day biodiversity. For blue whales (*Balaenoptera musculus*), three lineages representing six populations were recently identified. This study builds on these findings by using the same extensive genomic dataset (16,661 SNPs from 275 individuals) to investigate the roles of past climatic and oceanographic events on the diversification of blue whales. Coalescent-based approaches were employed to assess how major environmental events between the late Miocene and the Last Glacial Maximum affected genetic diversification. The diversification of the three lineages was dated for the Pleistocene, with one lineage diverging around 300,000 years ago, and the other two at around 200,000 years ago. This coincided with global ice accumulation and decreased primary productivity, which may have affected connectivity and led to the observed genetic divergence. Additionally, decreases in population sizes were detected in two of the three lineages. Major historical climatic oscillations and reductions in food availability might have affected genomic variation and connectivity in blue whale populations. These findings are particularly relevant when predicting range shifts, population persistence and adaptive potential of the world's largest animal under ongoing climate change.

3.3 Introduction

Extensive climatic changes throughout the last 20 million years have played a central role in shaping contemporary patterns of biodiversity in both terrestrial and marine environments. Historical events have influenced the distribution and connectivity of organisms, resulting in species extinctions as well as biodiversity increases (Eastman & Clarke, 1998; Hewitt, 2000; Maggs et al., 2008; Obura, 2012; Petuch, 2013). Past climatic processes shaped the distribution of primary productivity, prey resources, and suitable habitats, which are known to affect the demographic history of many species, including marine organisms (e.g. Buckley et al., 2022; Cabrera et al., 2018; Ulloa et al., 2017). Clarifying associations between past environmental change and species' evolutionary history can help to predict future biological impacts, such as range shifts and genomic targets of selection related to dispersal strategies and adaptive capacity (Elith et al., 2006; Liu et al., 2015).

During the late Pleistocene, climatic changes caused several glaciation events over approximately 740,000 years, including the Last Glacial Maximum (LGM) (Augustin et al., 2004). These climatic fluctuations led to profound changes in sea surface temperatures and ocean currents worldwide (Gupta & Thomas, 1999; Hewitt, 1996). Pleistocene glacial oscillations had a substantial impact on the genetic structure, distribution, and connectivity in many groups of marine organisms, including marine mammals (e.g., Amaral et al., 2012; Harlin-Cognato et al., 2007). Evidence of this can be seen in the Antarctic Ocean due to the formation of the circum-Antarctic water circulation and the Antarctic Polar Frontal Zone (Kennett, 1977). These palaeoceanographic events led to the divergence of marine organisms, such as Antarctic krill *Euphausia superba* (Patarnello et al., 1996) and possibly baleen whales (Berger, 2007). In addition, divergence due to vicariant events (e.g. the closure of the Isthmus of Panama) or long-distance dispersal followed by colonisation (e.g. crossing of ocean currents) have contributed to the genetic divergence and formation of new marine species (e.g. Cowman & Bellwood, 2013; Luiz et al., 2012; Sales et al., 2017).

Given the current rate of environmental changes, monitoring widespread sentinel species using genomic methods can advance conservation management (Dobrynin et al., 2015). Genomic information derived from periods of significant environmental and geological change can be used to explain contemporary patterns of species diversity, divergence, and adaptive capacity (Jensen et al., 2019; Vianna et al., 2020). This approach allows for an understanding of the intricate relationships between past events, genetic structure, and the current state of marine ecosystems, aiding in the formulation of conservation strategies.

Blue whales are a sentinel species (Lavery et al., 2014) composed of four recognised subspecies that differ morphologically: the Northern blue whale (*B. m. musculus*), the Antarctic blue whale (*B. m. intermedia*), the Northern Indian Ocean blue whale (*B. m. indica*), and the pygmy blue whale (*B. m. brevicauda*) (Committee on Taxonomy, 2022; Cooke, 2018; Ichihara, 1966; Pastene et al., 2020), with population structure apparently present within some subspecies (Attard et al., 2024; Leroy et al., 2021; Stafford et al., 2001; Torterotot et al., 2020). Recent research by Attard et al. (2024), which used the largest and most geographically comprehensive DNA dataset for blue whales to date, revealed significant genetic divergence among populations in the Eastern Pacific, Indo-Western Pacific, and Antarctic regions. Within these broader regions, further genetic differentiation was observed between populations in the Eastern North and South Pacific, as well as among populations in the Eastern Indian Ocean, Western South Pacific, and northern Indian Ocean. Interestingly, no genetic divergence was detected within the Antarctic population, adding further complexity to the current classification of blue whales.

The oldest fossil tentatively identified as a blue whales, to date, is from a specimen from the early Pleistocene (1.5 - 1.25 Mya) in Italy (Bianucci et al., 2019). Like many marine mammals, blue whale distribution is likely influenced by food availability, and, to a lesser extent, by sea ice and predation risk (Lesage et al., 2017). In general, they migrate between summer feeding grounds and winter breeding grounds, and the location of these feeding grounds varies according to subspecies. For example, feeding grounds of Antarctic blue whales have been identified in Antarctic waters (Corkeron et al., 1999), while pygmy blue whales feed in temperate waters (Attard et al., 2010; Gill, 2002; Hucke-Gaete et al., 2004; Torres, 2013). However, there is evidence that some individuals may not migrate (Barlow, Klinck, Ponirakis, Branch, et al., 2023; Barlow, Klinck, Ponirakis, Holt Colberg, et al., 2023; Branch, Stafford, et al., 2007). Distribution and movement information varies by location and most migration routes are unclear (Andrews-Goff et al., 2013; Möller et al., 2020; Thums et al., 2022).

Contemporary patterns of divergence and population structure in blue whales could be also associated with their over-exploitation. They were hunted to very low numbers, particularly the Antarctic population, during the 19th and 20th centuries, which led to their current status of Endangered by the IUCN (Cooke, 2018), but the impact varied across different subspecies and populations (Thomas et al., 2016). Antarctic blue whales are known to be the most exploited subspecies during the above period (Branch et al., 2008). Their populations were reduced from thousands to only a few hundred individuals, with the Antarctic subspecies currently classified by the IUCN as 'Critically Endangered' (Branch, 2007; Cooke, 2018). Other subspecies were also heavily hunted, but to a lesser extent. However, it remains unclear how whaling affected blue whales, as some populations are still classified regionally as 'Data Deficient' (e.g. Baker et al., 2016). Previous studies in cetaceans have shown how past oceanographic changes yield the current complex population histories of species with low levels of genetic differentiation (Brüniche-Olsen et al., 2018; Carroll et al., 2019; Foote et al., 2019). Thus, considering the current concern over their recovery and the possibility that inevitable climate change could exacerbate the decline of the species, a clear understanding of how environmental changes could impact their occurrence is imperative.

This study uses genomic data to test the influence of historical events in the phylogeography, migration, introgression and diversification of blue whales. The work builds on the SNP dataset produced by Attard et al. (2024) for 275 individuals representing six populations (Figure 3.1). This extensive dataset allows for a comprehensive examination of how historical events, such as the Last Glacial Maximum (LGM) (Clark et al., 2009), the Pleistocene glaciations, the late Miocene-early Pliocene transitions, the transition between Pliocene and LGM, and the potential effects of a bottleneck caused by over-exploitation from whaling, have shaped the genetic structure and evolutionary history of blue whales.

3.4 Methods

The study compiled a total of 275 biopsy samples from blue whales, the same samples used in Attard et al. (2024). These samples were collected from various regions, including the North and South Eastern Pacific (Attard et al., 2024; Galletti Vernazzani et al., 2017), Indo-Western Pacific in Australia (Attard et al., 2012, 2018), New Zealand (Barlow et al., 2018), Madagascar, the Maldives (LeDuc et al., 2007), and the Antarctic (Attard et al., 2012, 2016; Sremba et al., 2012) (Figure 3.1). Due to the small sample size from the Northern and Western Indian Ocean, and in line with the findings of Attard et al. (2024), this study collectively refers to all individuals from these regions, as well as the Eastern Indian Ocean, as 'Indian/West Pacific'. The samples were obtained using a non-lethal biopsy dart launched from a pneumatic rifle or crossbow (*e.g.* Kershaw et al., 2019) and were then preserved in either 20% dimethyl sulfoxide saturated with NaCl or 70–100% ethanol. Genomic DNA was extracted using a modified salting-out protocol (Sunnucks & Hales, 1996), and its quality,

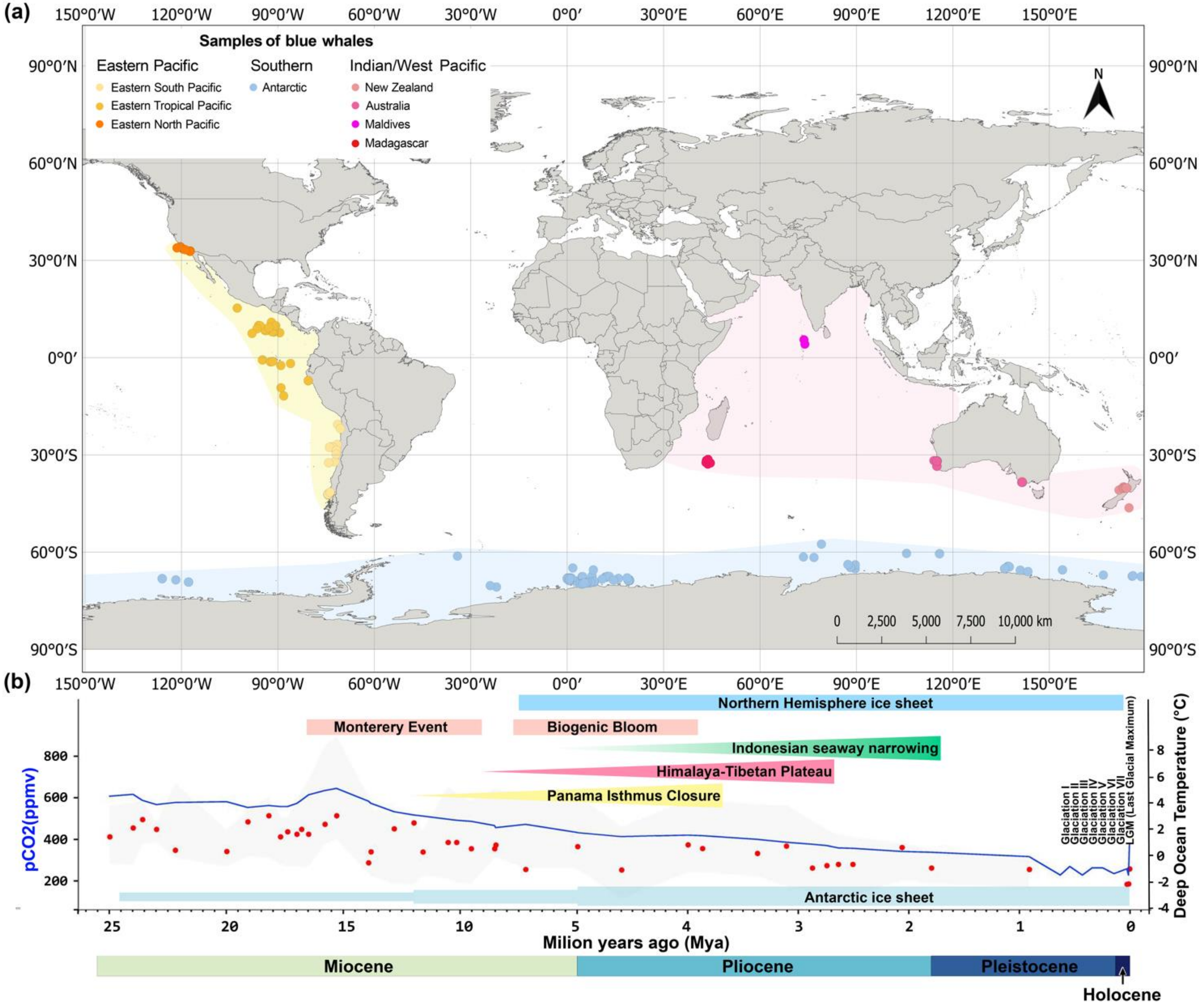


Figure 3.1. (a) Map of approximate sampling localities for 275 individual blue whales (*Balaenoptera musculus)*. Samples are coloured based on identified lineages (Figure 3.2; also see Attard et al., 2024). Pink shading: Indian/West Pacific Ocean; yellow shading: Eastern Pacific Ocean; blue shading: Southern Ocean. **(b)** Timeline showing variations in global climate with pCO2 in ppmv on the left axis and deep ocean temperature in Celsius on the right axis, with the main Events on the x-axis

integrity, and quantity were checked using a Nanodrop spectrophotometer (Thermo Scientific), 2% agarose gels, and a fluorometer (Qubit, Life Technologies), respectively.

The research team prepared libraries in-house, following the ddRAD protocol of Peterson et al., (2012) with modifications described in Brauer et al. (2016) and Sandoval-Castillo et al. (2018). The sequencing was performed single-end 100 bp, using 76 samples per lane on a HiSeq 2000 Illumina. To estimate calling error, 17 samples were replicated across lanes.

3.4.1 Sampling and Bioinformatics

All the bioinformatics were executed using DeepThought HPC (Flinders University, 2021). Over 888,000 filtered reads per sample were mapped to the *B. m. musculus* genome obtained from the National Centre for Biotechnology Information (NCBI) (GCF_009873245.2). Single Nucleotide Polymorphisms (SNPs) were called using BCFTOOLs version 1.16 (Li, 2011), and using VCFTOOLS version 0.1.17 (Danecek et al., 2011). All the scripts used are available at https://github.com/Yuma248/SNPcallingPipe, and full parameter details are described in Attard et al (2024). The final dataset consisted of 16,661 SNPs from 275 individuals. The high-quality SNPs were biallelic, present in at least 80% of individuals, and had minor allele frequencies >0.03. The average missing data per individual was 1.8% and the average coverage per locus per individual was 33.43X. Based on population allele frequencies, missing data were imputed using Genodive 3.05 (Meirmans, 2020).

3.4.2 Phylogenetic Inference

In addition to the 275 samples above that were sequenced and assembled (Attard et al., 2024), genomic data from two other samples were used; one from the North Atlantic corresponding to a Northern blue whale (*B. m. musculus*) (GCA_009873245.3 Árnason et al., 2018) (Figure 1 and Table S1 from supplementary information in Attard et al., 2024), and one humpback whale (*Megaptera novaeangliae*) (Seeary et al., 2022) used as the outgroup, leading to a total of 277 samples used for phylogenetic analyses.

A maximum likelihood (ML) phylogenetic inference was performed to investigate the evolutionary history of blue whale populations using all 277 individuals. The analysis was conducted in RAxML version 8.0.19 (Stamatakis, 2014) employing the GTRGAMMA substitution model (Rannala & Yang, 2007) used with the Lewis ascertainment bias correction (Lewis, 2001). To find the optimal maximum likelihood (ML) tree, 1000 replicate searches were executed, followed by bootstrapping employing the autoMRE option, which automatically determined the required number of bootstrap replicates (Pattengale et al., 2010). The tree was rooted using *M. novaeangliae*.

A different ML phylogenetic inference was performed using the IQ-TREE2 software version 2.1.2 (Minh et al., 2020) for validation purposes. This was based on a general time-reversible model (GTR) with ascertainment bias correction (ASC) (Lewis, 2001) to account for SNP data that typically do not contain constant sites in other words to evaluate if the heterozygous sites are irrelevant for the inference. iTOL version 6.5 (Letunic & Bork, 2021) was used to visualise the resulting phylogenetic tree.

Additionally, the SVDQuartets method within the software PAUP*(Chifman & Kubatko, 2014), was used, evaluating 100,000 random quartets and performing 100 bootstraps. SVDQuartets, along with the maximum likelihood (ML) methods, were used to investigate the evolutionary history of the blue whale lineages. ML methods have been found to be consistent with true gene trees but may exhibit inconsistency when gene trees are estimated from data for loci of finite length (Chou et al., 2015). This allowed the exploration of the question of statistical consistency for the lineages with the use of SVDQuartets and ML methods.

3.4.3 Migration Events and Introgression

Historical population events, such as divergence between population groups or lineages, and migration could leave a genetic signature in the genomes of current populations. To investigate blue whale population history, a TreeMix analysis (Pickrell & Pritchard, 2012) was implemented to detect migration events between populations rather than to estimate introgression. TreeMix uses genome-wide allelic data and a Gaussian approximation to estimate genetic drift and fit a tree model to observed genetic patterns using maximum likelihood. Then, based on allele frequency residual covariance among populations, TreeMix identifies admixture events and their directionality, which increment the likelihood of the tree model. Due to a lack of multiple samples from the North Atlantic and the outgroup, these unique samples were excluded from the analysis, with the initial 275 individuals remaining. The analysis was run with a window size of 500 SNPs. Zero to 10 migration events were tested with 10 iterations per event.

To assess historical gene flow between the three lineages, we calculated Patterson's D-statistic, also known as the ABBA-BABA test, and the related f4-ratio test (Durand et al., 2011; Green et al., 2010). Patterson's D-statistic was used to detect the presence of introgression by examining patterns of shared alleles among lineages, given the tree ((East Pacific, Indian/West Pacific), Southern Ocean), Outgroup), specifying *M. novaeangliae* as the outgroup. The primarily interested here was on understanding regional connectivity over evolutionary time scales, rather than more recent migration patterns. Samples identified as migrants and early generation hybrids were therefore omitted. To additionally quantify the proportions of any admixture detected, the f4-ratio test was applied, which estimates the relative contributions from different source populations by comparing ratios of f4 statistics (Patterson et al., 2012). Both tests were performed using the Dtrios function in Dsuite v.04 with default parameters (Malinsky et al., 2021).

3.4.4 Demographic Analysis

Introgression analysis is relevant for demographic history because it can reveal information about gene flow between populations and the impact of hybridisation events on the genetic makeup of populations. The resulting hypotheses can then be investigated individually by more richly parametrised model-based inference framework, such as fastsimcoal2 (Excoffier & Foll, 2011). For the coalescence and demographic analyses, the folded site frequency spectrum (SFS) was generated with an *in-house* perl script available at https://github.com/Yuma248/MELFUtilities.

Fastsimcoal version 2.7 (Excoffier & Foll, 2011) was used to test hypotheses about the divergence of the three previously identified genetic lineages (Attard et al., 2024). Given the biology of the species, some of the coalescence models took into consideration migration. These estimates reflect the amount of genetic variation resulting from migration over many generations rather than instantaneous dispersal rates. In this regard, population sizes and migration rates are not intended to refer to the status of a subspecies, but rather to the coalescence-based average size of populations and migration rates over time. For each pair of lineages, the joint minor-allele frequency spectrum (joint-MAF) was estimated and used as input for each model. The joint-MAF was calculated using the allele frequency data generated by the perl script, which processes Genepop files to count the allele frequencies across different populations and markers. This script generates the frequency spectra directly from the raw allele counts across the populations, ensuring that all genetic variation is accounted for in the coalescence-based models. The mutation rate used was 7.7×10^{-10} per year (Westbury, Petersen, & Lorenzen, 2019) and generation time was 31 years as estimated for blue whales (Taylor et al., 2007).

Models with or without migration, with divergence occurring in the late Miocene, before the LGM or after the LGM, were tested. To account for uncertainty in historical population sizes, models were tested using both large and small prior population size ranges. Large population sizes were set between 1,000,000 and 10,000,000 individuals, while small population sizes ranged from 100 to 10,000 individuals. These alternative priors allowed for the evaluation of how different demographic assumptions influenced model likelihoods and parameter estimates. For the two best-performing models, given their similar values of AIC (see results), further model variations were tested. These included demographic contractions before the LGM and expansion after the LGM, with migration. Additionally, the two best-performing models were used to account for the whaling bottleneck that occurred between two and four generations ago, to test if the radical contraction has left any genetic signal that could be detected. The dataset excluded samples previously identified as first-generation hybrids and migrants between Southern Ocean and Indian/West Pacific Oceans lineages (Attard et al., 2012), a finding also supported by the ADMIXTURE analysis.

Each model was run 100 independent times (100,000 simulations per run and 40 ECM cycles) and assessed by the highest likelihood and Akaike’s Information Criterion (AIC). The maximum likelihood parameters estimated for the best model were used to simulate 1,000 bootstrap MAF spectrum and perform 100 runs for the simulated data. From these data, the 95% confidence intervals (C.I.) were calculated using the R function boot.ci from the package ‘boot 1.3’ (Canty & Ripley, 2021).

To estimate temporal trends of genetic diversity, the model-flexible stairway plot was also implemented (details in Appendix S2 – Demographic history analysis).

3.5 Results

3.5.1 Phylogenetic Inference

The phylogenetic analysis of blue whale populations identified the earliest diverging individuals from the Southern Ocean, with a clade consisting of the Eastern Pacific and Indo-Pacific populations nested within. For clarity and consistency throughout the text, these three distinct regional groupings will be referred to as 'lineages' defined as follows: (1) Southern Ocean, (2) Eastern Pacific Ocean (including Chile, the Eastern Tropical Pacific, and Eastern North Pacific), and (3) Indian/Western Pacific Oceans (including Australia, New Zealand, Madagascar, and Maldives) (Figure 3.2). These lineages were consistently observed across all phylogenetic reconstructions, including Maximum Likelihood (ML) trees and the SVDQuartet tree (Figure 3.2, S3.1, S3.2, S3.3 & S3.4). The Southern Ocean lineage shared individuals with both the Eastern Pacific and the Indian Oceans. The phylogenetic reconstructions identified an initial split from the Southern Ocean lineage, followed by a subsequent separation between the Eastern Pacific and the Indian/West Pacific Ocean lineages.

These lineages are not strictly monophyletic (Figure 3.2) due to inferred migration and hybridisation between regions. For example, individuals identified as first-generation hybrids and migrants were found in both the Southern Ocean and Indian/Western Pacific lineages (Attard et al., 2012), indicating recent admixture between them.

The majority of branches defining the three lineages showed strong bootstrap support (>70%), confirming the robustness of the within-ocean basin structure (Figure S3.1 & S3.2). Low bootstrap values were observed for some smaller clades, which is not unexpected in intraspecific studies of wide-ranging populations (Westbury et al., 2023). As our study focuses on broader relationships of the three major lineages, the lower bootstrap support do not affect the primary conclusions or overall robustness of the findings.

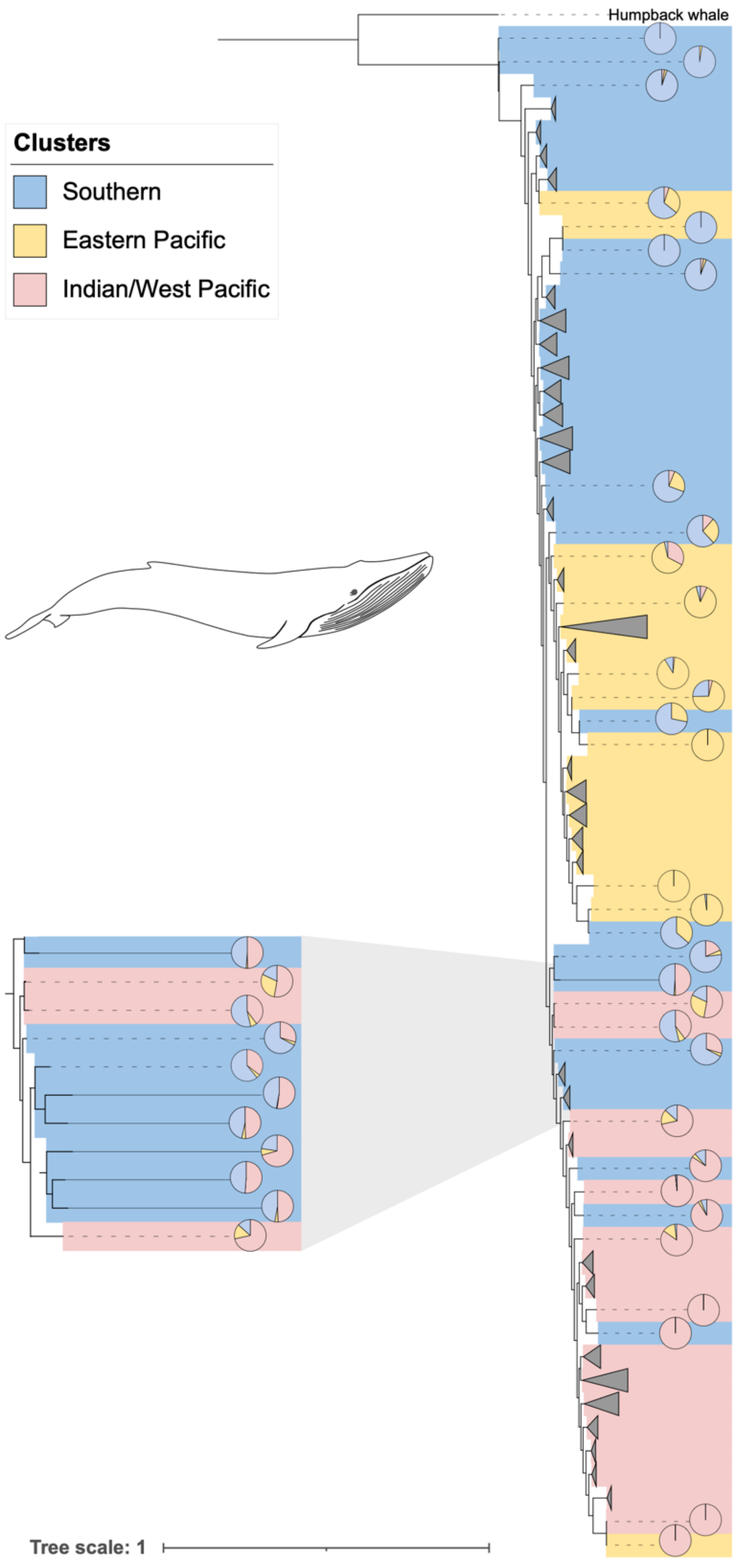


Figure 3.2. Maximum likelihood phylogeny of blue whales (*Balaenoptera musculus*) based on 16,661 SNPs estimated with IQTree2. The size of triangles represent collapsed clades consistent with the number of samples grouped within each clade. A detailed view of clades, including bootstrap values, is provided in Figure S3.1. Pie charts displaying Q-values derived from the Admixture analysis (see Attard et al. 2024) are shown for select samples. The zoomed area are identified hybrids excluded from the demographic history analysis. The tree is rooted with the humpback whale (*Megaptera novaeangliae*).

3.5.2 Introgression and Migration

The TreeMix analysis was used to evaluate introgression and migration among regional lineages and test for migration events. The analysis focused on historical gene flow, on events occurring after the divergence of the lineages. The unrooted tree supported no events of ancestral migration between the lineages and indicated genetic drift, between Indian/West Pacific and the other lineages (Figure 3.3). According to the TreeMix analysis, the final model demonstrated all branches with a strong drift of allele frequencies from their common ancestral gene pool.

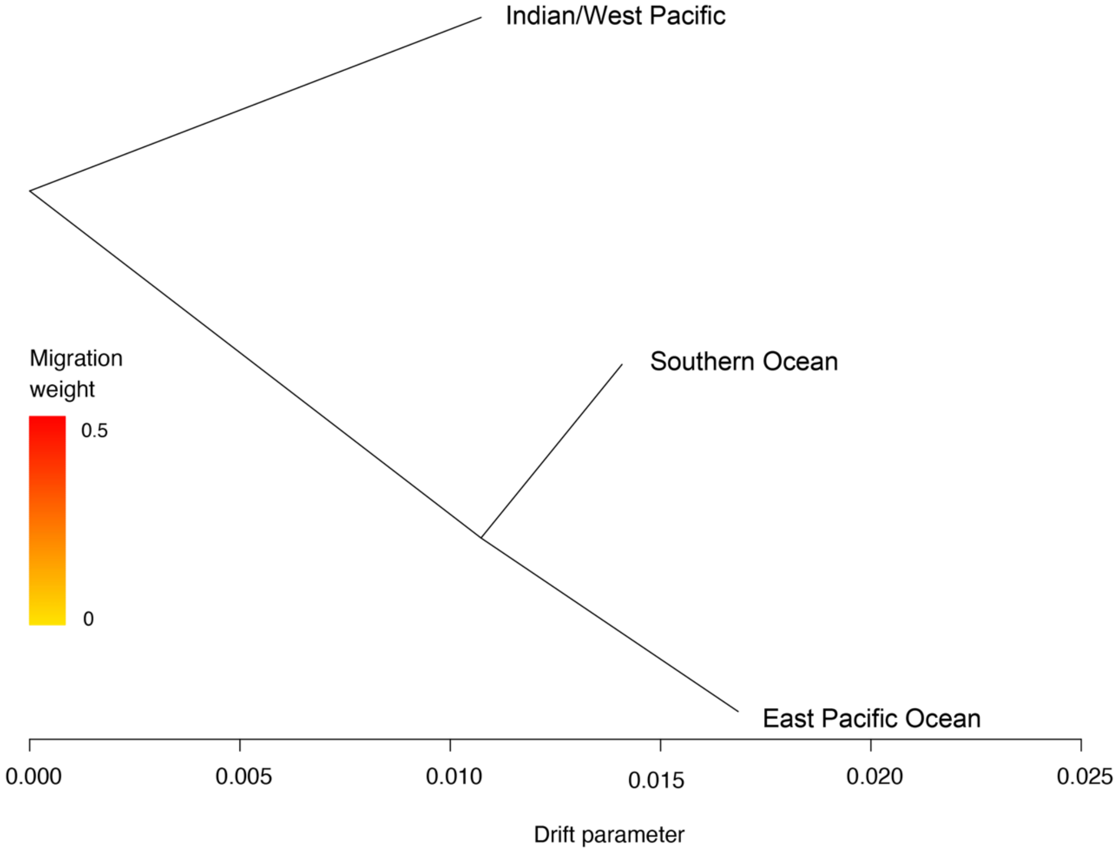
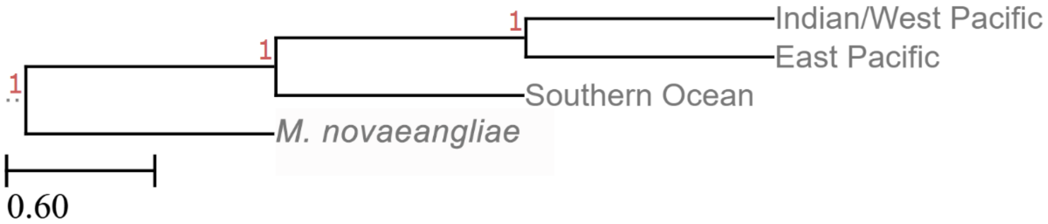


Figure 3.3. Unrooted maximum likelihood tree inferred with TreeMix for the three major lineages of blue whales (*Balaenoptera musculus*).

To assess any historical genome-wide signal of introgression, we calculated *D*-statistics and estimated *f*4-ratios for all combinations of lineage trios with Dsuite. Negligible evidence of introgression was found between lineages, with a non-significant *D* of 1% ($p = 0.14$) and $f4 = 0$ (Figure 3.4).

(a)



(b)

P1	P2	P3	D	Z-score	p-value	f4-ratio	BBAA	ABBA	BABA
East Pac.	Ind/West Pac.	Southern Oc.	0.010	1.091	0.138	0.000	651.195	574.691	563.697

Figure 3.4. (a) Phylogenetic tree (Newick format) representing the relationships used in the ABBA-BABA analysis. **(b)** *D* and *f4*-ratio statistics indicating minimal introgression between the Southern Ocean, Indian/West Pacific, and Eastern Pacific lineages. BBAA shows shared derived alleles between the Eastern and Indian/West Pacific lineages, ABBA between Indian/West Pacific and Southern Ocean, and BABA between Eastern Pacific and Southern Ocean.

3.5.3 Mid-Pleistocene Timing of Divergence of Blue Whale Lineages

The coalescence analysis favours divergence occurring during the Mid-Pleistocene, approximately 300,000-330,000 years ago (Table 3.1 and Figure 3.5). This timing was supported by the analysis of 26 different demographic models (Figure S3.5), with those incorporating migration showing the best fit to the data (Table S3.1). Specifically, the AIC values strongly supported the models with migration, simulating the Southern and East Pacific lineages as the ancestral lineages were most consistent with the observed data (Figure 3.5, Table 3.1 and S3.1). Consequently, focusing on these two best-fitting models, the estimated divergence time (T_{AN}) of the lineages was approximately 9,700-10,600 generations. This time corresponds to around 300,000-330,000 years ago considering the generation time of blue whales used in the analysis (Table 3.1). Although both the recent and historical migration rates between lineages were rather low, they were supported by the coalescence analysis (Table 3.1). The model aligns more closely with the younger end of the time estimates, and since Fastsimcoal is not skewing the estimates toward the older end, the divergence time is not believed to exceed 300,000 years. Also, recent migration rates between the Southern

Table 3.1. Maximum likelihood parameter estimates (and 95% C.I.) used to infer the evolutionary history of blue whale (*Balaenoptera musculus*) lineages. Results are for the two best-fitting models identified using fastsimcoal2: Model 1 with the Southern Ocean (SO) as ancestral lineage and Model 2 with the Eastern Pacific (EPac) as ancestral lineage. IWPac: Indian/West Pacific; SO: Southern Ocean; EPac: Eastern Pacific. Bold text highlights significant parameter estimates within each model. Historical and recent migration rates and effective population size estimates (Ne) are provided for each model.

Parameter	**Prior**	**Before LGM Southern Ocean as ancestral lineage**	**Before LGM East Pacific as ancestral lineage**
		Estimated (95% C.I.)	
Migration rate Historical			
IWPac vs. SO	1e-10 – 1e-3	**1.185e-04** (7.906e-05 – 1.579e-0.4)	-
SO vs. IWPac	1e-10 – 1e-3	**1.064e-0.3** (9.191e-0.4 – 1.208e-0.3)	-
EPac vs. SO	1e-10 – 1e-3	-	**2.504e-04** (1.618e-0.4 – 3.39e0.4)
SO vs. EPac	1e-10 – 1e-3	-	**1.542e-0.4** (1.135e-0.4 – 1.948e-0.4)
Migration rate Recent			
IWPac vs. SO	1e-10 – 1e-3	**2.841e-05** (2.4e-05 - 3.282e-05)	5.429e-05 (5.066e-05 - 5.793e-05)
SO vs. IWPac	1e-10 – 1e-3	4.053e-05 (1.143e-05 - 6.963e-05)	5.899e-07 (3.774e-07 - 8.023e-07)
EPac vs. SO	1e-10 – 1e-3	-	5.764e-0.4 (5.201e-04 – 6.326e-04)
SO vs. EPac	1e-10 – 1e-3	-	**2.751e-0.4** (1.959e-04 – 3.542e-04)
EPac vs. IWPac	1e-10 – 1e-3	8.398e-06 (6.575e-06 - 1.022e-05)	-
IWPac vs. EPac	1e-10 – 1e-3	8.333e-05 (5.064e-05 – 1.16e-04)	-
Effective Population sizes (Ne)			
Historical Ne SO	100 – 500,000	1,089,000 (1,031,000 - 1,146,000)	25,090 (23,090 – 27,090)
Historical Ne IWPac	100 – 500,000	22,280 (21,270 - 23,300)	72,870 (56,730 - 89,020)
Historical Ne EPac	100 – 500,000	64,410 (62,630 - 66,180)	681,700 (638,700 - 724,700)

Time (in generations)			
T_{Div} SO – EPac	7,500 – 15,000	10,600 (10,270 - 10,920)	**9,769** (9,457 - 10,080)
T_{DIV} SO – EPac – IWPac	4,000 – 7,500	5,992 (5,845 - 6,139)	**6,590** (6,464 - 6,717)

Ocean and the other two lineages were higher (recent migration rate: 2.841e-05 and 4.053e-05 between Southern Ocean and Indian/West Pacific; Table 3.1) than the migration rate occurring between Eastern Pacific and Indian/West Pacific (recent migration rate: 8.398e-06 and 8.333e-05; Table 3.1). This suggests that while the Southern Ocean lineage is not necessarily ancestral to the other lineages, it may be the main link connecting the southern hemisphere gene pools of the blue whale currently recognised subspecies.

The historical effective population size estimates for the Southern Ocean lineage ranged from 25,090 to 1,089,000 across the best supported models (Table 3.1). For the Indian/West Pacific Ocean lineage, estimates varied between 22,280 and 72,870, and for the Eastern Pacific Ocean lineage, estimates ranged from 64,410 to 681,700, according to the best supported models (Table 3.1). Given the high differences on the estimates of effective population sizes for both models, the findings cannot be interpreted as conclusive other than to suggest that all three lineages had high effective population sizes pre-whaling.

Additionally, the stairway plot analysis, as detailed in the Appendix S2 and Figure S3.6, revealed a continuous decline in effective population size for all three lineages—Southern Ocean, Eastern Pacific, and Indian/West Pacific Oceans—since the mid-Pliocene. This decline aligns with the estimated divergence time of approximately 300,000-330,000 years ago, supporting the coalescence analysis findings. Both analyses suggest that divergence occurred before the LGM, with a continuous decline observed for each lineage. However, given that stairway plots can be influenced by noise in the Site Frequency Spectrum (SFS) (Lapierre et al., 2017; Patton et al., 2019), these results should be interpreted with caution.

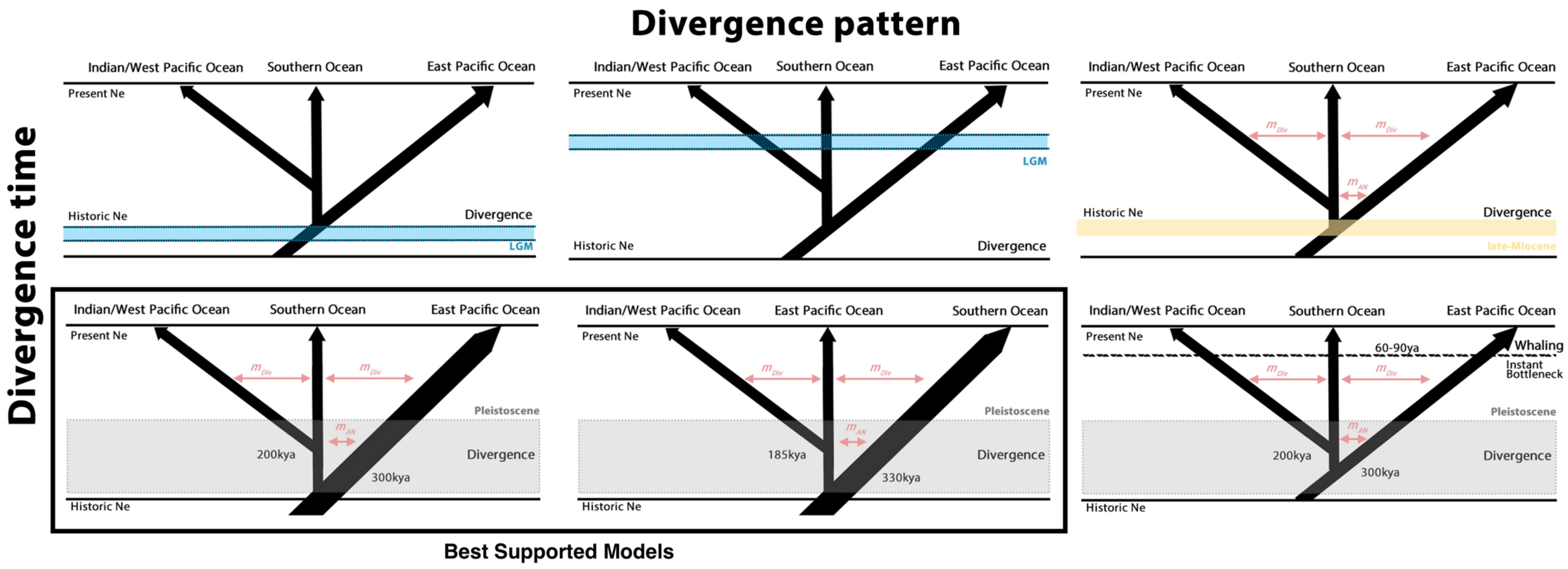


Figure 3.5. Representation of six models of demographic history compared using fastsimcoal2 with continuous gene flow. The lineages diverge at T_{AN} for the ancestral divergence and T_{Div} for the secondary divergence. Each model was tested with and without bidirectional migration (pink arrows) between lineages. The thickness of the arrows represents the magnitude of population size, with thicker arrows denoting larger populations. Coloured lines highlight specific divergence times: LGM (blue), late Miocene (yellow) and Pleistocene (grey). The two best-fitting models are enclosed within a square.

3.5.4 No Genomic Signal of Whaling Overexploitation

Using the results from the two best coalescence models—one with the Southern Ocean as the ancestral lineage and the other with the Eastern Pacific Ocean as the ancestral lineage—further tests of the bottleneck produced by whaling in the recent past were undertaken. These tests examined whether there was a significant reduction in effective population size indicative of a bottleneck. The likelihood values, based on the AIC, did not show significant improvements compared to the models that did not include bottleneck scenarios (Table S3.1 & Figure S3.5). This indicates that the data do not support a detectable bottleneck effect in the analysed genomic regions.

3.6 Discussion

Using the largest dataset of SNPs currently available for blue whales, the history of diversification and demography of this globally distributed species was reconstructed for the sampled populations. The phylogenetic analysis confirmed three distinct lineages: the Southern Ocean, the Indian and Western Pacific Oceans, and the Eastern Pacific Ocean lineages (Attard et al., 2024). The Southern Ocean lineage exhibited connections with both the Eastern Pacific and Indian Ocean lineages, suggesting recent or ongoing gene flow between these regions.

However, our data suggests the timing of the divergence of blue whales to be in the Mid-Pleistocene, suggesting that climatic and oceanographic changes from this period played a pivotal role in shaping the species' demography, dispersal, and speciation. Coalescence analysis unveiled a Mid-Pleistocene divergence of blue whale lineages, identifying the Southern and East Pacific lineages as ancestral. The estimated divergence time points to an approximate timeframe of 300,000-330,000 years ago. These data provide evidence that the Indian Ocean lineage is the most recently diverged lineage but provide insufficient evidence to clarify from which specific lineage or ancestral group the original blue whale lineage diverged.

Furthermore, the coalescence analysis indicated low recent migration rates, with the Southern Ocean lineage serving as a primary link between the gene pools of southern hemisphere blue whale subspecies. This suggests that while the Southern Ocean lineage plays a key role in gene flow between these populations, it is not necessarily the ancestral lineage but rather a conduit for recent gene exchange. Despite the overexploitation during the whaling era that dramatically impacted species abundance (Paarman et al., 2021; Pastene et al., 2020; Tulloch et al., 2019), the analyses were unable to detect a genomic signal consistent with anthropogenic bottlenecks based on this dataset. The genetic signatures of such bottlenecks can be complex and may not always be easily identifiable, especially if the demographic reduction did not persist for many generations and if population sizes recovered to some extent post-whaling.

3.6.1 Phylogeography

Previous molecular studies based on mitochondrial DNA and microsatellites suggested that the low levels of genetic diversity of Eastern Indian Ocean blue whales was likely the result of a founder event from the Southern Ocean lineage of Antarctic blue whales around the LGM, followed by evolutionary divergence (Attard et al., 2015). The present results corroborate the finding of separation of the Indian Ocean lineage from the Southern Ocean and Eastern Pacific lineages. However, all the coalescence simulations support an earlier divergence time around the Mid-Pleistocene. This can perhaps be attributed to the higher resolution of the genomic data used in the present study. Evolutionary processes affect certain regions of the genome differently, and discordances between studies with different genetic markers are not uncommon (Toews & Brelsford, 2012). The full coalescent method used here is based on genome-wide data and considers recombination and variation in mutation rate. Genome-wide markers also tend to provide better resolution and give a clearer understanding of a species' evolution (Zhang et al., 2020). If species diverge rapidly, or if alleles have long coalescence times, incomplete lineage sorting may lead to mismatches when using gene trees to estimate species trees (Funk & Omland, 2003). Therefore, future studies would benefit from whole-genome data and inclusion of all representative groups of this species to confirm the current results. By using whole-genome data, a more comprehensive view of genetic variation can be obtained, enhancing the detection of subtle signals and clarifying historical demographic events.

In the exploration of blue whale lineage dynamics, fastsimcoal2 offers detailed insights into the evolutionary and demographic processes shaping these populations, including estimates of historical population sizes, migration rates, and the effects of past demographic events. Fastsimcoal2 is based on Nielsen's approach (Nielsen, 2000), and proves itself as a flexible and resilient methodology, adept at estimating demographic parameters based on the site frequency spectrum (SFS) inferred from SNP data (Excoffier et al., 2013). The current coalescent analysis supports two northward dispersal events from the Southern Ocean. The first dispersal into the Indian/West Pacific and Eastern Pacific, with a subsequent split between whales from these two regions. The second dispersal occurred separately from the Southern Ocean into the North Atlantic. This pattern is supported by the phylogenetic and coalescence analyses presented here and is consistent with the findings of Bukhman et al. (2024) and Jossey et al. (2024). Bukhman et al. (2024) did not have Southern Ocean representation and proposed a cessation of gene flow between the Pacific and Atlantic Ocean blue whales at around 125,000 years ago, consistent with the end of the Saalian ice age. This agrees with the ML results (Figure S3.1), which showed that the Atlantic Ocean sample was positioned within the Southern Ocean lineage and suggests that the individuals from the Atlantic and Pacific Oceans are derived lineages from the Southern Ocean. Moreover, Jossey et al. (2024) found that the Antarctic blue whale they sequenced occupied a basal position relative to North Atlantic blue whales, with that Antarctic individual being the sister to all North Atlantic individuals. This placement is consistent with our results, where the Antarctic sample is similarly placed as basal. However, the North Atlantic sample, was unexpectedly positioned within the Southern Ocean lineage. The low bootstrap support for this placement is likely attributed to high interindividual variability within the dataset, which limited the resolution of relationships involving this single sample. Since there were no other North Atlantic samples to compare with, it is challenging to determine if the observed placement is representative of North Atlantic populations or an artifact of the limited sample size. The rest of the samples were grouped primarily by geographic region, with bootstrap values for these groupings generally supporting the established lineages. Nevertheless, this observation could be attributed to unsorted ancestral polymorphism, as suggested by Bukhman et al. (2024) and Jossey et al. (2024). Alternatively, they propose that females might exhibit dispersal behaviour involving occasional long-range matrilineal dispersals.

The Pleistocene is known to be a period in which several marine organisms either sought refuge in glacial areas or dispersed to new regions (Amaral et al., 2012; Liu et al., 2011). The coalescent simulations predicted a similar time of divergence of around 10,000 generations ago, which is equivalent to ~0.31 million years ago (Mya) for the Southern Ocean and Eastern Pacific lineages of blue whales. This estimation coincides with variations in temperature linked to numerous glaciations (Figure 3.1; Augustin et al., 2004; Figure 4 in Clark et al., 2009) and overturning oceanic circulation (Starr et al., 2021), potentially triggering divergence among the subspecies due to ice accumulation and food availability (Gupta & Thomas, 1999; Lawrence et al., 2006; Peterson et al., 2020). Oceanic circulation, is a critical factor in the feeding ecology of krill (Hamner, 1988; Patarnello et al., 1996; Schmidt, 2010). Blue whales, known for tracking areas of higher prey density over time (Abrahms et al., 2019; Fahlbusch et al., 2022), may have responded to Pleistocene oceanic circulations, potentially contributing to the divergence of distinct lineages. In the past 500,000 years, during periods of peak glaciations, prey/krill abundance have been shown to be reduced (Earle, 2015; Lisiecki & Raymo, 2005). This reduction in prey can contribute to the isolation of populations, reducing gene flow and promoting genetic differentiation (Jackson et al., 2014; Liu et al., 2011). Marine organisms, including other cetacean species such as common dolphins, dusky dolphins, humpback whales and gray whales, also appear to have diversified during this period (Alter et al., 2015; Amaral et al., 2012; Harlin-Cognato et al., 2007; Jackson et al., 2014).

Morphological features, such as body weight relative to the total body length, snout-eye measurements and nasal bone shape, vary across blue whale subspecies (Omura et al., 1970; Pastene et al., 2020). The pygmy subspecies, represented in this study by individuals from Australia, New Zealand, and Madagascar, is characterised by a shorter maximum body length and tail (Ichihara, 1966). The Eastern Pacific subspecies, represented by samples from Chile and the Eastern Tropical Pacific, exhibits an intermediate size between the pygmy and the Antarctic subspecies (Branch, Abubaker, et al., 2007; Pastene et al., 2020). The observed diversity among the blue whale lineages, coupled with their distinct foraging strategies (Buchan & Quiñones, 2016; Torres et al., 2020), likely played a crucial role in driving their diversification and suggest a relationship between physical characteristics and functional ecology. Long-distance migration capabilities of blue whales (Abrahms et al., 2019; Thums et al., 2022; Truong & Rogers, 2021) indicate that their isolation and diversification were likely influenced by climatic changes affecting foraging behaviour (Barlow, Bierlich, Oestreich, Chiang, et al., 2023). The divergence of blue whale lineages reported here coincided with a stage of high productivity in the Pacific Ocean. It is hypothesized that a major cooling event around 0.23 Mya (Lawrence et al., 2006), along with a decrease in Southern Hemisphere productivity, may have promoted the dispersal of blue whale morphotypes to other ocean basins in search of prey availability. This dispersal likely contributed to niche specialisations of the Indian/West Pacific Ocean lineage that led to the emergence of the pygmy blue whale morphotype. For example, it has been suggested that the small New Zealand blue whale potentially forfeited engulfment size and diving capacity for increased agility to capture patchier surface prey (Torres et al., 2020). These results add to Attard et al. (2015) findings about the effects of past climatic changes in pygmy blue whales.

The coalescent modelling approach did not reveal any genomic signal of the bottleneck that occurred about two generations ago due to the intense whaling period. Baleen whale genetic structure occurs at relatively large spatial scales, which generally relates to their migratory behaviour (Baker et al., 2013). Due to the long and overlapping generation time of blue whales (31 years) and the few generations since the end of whaling, it is difficult to interpret the impacts of human overexploitation based on genetic data (Alter et al., 2012; Sremba et al., 2023; Torres-Florez et al., 2014). If a blue whale population crashes, the effects on heterozygosity will probably not be detectable for approximately 0.2–4*Ne generations (Luikart & Cornuet, 1998). Although genetic diversity could have been lost during the whaling period, the long generation time of the species may have delayed its loss causing the signal to not yet be detectable. Also, fastsimcoal2's model selection process is contingent on several factors, including chromosome size, SNP density, and the degree of linkage disequilibrium among SNPs. A previous study (Johri et al., 2021) suggested that for smaller chromosome sizes (1 and 10 Mb), the correct model was identified with relatively higher accuracy. In contrast, larger genomic regions displayed a greater preference for alternative models, such as instantaneous size changes or bottlenecks. This phenomenon can be attributed, at least in part, to the nonindependence of SNPs in the data. The software's model selection procedure assumes SNP independence, yet SNPs are not completely independent in real-world genomic datasets. This leads to potential challenges in identifying signals of instant bottleneck in smaller genomic regions. These factors should be considered when interpreting the results, as the best “true” scenario may lie outside the set of models tested here.

3.6.2 Absence of Introgression and High Genetic Drift

The D-statistic and f4-ratio estimates revealed no significant evidence of historical admixture among the regional lineages. This finding contrasts with the presence of early-generation hybrids nested within each region, which provides definitive evidence of recent gene flow. These hybrid individuals likely reflect more recent, localised interbreeding between lineages that appear to have been largely isolated throughout their evolutionary history. It is possible that demographic declines due to recent whaling overexploitation have disrupted historical migration patterns, reducing opportunities for gene flow and further isolating regional lineages. Future studies employing more sensitive methods, such as haplotype-based approaches, may provide further insights into the timing, frequency, and genomic consequences of these very recent hybridisation events, as shown for other cetacean species (Carroll et al., 2019).

While the coalescence analysis supported some degree of migration between the lineages, the TreeMix analysis did not detect any migration. The lack of samples from the North Atlantic in the TreeMix may confound some of the results produced by limiting the ability to assess the extent of gene flow and migration involving populations in that region. The absence of data from the North Atlantic lineage may result in an incomplete understanding of historical migration patterns and potentially underestimate or overlook significant migration events involving other populations. Consequently, the TreeMix analysis may be limited and not fully capture the complexity of genetic exchange within the studied subspecies.

Moreover, the overexploitation of blue whale populations during the whaling period (19th and 20th century) may have reduced contemporary populations to a fraction of their ancestral size. Consequently, the genetic structure observed today might be more fragmented, and the historical patterns of gene flow and migration could be more complex than current data suggest. Additionally, when using TreeMix, it is important to consider that accuracy tends to drop considerably when migration occurs between closely related populations (Pickrell & Pritchard, 2012). As previously found by Attard et al. (Table S3; 2024),the lineages show low yet significant genetic structure with F_{st} values ranging between 0.040 and 0.233. Therefore, while the absence of introgression signal in the current analyses should be interpreted cautiously, it does not rule out the possibility of more subtle or localised gene flow, particularly when supported by evidence of hybrids within each region. This highlights the need for additional genomic data, especially from underrepresented regions like the North Atlantic, to provide a more complete understanding of historical and ongoing gene flow within blue whale populations.

3.6.3 Consequences of Climate and Anthropogenic Pressures

The demographic response from blue whales to past environmental changes shows that severe climate change can drive the isolation and potential population declines. For instance, during the 2014 winter off the coast of Newfoundland, Canada, several blue whales died due to ice entrapment after a particularly strong cold winter (Moors-Murphy et al., 2019). Adding to this factor, anthropogenic pressures, leading to habitat modification and over-exploitation, and climatic change can also increase the degree of hybridisation in natural species (Allendorf et al., 2022; Brauer et al., 2023). Blue whales have shown to successfully reproduce with other species such as fin whales (*Balaenoptera physalus*), possibly due to the limited number of available partners (Spilliaert et al., 1991; Westbury, Petersen, & Lorenzen, 2019). In this study, first generation subspecies hybrids recognised in previous studies (Attard et al., 2012, 2024) were confirmed between Southern Ocean and Indian/West Pacific Oceans lineages. This may be explained by recent anthropogenic stressors such as the large reduction in abundance of the different subspecies due to whaling, or the radical changes in climate, which may have prompted hybridisation events (Attard et al., 2012). There is clear ecological specialisation in blue whales illustrated by genomic, phenotypic and behavioural differences (Attard et al., 2018; LeDuc et al., 2017; Leroy et al., 2021; Möller et al., 2020; Sremba et al., 2012; Torres et al., 2020; Torres-Florez et al., 2014; Warren et al., 2021), suggesting that regional management based on ecological differences should be considered.

3.7 Conclusions

Past environmental changes have been possibly a major driver of diversification for blue whales. Using coalescence models, three distinct lineages of this species seem to have diverged during the Pleistocene at around 300,000 years ago, followed by demographic reductions in two of the three regions analysed. This diversification period coincided with accumulation of ice and decreased primary productivity at a global level, which might have affected connectivity and promoted lineage splits.

Future studies will benefit from whole-genome datasets and inclusion of a good representation of samples from the North Atlantic Ocean to fill the knowledge gaps about this species' evolutionary history. This understanding of their evolutionary history, will allow predicting the genomic adaptability and putative responses of the species to future climatic and anthropogenic changes. Such understanding and predictions are crucial to guarantee appropriate management and conservation of blue whales, particularly when considering significant population declines and global warming of the oceans.

3.8 Data Availability Statement

The supporting data generated in this study (VCF file, phylip file, treemix file, geographic coordinates for all samples, blueprint files, input files for coalescence models and R scripts) are available from Zenodo: 10.5281/zenodo.10704669. Original genomic data from Attard et al. (2024) are available from FigShare at https://doi.org/10.6084/m9.figshare.25241827.

Chapter 4

DEEP INTO THE BLUE: Unravelling the Demographic and Genomic Health of Blue Whales (*Balaenoptera musculus*) Post-whaling

Geographe Marine Research

4.1 Contributions

Gabrielle Genty – conception of study design, DNA extractions and whole-genome preparation, bioinformatics, data analysis and interpretation, writing of manuscript.

Luciana Möller – primary supervisor – conception of study design and interpretation and drafting and revision of manuscript.

Luciano Beheregaray – associate supervisor – guidance in study design and interpretation, drafting and revision of manuscript.

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4.2 Abstract

The blue whale (*Balaenoptera musculus*) faced dramatic population collapses due to intensive whaling during the 19th and 20th centuries, with some populations estimated to have dropped from approximately 300,000 to only 2,000 individuals. The genomic health of this species remains uncertain as the impact of these severe bottlenecks may be masked by the species' long generation time. In this study, whole-genome data from three lineages and five populations of blue whales were analysed to assess the genetic effects of their recorded extensive population declines. Phylogenetic and population structure analyses suggested four distinct lineages represented by three of the recognised subspecies. Estimates of recent effective population size in blue whale lineages indicated dramatic demographic reductions followed by recent recoveries, except for the Antarctic lineage. Antarctic blue whales exhibited the most severe recent reduction, likely associated with its extensive exploitation, and negligible demographic recovery. Low levels of inbreeding were disclosed by runs of homozygosity (ROH), except for the pygmy blue whale lineage, which exhibited higher and longer ROHs likely due to a natural founder effect. Although a crash in effective population size was observed for all lineages, they exhibited relatively low levels of genetic load. This suggests that despite recent historical overexploitation, the species retained enough genetic diversity to support recovery, providing a positive outlook for the long-term survival of blue whales. These findings emphasise the importance of ongoing conservation efforts and population monitoring, particularly the protection of migratory routes and feeding grounds for these oceans' giants, which will be critical to ensure the future of the species.

4.3 Introduction

The overexploitation of species and other human-driven environmental pressures have led to dramatic reductions in population sizes across many taxa (Dulvy et al., 2003; Rosser & Mainka, 2002; Rowcliffe et al., 2003). Such population declines can have profound genetic consequences, including reduced genetic diversity, increased inbreeding, and a loss of adaptive potential (Amos & Harwood, 1998; Robinson et al., 2023; Van Dyke, 2008). Genetic effects may persist long after populations stabilise or begin to recover, potentially compromising the long-term survival and evolutionary potential of a species (Gaggiotti, 2003). Understanding the genetic legacy of overexploited populations is crucial for predicting how species may respond to future environmental changes and for informing effective conservation management strategies.

The largest animal on Earth (the blue whale, *Balaenoptera musculus*) serves as example of how economic forces can drive a species to the brink of extinction. During the 19th and 20th centuries, blue whales were extensively hunted due to the high value of their oil, meat, and baleen (Tønnessen & Johnsen, 1982). The Antarctic population experienced a catastrophic decline, with approximately 293,000 individuals killed by commercial whaling between 1928 and 1972, despite the international protection established in 1966 (Olson & Kinzey, 2024). Illegal hunting by the Soviet Union persisted until 1972, depleting the population by an additional 760 individuals (Yablokov, 1994; Zemsky et al., 1995). As of recent estimates, between 1,800 and 3,500 whales remain in the Antarctic population (Branch, Stafford, et al., 2007; Olson et al., 2024). Similar patterns of exploitation were observed in other blue whale populations. In the North Atlantic, it has been estimated that over 10,730 blue whales were killed between 1868 and 1965, leading to a dramatic reduction in their numbers (Allison, 2020). Although there are indications of a slow recovery, with the population currently estimated at 1,000 and 3,000 individuals, the impact of past exploitation remains unclear (Pike et al., 2019). In the North Pacific, an estimated 9,000 blue whales were caught, along with an additional 7,000 unspecified whales, some of which were likely blue whales (Allison, 2020). The current population in this region is estimated at 3,000-5,000 individuals (Calambokidis & Barlow, 2004; Calderan et al., 2020; Galletti Vernazzani et al., 2017; Williams et al., 2011).

The pygmy blue whale population, primarily found in the Indian and southwestern Pacific Oceans, also suffered heavy exploitation. Approximately 12,000 pygmy blue whales were killed, with 97% of these catches occurring between 1959/60 and 1971/72, largely by Japanese and Soviet pelagic whalers (Branch et al., 2021). Current population estimates for pygmy blue whales are uncertain but may range between 2,000 and 5,000 individuals. Estimates along the Western Australian coast suggest a population size of 662-1,559 individuals (McCauley & Jenner, 2010), while the New Zealand population was estimated at 718 individuals based on a closed capture-recapture model (Barlow et al., 2018).

The drastic reduction in blue whale populations due to intensive whaling has likely led to significant demographic bottlenecks across these populations. The loss of genetic diversity associated with bottlenecks can have lasting negative impacts, which may hinder the species' ability to fully recover and adapt to changing environmental conditions (Gautschi et al., 2024; Imamoto et al., 2024). Despite their heavy depletion, blue whales are long-lived animals, with lifespans that can exceed 80-90 years (Sears & Perrin, 2018). This longevity complicates the assessment of the full impact of population bottlenecks, as the effects may take several generations to become apparent. Previous studies have struggled to detect the genetic consequences of these recent bottlenecks, likely due to the extended generation times of blue whales, limited regional sampling and, in some cases, the use of low-resolution genetic datasets (Attard et al., 2015; Chapter 3 of this thesis; Jossey et al., 2024; Westbury, Petersen, & Lorenzen, 2019).

This study aims to address these challenges by using whole-genome data of contemporary blue whales and powerful analytical tools to (1) re-assess phylogenetic relationships and population structure, (2) investigate recent demographic history, and (3) estimate genomic diversity, genomic load, and inbreeding. The results are interpreted in the context of evidence of whaling-induced bottlenecks and used to perform the first assessment of the genomic health for the species.

4.4 Methods

4.4.1 Data Sampling and Whole Genome Sequencing

Samples from 24 adult blue whales (*Balaenoptera musculus*) were selected from an existing genetic dataset of 276 whales based on tissue availability, DNA extraction quality, and population origin. The samples were previously utilised in a global study of population structure, where a small proportion of the genome was analysed using double digest Restriction site Associated DNA (ddRAD) sequencing (Attard et al., 2024, Chapter 3 of this thesis). Representatives of key genetic clusters previously identified by PCA and admixture analyses (Attard et al., 2024) were selected for this study. These samples were skin biopsies obtained following a standard protocol for free-ranging cetaceans, employing a small stainless-steel biopsy dart deployed via crossbow or rifle. Samples were chosen from across the Indian Ocean (pygmy subspecies; n = 10, including individuals from the coasts of Western Australia (AUS population) and New Zealand (NZ population); and Antarctic subspecies (ANT; n = 14) (Attard et al., 2024). Additionally, whole genome data from nine North Atlantic individuals (Jossey et al., 2024), one collected in 1938 in Antarctica (Jossey et al., 2024) and one from the North Pacific (Bukhman et al., 2024) were acquired from NCBI (www.ncbi.nlm.nih.gov) for a total number of 35 individuals (Table S4.1). These represent five populations of blue whales; noting only one representative for the North Pacific.

For samples representing the pygmy and Antarctic subspecies, genomic DNA was extracted using a modified salting-out protocol (Sunnucks & Hales, 1996), and their quality, integrity, and quantity were checked using a Nanodrop spectrophotometer (Thermo Scientific), 2% agarose gels, and a fluorometer (Qubit, Life Technologies), respectively. Libraries were prepared with the NEBNext Ultra II DNA library prep kit (New England Biolabs) and sequenced on the Illumina NovaSeq 6000 S2 platform (150 bp paired ends) using the commercial service provided by Novogene (Singapore). Sequencing coverage ranged from approximately 5x to 7x across most samples, except for one sample per population which was sequenced at a higher depth (~30×).

4.4.2 Bioinformatics

All the bioinformatics were done using DeepThought HPC (Flinders University, 2021). Reads from each individual were processed with the pipeline MELFUwgrs available from https://github.com/Yuma248/MELFUwgrs- using the blue whale chromosome reference genome of the Vertebrate Genome Project (GenBank assembly ID: GCF_009873245.2) (Bukhman et al., 2024). The reference genome was first indexed and based on the chromosome statistical assembly, the contigs and scaffolds that were not in the chromosomes were ignored as this represented less than 0.017% of the genome. Individual reads were processed by first merging files of the same sample, followed by using AdapterRemoval (Schubert et al., 2016) to trim residual adapter sequences, and eliminate low-quality stretches (Q < 20) at read ends. Filtered reads longer than 30 bp were aligned to the reference genome using the SNAP aligner (Zaharia et al., 2011). The resulting alignments were sorted by name, corrected for mate-pair information, sorted by coordinates, and duplicates were marked using SAMtools in parallel (Li et al., 2009). Repeat regions were then masked using BEDTools (Quinlan & Hall, 2010). Single nucleotide polymorphisms (SNPs) were genotype-called with ANGSD (Korneliussen et al., 2014) using a likelihood approach suitable for low-coverage genome sequencing, across multiple samples for a total of 4,988,583 linked SNPs.

4.4.3 Population Structure and Phylogenomic Analysis

Linkage disequilibrium (LD) decay is useful for inferring historical variations in population size (Park et al., 2021; Rosenberg & Nordborg, 2002). However, when analysing population structure, it is necessary to use unlinked SNPs to avoid redundant information and ensure an accurate representation of genetic differences within the population (Liu, Shringarpure, Lange, & Novembre, 2020). Linked SNPs were pruned using ngsLD and prune_graph.pl, considering a maximum distance of 50 kbp and a minimum r^2 of 0.8. We then removed SNPs with more than 20% missing data, resulting in a final dataset of 1,520,428 unlinked, pruned SNPs per individual.

To investigate genomic diversity and population structure among the 35 blue whale individuals, principal components analysis (PCA) was conducted using ANGSD (Korneliussen et al., 2014). The PCA, which summarises species identification and population structure, was derived from classic eigenvalue decomposition and visualised using the dplyr and ggplot2 packages in R (R Core Team, 2021; Wickham et al., 2016, 2023).

To assess admixture among the blue whale populations, ancestry proportions were inferred in ANGSD using NGSadmix, chosen for its robustness in handling low-depth sequencing data, reducing errors associated with potential ascertainment bias and uncertainty (Skotte, Korneliussen, & Albrechtsen, 2013). A minor allele frequency threshold of 0.05 was used, with the number of assumed populations (K) ranging from 1 to 6, with 10 iterations per K. Delta K was estimated in R and used to select the optimal number of ancestral populations. Individuals that did not clearly resolve into a single population were identified as putative hybrids if they exhibited a genetic composition roughly equal to half of each ancestral population in the admixture analysis.

To quantify genetic diversity, an in-house script 'callSFS.pl' available at https://github.com/Yuma248/MELFUwgrs-/tree/main/scripts was used to estimate the site frequency spectrum (SFS). This script employs ANGSD to calculate the SFS for each individual and population, and subsequently computes heterozygosity (He) and pairwise Fst values based on these SFS estimates. The script requires a popmap file to assign individuals to populations and an input folder containing the BAM files for all samples. Additionally, a list of pruned SNPs (e.g., Somatic_prunedSNPs.list) was provided to ensure that the SFS calculations were based on the unlinked SNPs, thereby minimising the effects of LD. This approach was critical for generating accurate estimates of genetic diversity across the sampled blue whale populations.

For the phylogenomic analysis, gene prediction and comparative genomics were conducted using the BUSCO v5 pipeline (Manni et al., 2021) with the Cetartiodactyla orthologous database OrthoDB v10 (Kuznetsov et al., 2023), following Genty et al. (2024; Chapter 2 of this thesis). BUSCO was employed using default parameters to identify gene sequences present in each genome.

Completeness of the genome assemblies was assessed by the proportion of detected orthologous genes. Only single copy genes present in all individuals were included, yielding a final set of 9,282 genes. Multiple nucleotide alignments of the identified genes were generated using PRANK (Löytynoja, 2014), and a partitioned file in nexus format was created to facilitate subsequent phylogenomic inferences.

To reconstruct evolutionary relationships among samples, a maximum likelihood phylogenomic tree was constructed using IQ-TREE (Minh et al., 2020), as per Genty et al. (2024; Chapter 2 of this thesis). The tree was rooted with Rice's whale (*Balaenoptera* ricei; available at DNAzoo.org: Balaenoptera_ricei_HiC) as the outgroup, and the best-fit model for each gene was determined using ModelFinder. The robustness of the resulting tree was evaluated with 1,000 UFBoot bootstrap replicates, and the tree was visualised using iTol (Letunic & Bork, 2021).

4.4.4 Demographic History and Inbreeding

Demographic history was analysed using two methods, Stairway Plot and GONe. Long-term (> a few hundred generations) historical changes in population size were inferred using Stairway Plot (Liu & Fu, 2020), which is a coalescent approach that reconstructs demographic history from the folded SFS data for each lineage. The analysis was run with default parameters, including singletons in the SFS, to maximise resolution for recent demographic events. The total number of SNPs was set as the parameter L and the number of generations to 150. The median effective population size (Ne) and 95% confidence intervals were plotted using the ggplot2 package in R.

To infer more recent (< 200 generations) changes in population size, the GONe software (Coombs et al., 2012) was employed. This analysis computes recent demographic history from patterns of linkages disequilibrium for each population. Each run of GONe used the recommended 40 replicates and maximum analysed value of c of 0.05. Given the unavailability of a cetacean rate, the recombination rate was set to 1 cM/Mb (Dumont & Payseur, 2008).

The average length and number of runs of homozygosity (ROH) were estimated to quantify inbreeding potentially related to the major demographic declines experienced by blue whale populations. The ROH were detected using a window-based approach in PLINK (Purcell et al 2007). A minimum length of 1 Mbp, containing at least 50 SNPs, with a minimum density of one SNP per 50 kb were required to identify a ROH. Scanning windows containing 10 SNPs were used, allowing up to two heterozygote sites and five missing calls to account for genotyping errors. The genomic inbreeding coefficient (FROH) was then calculated by dividing the total length of ROHs by the total length of autosomes, estimated at approximately 2371 Mb (Bukhman et al., 2024). This metric provides an estimate of the proportion of the genome that is autozygous and is indicative of recent inbreeding.

4.4.5 Genomic health

To assess genetic load, SNPs were called using the pipeline MELFUwgrs available from https://github.com/Yuma248/MELFUwgrs- and the Rice's whale genome (Table S4.1) as the reference, enabling the identification of derived and ancestral alleles. A total of 619,135 filtered, somatic, and unlinked SNPs were obtained. SnpEff (Cingolani, 2012) was employed to annotate and classify these SNPs based on their predicted functional impact on protein-coding genes. Deleterious alleles were defined as the derived alleles that cause a loss of function (LOF). Individual total genetic load was calculated as the proportion of deleterious alleles among the genotyped SNPs with predicted deleterious effects. Total load was divided into realised and masked components: realised load was calculated as the proportion of deleterious alleles in homozygosity, while masked load was calculated as the proportion of deleterious alleles in heterozygosity. The results were plotted with the ggplot2 package in R.

A polygenic risk score approach was also applied to evaluate the potential fitness impact of deleterious alleles, with masked alleles weighted less (30%) than realised alleles (70%). This weighting scheme was chosen based on the assumption that masked alleles (in heterozygotes) are less likely to affect fitness compared to homozygous realised alleles. To calculate the PRS, the effect sizes for each deleterious allele were summed across loci, following the method outlined in Dudbridge (2013), where PRS is calculated as the sum of the weighted effects of risk alleles at each locus. This method uses the Genome-Wide Association Study (GWAS) correlation between markers and traits. However, in the absence of GWAS data, there is no available correlation between individual markers and specific fitness traits, therefore an assumption of full correlation, where every deleterious allele is assumed to impact the trait equally, was used. Here, the assumption of full correlation produces a generalised load estimate, reflecting cumulative deleterious alleles without trait-specific nuances. Consequently, this PRS approach reflects an aggregate load pattern, rather than a trait-specific PRS based on empirical effect sizes. The assumption of normality was checked using visual inspection of Q-Q plots, which indicated that the PRS distributions for each population were approximately normal. The homogeneity of variances was assessed using Levene's test, which returned a non-significant result (p-value = 0.789). This confirmed that the variances across populations were roughly equal, justifying the use of an analysis of variance (ANOVA). An ANOVA was then performed to test for differences in PRS means across populations. Following this, Tukey's Honest Significant Difference (HSD) test was conducted for pairwise comparisons. These analyses allowed for a more accurate estimation of the effect of genetic load across populations.

4.5 Results

4.5.1 Phylogenetic Relationships and Population Structure

The analysis of whole-genome sequences confirmed the differentiation among known blue whale subspecies included in this study (Figure 4.1). Phylogenomic reconstructions based on orthologous genes (Figure S4.1 & S4.2) showed that the North Atlantic, North Pacific, Antarctic, and pygmy lineages form a single clade with smaller sequential groupings that largely correspond to individual subspecies. Most such groupings are supported by high bootstrap values (95-100%, with the lowest 74%) (Figure 4.1). Notably, the North Pacific sample appears to be more related to the Antarctic samples than the North Atlantic samples. Despite most samples from a region falling within the same clade, paraphyly was detected in some lineages, particularly between Antarctic and pygmy blue whales, suggestive of some gene flow between blue whales from these different regions (Figure 4.1).

The Principal Component Analysis (PCA) results provided support for five groups: Antarctic, North Atlantic, North Pacific, pygmy, and Antarctic vs pygmy hybrids, with the first 2 PCs representing 11.7% and 5.7% of the genetic variance (Figure 4.1). The greatest differentiation was observed between the North Atlantic and the pygmy lineages (see PC1, Figure 4.1). The PCA also indicated that the North Pacific sample is closer to the Antarctic than to the North Atlantic lineage.

The admixture analysis from NGSAdmix suggested that the most likely number of clusters was K = 6, based on the highest mean likelihood value of -53604131 (Figure S4.3). However, following the Evanno method, ΔK suggested K = 2, with the hybrids positioned between Antarctic and pygmy blue whale lineages (Figure S4.3). The Fst values ranged from 0.04 between Antarctic and Atlantic blue whales, to 0.09 between pygmy and Atlantic blue whales (Figure S4.4).

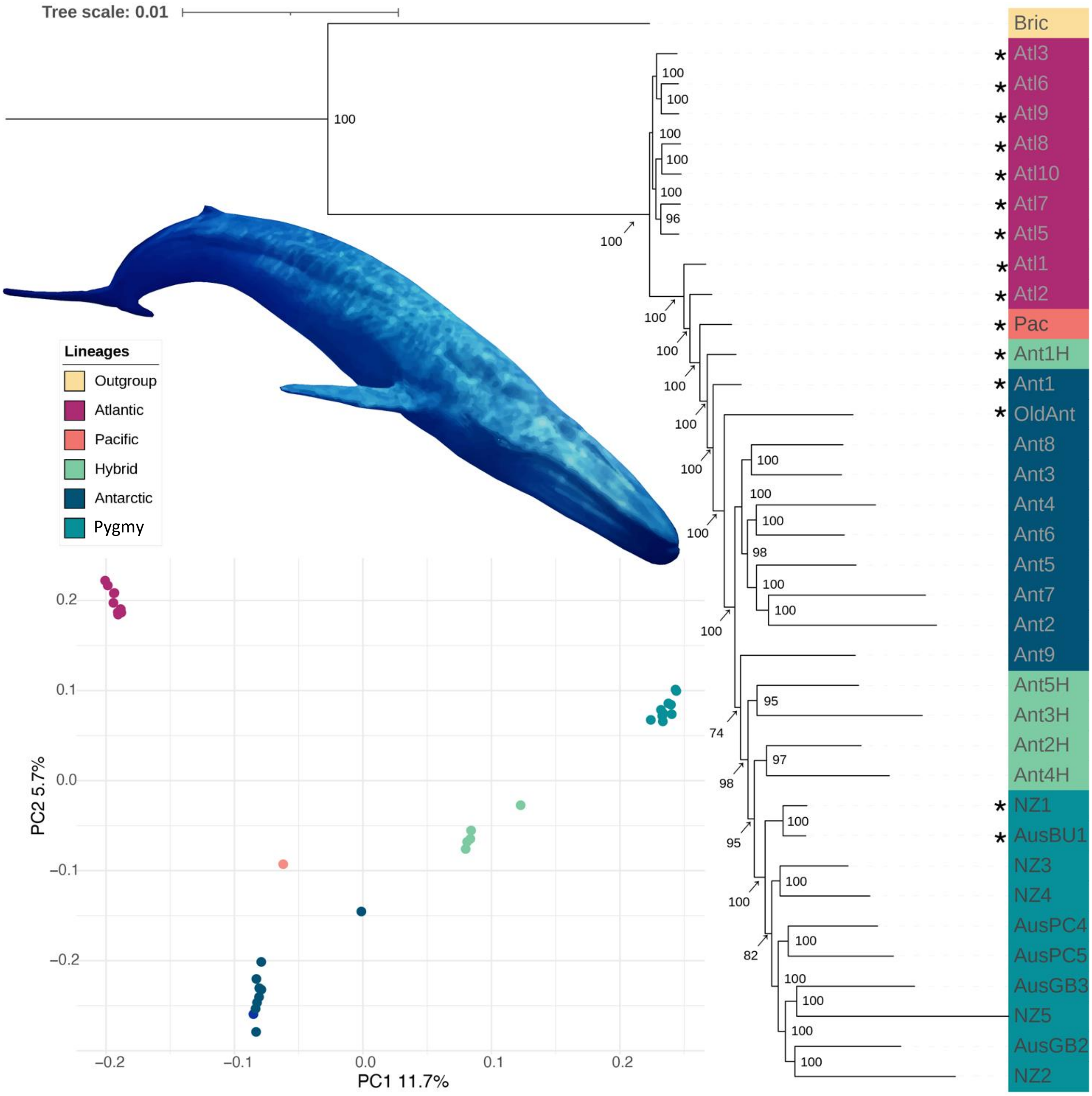


Figure 4.1. Phylogenomic tree and PCA results illustrating genetic relationships among blue whale lineages, which includes 9 Atlantic individuals, 1 Pacific, 10 Antarctic, 10 pygmy, and 5 hybrids (Antarctic and pygmy). **Phylogenomic Tree:** Based on 13,041 orthologous genes, using *B. ricei* as the outgroup. This tree shows the genetic relationships between the different lineages, highlighting the distinct clustering of each subspecies with the corresponding bootstrap values for each branch. The asterisk denotes samples with higher coverage (30x). **PCA:** Based on 1,520,428 unlinked pruned SNPs. The PCA plot visually shows the genetic differentiation between the lineages, showing clear separation among the Atlantic, Pacific, Antarctic, and pygmy blue whales, as well as the hybrids as an intermediate group.

4.5.2 Effective Population Size, Genetic Diversity and Inbreeding

The GONe analysis revealed a severe bottleneck in the Antarctic blue whale lineage, with the most pronounced decline in effective population size (Ne) coinciding with the peak of whaling activities at around 7-10 generations ago (Figure 4.2a). Additionally, there was no evidence of recovery in the Antarctic lineage, as they show little rebound in population size. In contrast, both the North Atlantic and pygmy blue whale lineages, while also showed steep reductions (between 13-27 generations ago in the North Atlantic, and between 27-38 generations ago in the pygmy), their Ne appear to be recovering in numbers. The North Atlantic blue whale, which initially experienced a sharp decline, showed signs of stabilisation in their Ne since around 9 generations ago (Figure 4.2b).Similarly, the pygmy blue whale, which include both the Australian and New Zealand populations, showed signs of recovery in Ne (Figure 4.2c), with stable values since around 20 generations ago. The Stairway plot analysis also provided evidence for distinct demographic trends among blue whale lineages (Figure S4.5), with the Antarctic displaying a continuous decline since the estimated divergence from the other lineages (Chapter 3 of this thesis) while the pygmy remained stable, and the North Atlantic exhibited recovery after the observed decline, before stabilising at approximately 100,000 years ago.

The average heterozygosity ranged from 0.19 (95% CI: 0.185 – 0.192) in pygmy blue whales to 0.24 (95% CI: 0.238 – 0.241) in Atlantic blue whales and 0.26 (95% CI: 0.255 – 0.264) in Antarctic blue whales. The highest value of 0.275 was observed for a sample collected in Antarctica in 1938 (OldAnt), during the peak of whaling (Figure S4.6). Moreover, the signals from the total number of ROH (NROH) and the sum of ROH lengths (SROH) (Ceballos et al. 2018) offered further insights into the demographic history and genetic diversity of blue whale lineages (Figure 4.2d). The SROH and NROH values show a high correlation coefficient ($\rho = 0.98$), with relatively low values for Antarctic, North Atlantic, and potentially North Pacific (noting that only one sample was used for this lineage). Overall, blue whales exhibited low FROH (mean across all lineages = 0.0232), with the pygmy lineage exhibiting a greater FROH (mean = 0.073, range:0.061-0.086) compared to the other lineages (Figure S4.7).

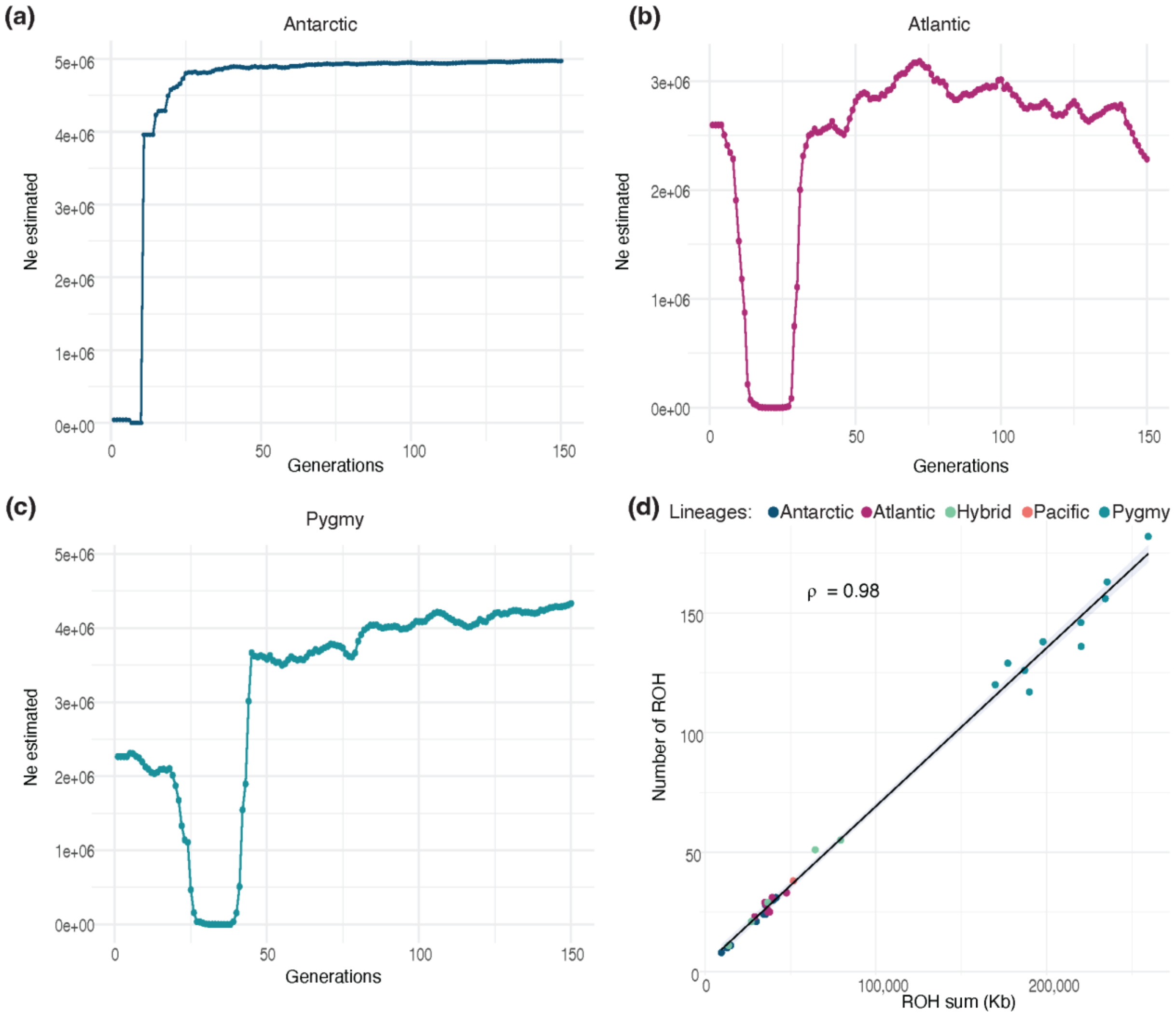


Figure 4.2. (a), (b) and **(c)** Effective population size estimates on recent generations using software GONe, showing the Ne estimates across generations, providing insights into the demographic history of the different lineages. **(d)** Comparison of the number of Runs of Homozygosity (ROH) against the total sum length of ROH across autosomes. Each dot represents an individual sample, with lineage indicated by different colours. The pygmy lineage, with more and longer ROHs, suggests a smaller population size relative to the other lineages**.**

4.5.3 Genetic Load

Low levels of genetic load were detected for all blue whale lineages, with mean values of 0.129 for masked load, 0.026 for realised load, and 0.155 for total genetic load (Figure 4.3a). Pygmy blue whales exhibited a slightly higher masked load (mean = 0.135, range = 0.121–0.148) and realised load (mean = 0.028, range = 0.014–0.0461), resulting in a higher total genetic load (mean = 0.1627) than Antarctic (mean = 0.147) and North Atlantic (mean = 0.150) blue whales. The hybrid individuals also showed a higher total genetic load (mean = 0.162), primarily driven by the proportion of masked alleles (mean = 0.1376). Nonetheless, the ANOVA test revealed no significant differences in PRS across the different lineages (p-value = 0.323). The relationship between the inbreeding coefficient (FROH) and genetic load (Total, Realised, and Masked) across blue whales (Figure 4.3b), indicated that as FROH increases, total and masked genetic load decrease. In contrast, realised genetic load slightly increased with higher FROH values, suggesting that as inbreeding rises, more deleterious alleles become exposed.

4.6 Discussion

4.6.1 Evolutionary Lineages and Genetic Structure

The observed genetic differentiation among blue whale lineages aligns with previous studies that identified distinct subspecies and populations based on genetic, morphological and ecological research (Attard et al., 2024; Committee on Taxonomy, 2022; Cooke, 2018; Ichihara, 1966). Phylogenomic reconstructions support the presence of multiple blue whale lineages, but evidence of paraphyly suggests that gene flow among them have persisted through time, indicating incomplete reproductive isolation and confirming their classification at subspecies level. However, for the North Atlantic and North Pacific blue whales, which are currently classified as a single subspecies, the genetic distinctiveness was comparable to that between recognised subspecies. Further studies based on a larger North Pacific sample are required to substantiate these findings. The close relationship between Antarctic and pygmy blue whales suggests a more recent divergence between them compared to other lineages (Attard et al., 2024; Chapter 3 of this thesis) and that gene flow has persisted despite their geographical and ecological distinctions. The presence of hybrids between these lineages further supports incomplete reproductive isolation, even though these lineages have been historically separated. For example, sample AntH1, classified as a hybrid, appears to be closely related to Ant1 (an Antarctic individual).

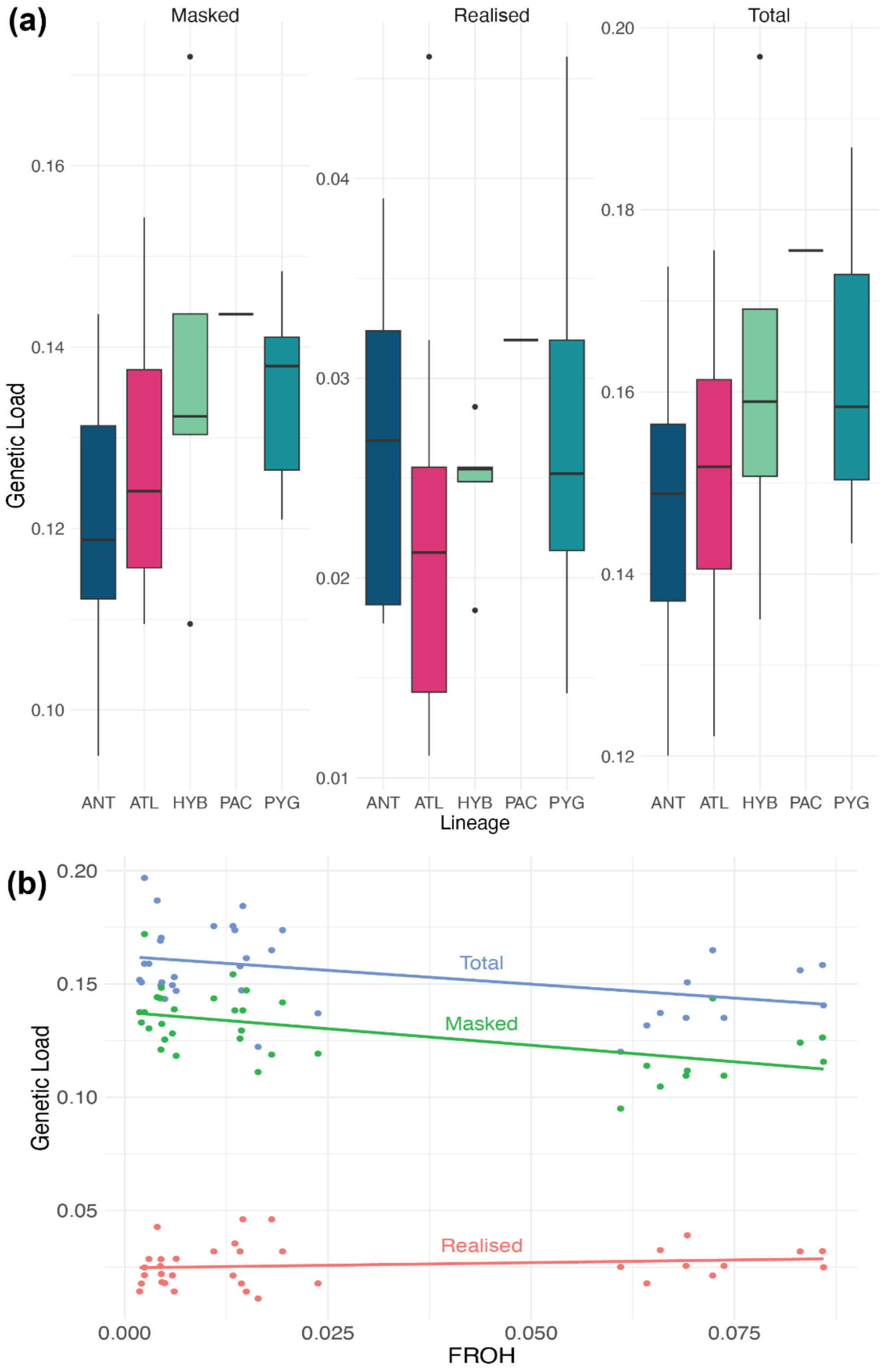


Figure 4.3.(a) Boxplot of the distribution of genetic load (Total, Realised, and Masked) across the different blue whale lineages. Each population (Antarctic, Atlantic, Australian and New Zealand) is grouped per geographic area to highlight differences in the genetic load across lineages, with ANT being Antarctic, ATL being Atlantic, HYB being Hybrid, PAC being Pacific and PYG being the Pygmy. **(b)** Scatter plot showing the relationship between the FROH (inbreeding coefficient) and genetic load (Total, Realised, and Masked) in blue whales. Linear regression lines represent the trend for each genetic load component across increasing FROH values. Total load is represented by the blue line, Realised load by the coral line, and Masked load by the green line.

The retention of Antarctic ancestry in hybrids like AntH1 could contribute to the observed pattern (Schumer et al., 2014). Alternatively, this could be due to the higher coverage of these particular samples (both ~30x), which might influence their alignment and cause them to cluster more closely in the phylogeny (Taylor & Piel, 2004). Another potential explanation is due to the stochastic nature of the admixture process, which might result in hybrids aligning more closely with one parental lineage in certain genomic regions (Gogoi et al., 2013; Schumer et al., 2020). The presence of hybrids and paraphyletic lineages indicates occasional gene flow between them, suggesting that while these groups might have been historically isolated (Attard et al., 2015), complete reproductive isolation has not occurred. This finding is consistent with broader patterns observed in other migratory cetacean species, where geographic separation does not necessarily prevent genetic exchange (Louis et al., 2021; Pratt et al., 2022), and their classification at the subspecies level. This underscores the complexity of blue whale population structure, where genetic lineages may not always correspond to clear-cut clusters due to relatively recent divergence (Ferguson, 2002) and/or continuous gene flow (Chesser, 1991) at low to moderate levels across populations.

The genetic structure found in this study, likely reflects historical isolation due to distinct geographic ranges and ecological niches (Attard et al., 2015; Jossey et al., 2024). However, the high conservation of ancestral polymorphism in cetaceans has been shown to contribute to complex genetic structure (Foote et al., 2019; Jamie & Meier, 2020). This is further supported by the low Fst values and the difference between K values observed in the admixture analyses. Additionally, the PCA results and phylogenomic tree highlight a genetic affinity between the North Pacific and Antarctic blue whale populations, suggesting a closer relationship than that observed with the North Atlantic animals. Ancient genetic variants shared by a common ancestor may persist across lineages, leading to unclear contemporary relationships. However, it is important to consider the possibility of ongoing gene flow between the North Pacific and Antarctic lineages. These populations are not entirely geographically isolated, and occasionally interbreed in the South Pacific, which to some extent may contribute to their genetic similarities (Attard et al., 2024; LeDuc et al., 2017).

4.6.2 Impact of Whaling on Effective Population Size and Inbreeding

The reduction in Ne observed across all lineages, particularly in the Antarctic, supports historical records of severe overexploitation due to whaling. This reduction is evident based on the linkage disequilibrium method in GONe (Coombs et al., 2012), which reconstructed the recent demographic history of the populations. The timing of genetic bottlenecks in this analysis corresponds to the peak of whaling activities, further underscoring the significant impact that whaling has had on the long-term genetic make-up of blue whales. The Antarctic blue whale lineage exhibited the most pronounced bottleneck, with a steep decline in Ne estimates around the time of peak whaling activities. The Ne of the North Atlantic and pygmy blue whale lineages also displayed notable reductions, although to a lesser extent than the Antarctic. The pygmy blue whale, though historically smaller and more isolated, appears to be recovering more steadily. The North Atlantic and pygmy lineages seem to have higher Ne to support a gradual rebound, possibly due to the less severe impacts from historical whaling compared to the Antarctic. These findings align with historical records documenting the near-extirpation of the Antarctic blue whale due to whaling (Branch et al., 2008) and the reported number of catches for this lineage from the IWC database (Allison, 2020). Their slow recovery is likely supported by the relatively high levels of heterozygosity, low inbreeding rates and low genetic load observed, all of which may have helped preserve enough genetic diversity to allow for the gradual recovery. In the case of Antarctic blue whales, for instance, an estimated annual recovery rate of 7.3% has been observed since protections were implemented (Branch et al., 2004).

Long-lived mammals like blue whales have long generation times and overlapping generations (Sears & Perrin, 2018). The extended period between generations means that changes in genetic diversity, such as those caused by reductions in population size, are reflected more slowly in the genetic variation of a population, subspecies or species (Luikart & Cornuet, 1998). This slow turnover can buffer the immediate effects of genetic drift or inbreeding, thereby maintaining higher levels of heterozygosity even after significant population declines (Hoffmann et al., 2017). In fact, while previous studies on other mammals have indicated lower levels of heterozygosity, blue whales exhibit much greater diversity on a per-site basis compared to bowhead whales, belugas, pilot whales, and other cetaceans (see Figue 4 from Cerca, 2022). The heterozygosity values estimated here suggest that the effects of the bottleneck may not yet be fully visible in the lineages. With long generation times, it may take several more generations for the consequences of historical exploitation to be reflected in the genetic diversity of the species (Torres-Florez et al., 2018). The results from the GONe analysis further support this notion, demonstrating a significant bottleneck in the Antarctic lineage that aligns with the whaling period. While the low presence of masked and realised deleterious mutations suggests a level of genetic resilience, it does not negate the overall impact of the bottleneck. Rather, it highlights a complex interaction between historical population declines and the current genetic structure, indicating that while some genetic diversity may have been retained, the long-term consequences of past exploitation are still critical to understanding the future of blue whale populations and subspecies.

Additionally, the ROH values observed for Antarctic, North Atlantic, and potentially Pacific lineages, suggest that these groups may have retained greater genetic diversity over time, reflecting larger historical population sizes compared to the smaller population of pygmy blue whales. For pygmy blue whales, previous studies indicated that their lineage likely diversified after splitting from the Antarctic blue whale, a process thought to have been driven by geographic isolation due to various periods of glaciations (Attard et al., 2015; Chapter 3 of this thesis), which may have led to lower genetic diversity and/or inbreeding within this lineage. Relatively small populations tend to exhibit higher ROH levels due to reduced genetic diversity (Ceballos et al. (2018). This is expected in populations that have experienced bottlenecks or prolonged periods of reduced population size. Despite this elevated number and longer ROH, it is notable that the ROH distribution in pygmy blue whales does not show a plateau suggestive of inbreeding, where long ROH segments accumulate as a result of mating between closely related individuals. This pattern was further investigated in the current study by assessing FROH values of each lineage, which were found to be relatively low across all of them, indicative of negligible inbreeding at the population and subspecies level.

4.6.3 Genetic Potential for Recovery

Pygmy blue whales exhibited a higher proportion of total genetic load compared to other lineages, which may be attributed to their smaller population size and relative geographic isolation (e.g., van der Valk, de Manuel, Marques-Bonet, & Guschanski, 2019). In smaller populations, the efficacy of natural selection in purging deleterious variants decreases, allowing these alleles to persist (Dussex et al., 2023). The absence of significant inbreeding and high heterozygosity levels indicates that pygmy blue whales have so far avoided the detrimental effects often associated with reduced genetic variation.

Genetic load was low for all blue whale lineages, which is consistent with findings in other cetaceans. For example, the vaquita (*Phocoena sinus*), a species that experienced a severe population decline, showed a low burden of deleterious variation due to its historical rarity, suggesting that such species may be less impacted by inbreeding depression than expected (Robinson et al., 2022). Similarly, in the Gulf of California a population of fin whales (*Balaenoptera physalus*), which was not targeted by industrial whaling, retained sufficient genetic diversity despite being small and isolated, demonstrating the importance of migration in maintaining population viability (Nigenda-Morales et al., 2023). These examples and the low genetic load found in blue whales suggest that, despite their historical overexploitation, these lineages retained enough genetic variation to support recovery, especially with ongoing conservation. However, an increase in realised genetic load with higher levels of inbreeding (FROH) could expose more deleterious alleles in the future. This highlights the importance of maintaining effective population sizes and limiting further inbreeding to ensure the long-term viability of these subspecies and populations (Amos & Balmford, 2001).

Hybrid individuals, which contain genetic material from both Antarctic and pygmy lineages, could play a role in the recovery of these groups. Hybridisation between the Antarctic and pygmy blue whale lineages could mitigate the genetic load in the pygmy population and could help alleviate some of the genetic losses incurred during the bottleneck, potentially enhancing the adaptive capacity of the Antarctic population. Since the Antarctic population maintains higher genetic diversity, hybridisation could introduce beneficial alleles into the pygmy lineage and reduce the impact of harmful variants. However, hybrids were found to have a higher proportion of masked deleterious alleles than the Antarctic, posing a potential risk to their fitness (Bertorelle et al., 2022; Björklund, 2013). Continued genomic monitoring will be essential to track changes in genetic diversity, gene flow, and population health, providing valuable insights into the long-term viability of blue whale recovery and informing conservation management strategies..

4.6.4 Conservation Implications

Findings from this study highlight the critical need for comprehensive conservation strategies to protect blue whale subspecies and populations, emphasising the preservation of genetic diversity and the protection of their critical habitats. The severe reduction in population sizes underscores the urgency of continued protection, such as the moratorium on commercial whaling implemented by the International Whaling Commission (IWC) in 1986 (Smith, 1983), which remains vital for species recovery. Moreover, understanding migratory routes and protecting feeding areas, such as those dependent on Antarctic krill and other species important for the subspecies, is crucial as overfishing currently threatens key feeding grounds (Savoca et al., 2024). The evidence of admixture between lineages suggests that maintaining gene flow could benefit the long-term survival of blue whales. Conservation strategies should therefore prioritise protecting habitats that facilitate inter-lineage interactions.

Considering blue whales' long lifespan and slow reproductive rate, the genetic consequences of historical whaling may continue to affect populations for generations. This reinforces the importance of long-term conservation efforts that account for the slow pace of genetic recovery. Other cetacean studies have shown the impact of whaling on species' genetic diversity. For example, in the eastern Pacific grey whale, a significant population decline during commercial whaling led to a substantial reduction in genetic diversity (Alter et al., 2012). Alter and colleagues (2012) demonstrated that the eastern Pacific gray whale population was reduced by up to five times its historical abundance due to whaling, resulting in a genetic bottleneck that continues to affect the species' recovery today. Similarly, Crossman and collaborators (2023) found that while the Southwest Atlantic southern right whale population has experienced less genetic impact, the North Atlantic population remains severely affected by historical whaling, exhibiting reduced genetic diversity and higher inbreeding levels. However, some other species, such as the North Atlantic fin whale population, show that while the genetic diversity of the population has remained relatively stable, it still presents signs of past genetic bottlenecks (Wolf et al., 2022). Despite this, the fin whale’s genome is less impacted compared to other whale species. These studies highlight the importance of ongoing conservation efforts to ensure the recovery of species with similar demographic histories. Continued genomic monitoring will be essential to assess the ongoing impacts of past exploitation and to inform adaptive management strategies that can support the resilience of subspecies and populations of species such as the blue whale, as well as other baleen whales in a rapidly changing world.

4.7 Conclusions

The genetic structure of blue whales reflects a complex interplay of historical isolation and ongoing gene flow among distinct lineages. The presence of hybrids and the paraphyletic nature of these lineages suggests that while geographical and ecological distinctions exist, complete reproductive isolation has not occurred. This gene flow may play a crucial role in mitigating genetic load, enhancing their adaptive capacity in the face of ongoing environmental changes.

The impacts of past whaling on the effective population size and genetic diversity of blue whale populations highlight the need for ongoing conservation efforts. Despite the severe declines, the observed genetic diversity provides a foundation for potential recovery, but the slow generation times of these mammals need a long-term commitment to monitoring and protecting their habitats. Therefore, sustained genomic monitoring and adaptive management strategies should be prioritise to ensure the resilience and long-term survival of blue whale populations in an ever-evolving marine ecosystem.

4.8 Data Availability Statement

The genomic data produced in this chapter will become available in figshare and NCBI at the time of manuscript acceptance: link to be provided.

Chapter 5

BEYOND THE BLUE: General Discussion

Geographe Marine Research

Marine ecosystems, with their few physical barriers, offer unique opportunities to study evolutionary adaptations in the sea (Räsänen & Hendry, 2008). These adaptations are vital for improving a species' fitness in the marine environment (Rose & Lauder, 1996). Cetaceans exemplify these adaptations, having transitioned from terrestrial to aquatic environments over millions of years (McGowen et al., 2014). Despite significant advancements in genomics of cetaceans (e.g. Endo et al., 2018; Tian et al., 2016; Tsagkogeorga et al., 2015; Yim et al., 2014), research gaps remain in understanding various aspects of their evolution. These include knowledge gaps about genomic drivers of adaption in baleen whales, impacts of past environmental changes and threats such as exploitation and climate disruptions, and population resilience in face of emerging challenges.

This thesis investigated the evolutionary genomics of the Balaenopteridae family, with a focus on the blue whale (*Balaenoptera musculus*). The aim was to explore the molecular mechanisms driving species diversification and genomic responses to both environmental and anthropogenic pressures. It builds on previous studies to assess how genomic regions subject to natural selection promoted adaptation to varying environments for balaenopterids. Altogether, this study addressed several gaps in our understanding of the evolutionary and conservation genetics of balaenopterids, particularly the blue whale, including how past environmental changes and more recent threats have impacted on this species.

5.1 Evolutionary Genomics and Adaptive Radiation in Baleen Whales

The evolutionary history of cetaceans, particularly the Mysticeti suborder, is characterised by significant radiation events, including the transition to an aquatic environment and the emergence of baleen filtering (Steeman et al., 2009; Stiefel, 2021). These evolutionary shifts have facilitated the diversification of cetaceans across a wide range of habitats, making them a valuable system for studying the genomic basis of adaptive radiation. Additional evolutionary radiations have been proposed within specific lineages, such as balaenopterids, which further contributed to their ecological diversity (Árnason et al., 2018; McGowen, Tsagkogeorga, Williamson, et al., 2020).

Comparative genomic studies between cetaceans and terrestrial species have identified specific genetic adaptations enabling cetaceans to overcome the challenges associated with aquatic life, such as adaptations related to locomotion, sensory systems, thermoregulation, osmoregulation, and pathogen resistance (Arregui et al., 2021; Tian et al., 2019; Wang et al., 2008; Yim et al., 2014; Yuan et al., 2021). The often large and diverse oceanic populations of cetaceans allow for significant genetic variation, although high gene flow may limit the segregation of adaptive alleles, complicating local adaptation and speciation, particularly in species inhabiting divergent environments (Kelley et al., 2016; McGowen et al., 2014). This intricate interplay between genetic variation, adaptation, and environmental pressures, underscore the complexity of evolutionary processes in cetaceans. Additionally, genes under positive selection have been shown to drive adaptive phenotypic evolution and contribute to fitness outcomes (Chase et al., 2021; Fan et al., 2019; Qiu et al., 2012). High-quality genomes from a range of evolutionary lineages have further enabled studies of adaptation at multiple levels, including lineage, gene, and molecular pathways (Themudo et al., 2020). However, understanding the diversification processes in the radiation of balaenopterid remains challenging (Norris & Hull, 2012).

The Balaenopteridae family demonstrates remarkable evolutionary adaptation to marine environments, with specific genomic changes supporting their diversification (McGowen, Tsagkogeorga, Williamson, et al., 2020; Steeman et al., 2009; Sun et al., 2022). Previous studies have identified positively selected genes (PSGs) for the immune system, lipid metabolism, and thermoregulation (Shen et al., 2012; Tejada-Martinez et al., 2021; Yim et al., 2014), as well as metabolism and sensory perception (Chikina et al., 2016; Yuan et al., 2021). However, many of these studies focused on small gene sets. The current study (Chapter 2) expands the analysis to a larger set of orthologous genes, using 10,159 orthologous genes from 17 high-quality artiodactyl genomes. This broader approach has significantly increased the identification of genes likely to be adaptive, with 2,659 novel PSGs disclosed, representing more than a twentyfold increase compared to the 120 previously known PSGs (Table S2.2). The results provide greater insights into bodily systems impacted throughout the evolution in balaenopterids.

The findings of Chapter 2 expand on previous studies (Cao et al., 2021; Ding et al., 2022; Keane et al., 2015; Kishida & Thewissen, 2012; McGowen, Tsagkogeorga, Williamson, et al., 2020; Nam et al., 2017; Seim et al., 2014; Sun et al., 2019; Tollis et al., 2019; Tsagkogeorga et al., 2015; Wang et al., 2015; Xu et al., 2012; Yim et al., 2014; Zhou et al., 2018) by outlining how *Balaenopterids'* species have evolved by undergoing significant positive selection on genes linked to gigantism, enhanced diving capacities, migration, and specific reproductive strategies. These adaptations enable these species to manage environmental challenges and reproductive demands, offering a comprehensive view of the evolutionary processes that have shaped their survival in distinct marine ecosystems. The findings highlight broader evolutionary patterns, such as the recurrent selection of genes related to stress resistance, oxygen management, and immune function, for example BZW2, CD55, EPO, ERG, GIF and SETX. These genomic changes have enhanced the species' ability to adapt to environmental challenges like hypoxia and food scarcity, reinforcing their ecological significance (Beichman et al., 2019; Kelley et al., 2016).

The evolution of body mass in cetaceans, driven by factors such as aquatic buoyancy, diet, and thermoregulation, has proceeded more rapidly than in their terrestrial relatives (Evans et al., 2012; Montgomery et al., 2013). Therefore, the identification of PSGs associated with traits like gigantism and cancer resistance underscores the role that body size has played in the diversification of balaenopterids (Chapter 2). Large body sizes in species such as the blue whale appear to be linked to evolved mechanisms for DNA repair and cell cycle regulation, supporting Peto's paradox, which posits that larger animals tend to exhibit lower cancer rates despite their size (Peto et al., 1975). Research into cetacean genomes has suggested that whales may possess unique cancer resistance mechanisms, providing a model for studying the genetic basis of body size, aging, and cancer resistance (Sun et al., 2022; Tejada-Martinez et al., 2021; Tollis et al., 2019). The identification of PSGs related to DNA repair and cell cycle regulation (Chapter 2) related to the term of 'growth/size/body region phenotype' further supports the hypothesis that blue whales may have evolved unique anti-cancer mechanisms. However, the absence of enrichment for body size-related genes in other species may be due to incomplete genome assemblies, differences in annotation quality, or the possibility that large body size in baleen whales may have originated multiple times during their evolutionary history (Fordyce & Marx, 2018), highlighting areas for future research.

Diving capabilities in cetaceans are another adaptive trait in their evolution, with baleen whales exhibiting varying depths and durations of dives (Würsig et al., 2018), which expose them to cellular stress, including hypoxia and oxidative damage (Fabrizius et al., 2016). Several PSGs related to stress resistance, such as PRDX6, SOD1, and GSTP1, have been identified in cetaceans, enabling them to cope with hypoxic conditions during deep dives (Yim et al., 2014). Enrichment in genes related to homeostasis and blood regulation, for instance ABCG8, ADORA2B, DOK7 and YTHDF2 genes, observed in blue, fin, gray, and edeni whales, further indicates that these species have evolved specialised adaptations to maintain physiological stability during prolonged dives (Chapter 2; Yim et al., 2014). While odontocetes have superior diving abilities, baleen whales may have evolved different adaptations—such as enhanced myeloid cell morphology and haematopoiesis-related genes—to strengthen their homeostasis response and cope with the pressures of diving in pursuit of their prey (Tian et al., 2016; Yim et al., 2014). Positive selection was also observed on genes related to preweaning lethality, such as ACO2, AIFM1, DENND1C and TAT, reflecting adaptations aimed at reducing calf mortality during vulnerable developmental stages. Preweaning lethality represents a significant risk for balaenopterid populations, as calves depend heavily on maternal energy reserves during nursing (Agbayani et al., 2020; Fortune et al., 2021). The presence of PSGs linked to this trait highlights the evolutionary pressures acting on reproductive success and the importance of maternal condition in shaping offspring survival.

The use of whole-genomes facilitated the investigation of species-specific adaptations, such as lunge-feeding and skim-feeding techniques, as potential drivers of further speciation within the group. For instance, gray whales display positively selected genes associated with renal function and mineral homeostasis, including AKAP11, BMP7, BTD and CCK genes, which may help them maintain water and salt balance in their benthic feeding habitats, despite the challenges posed by high salinity and sediment-laden environment (Ortiz & Suzuki, 2024). Humpback whales also exhibit PSGs related to sensory coordination and movement, like the genes ANK, ARHGAP32, DMRT3, PUM2 and RAB10, which may support their complex foraging techniques and social behaviours, including vocal communication and mating strategies (Chu & Nieukirk, 1988; Fish & Battle, 1995), both of which are essential for their survival and reproductive success.

The specialisations observed within the balaenopterids draw attention to how environmental pressures have shaped the genomic adaptations of species, enhancing their fitness. However, as climate change accelerates alongside various anthropogenic factors—such as pollution and other human-induced stressors (Kennish et al., 1984; Kovalenko, 2019; Popper & Hawkins, 2016)—a deeper understanding of these adaptive mechanisms becomes essential. The survival of these species in the face of rapid environmental changes is increasingly linked to their genetic diversity and adaptive capacity (Franks & Hoffmann, 2012; Grummer et al., 2019). Therefore, gaining greater insight into how historical and contemporary environmental factors, along with human activities, influence the genetic diversity and population structure of a species such as blue whales—examined in this thesis—underlines the importance of continued research and conservation efforts.

5.2 Speciation and Divergence of Blue Whales

Historical climatic events and human-induced changes have profoundly influenced the genetic divergence and population structure of cetaceans (e.g. Amaral et al., 2012; Ben Chehida et al., 2020; Cabrera et al., 2022; Carroll et al., 2019). Geographic isolation due to environmental shifts, combined with reduced gene flow between populations, is a primary driver of speciation (Räsänen & Hendry, 2008), and this is evident in the divergence of blue whale subspecies. Chapter 3 demonstrates how environmental fluctuations, such as those during the Pleistocene, have shaped the diversification of blue whale lineages by affecting genetic connectivity. These processes highlight how species' responses to past environmental pressures, such as ice accumulation and reductions in primary productivity, have led to distinct genetic patterns in three modern blue whale lineages (Chapter 3). Through a coalescence analysis of 16,661 SNPs from 275 individual blue whales, this study identified three distinct lineages, with divergence events occurring approximately 300,000 and 200,000 years ago. This divergence aligns with periods of global ice accumulation and diminished primary productivity, which likely impacted connectivity and contributed to the observed genetic differences among lineages. The results stress the influence of historical climatic oscillations and decreased food availability on the genetic variation and connectivity of blue whales. Understanding these historical influences is crucial for assessing the adaptive potential of blue whales in the face of ongoing climate change. For example, protecting dynamic marine habitats, including migratory corridors and feeding grounds essential for seasonal behaviours, is crucial for mitigating the impacts of shifting temperatures and prey distributions while maintaining population connectivity (McGuire et al., 2023; Theodoridis et al., 2020). Additionally, genetic and ecological monitoring enables conservationists to track adaptive capacity, implementing flexible management practices that can respond to rapid ocean changes (Theodoridis et al., 2020). By recognising the role of past climatic events in shaping genetic diversity, conservation strategies can be better informed to mitigate the impacts of current and future environmental shifts.

Moreover, the genetic structure observed in blue whale populations reveals complex evolutionary patterns driven by both historical isolation and ongoing gene flow. Despite the distinct lineages identified—such as Antarctic, pygmy, North Atlantic and North Pacific—evidence of incomplete reproductive isolation, particularly between Antarctic and pygmy blue whales, suggests that gene flow has persisted throughout their divergence (Chapter 3 & 4). The presence of hybrid individuals further illustrates that geographic and ecological boundaries do not completely prevent genetic exchange, indicating that evolutionary divergence is ongoing rather than fully complete. Additionally, the close genetic relationship between Antarctic and North Pacific blue whales raises questions about some of the current taxonomic classifications (Chapter 4). The genetic proximity may reflect a combination of ancient, shared ancestry and sporadic interbreeding through intermediary populations in the South Pacific, reinforcing the need for a re-evaluation of blue whale subspecies distinctiveness by including genomic evidence.

5.3 Genomic Health and Adaptation of Blue Whale Lineages

Human activities, particularly the intensive whaling in the 19th and 20th centuries, led to dramatic blue whale population declines that have left lasting imprints on the species' genetic landscape, and as presented in Chapter 3, environmental factors have also shaped the species’ history. By using whole-genomes, further evaluation on the species’ genomic health reveals that while blue whales generally retain relatively high diversity, certain populations, such as the pygmy blue whales, exhibit reduced genetic variation and small population size (Chapter 4). These patterns suggest that some lineages may be more vulnerable to future challenges, such as inbreeding, disease outbreaks or prey shortages, making them particularly susceptible to environmental and stochastic events (Lancaster et al., 2022; Chapter 4).

The analysis of PSGs in blue whales uncovered traits likely critical to their long-term survival (Chapter 2). Genes involved in immune function, stress response, and body size regulation show evidence of positive selection, possibly reflecting adaptations to deeper diving, long migrations, and the cold-water environments they inhabit (Tollis et al., 2019; Chapter 2). For instance, enhanced oxygen management and hypoxia tolerance—traits linked to the PSGs disclosed here—might enable blue whales to dive at depths of up to 500 meters (Calderan et al., 2023), which is rare within the balaenopterid family (Table 1.1 of Chapter 1). Additionally, the discovery of PSGs associated with gigantism and increased immune health supports the hypothesis that blue whales, as the largest animals on Earth, have evolved unique mechanisms for DNA repair, immune responses and cell cycle regulation. These adaptations may help mitigate cancer risks, support tumour suppression, and protect against other diseases—an unexpected advantage considering their massive size and long lifespan (Sun et al., 2022; Chapter 2). These findings emphasize the need for ongoing genomic research, particularly in smaller populations like the pygmy blue whales, which are characterised by their smaller body size compared to other subspecies and display reduced genetic diversity compared to the other lineages (Chapter 4). Despite these challenges, the pygmy blue whale lineage, while smaller and more geographically isolated, appears to have been less severely impacted by historical whaling. Elevated runs of homozygosity (ROH) in this population more likely reflect their long history of isolation and smaller population size (Chapter 4). Yet, despite these limitations, the absence of a signal of inbreeding suggests that their genetic structure has remained stable, with no widespread deleterious effects emerging so far.

Encouragingly, the potential for genetic recovery in blue whale populations is supported by a low realised genetic load across all lineages, even in the face of past severe decline of the populations. Natural hybridisation between Antarctic and pygmy blue whales might offer a potential mechanism to enhance genetic diversity, especially for the smaller pygmy population. By introducing beneficial alleles from the more genetically diverse Antarctic lineage, hybridisation could help mitigate the effects of historical genetic drift and loss of genetic diversity (Adavoudi & Pilot, 2021). However, there are risks associated with hybridisation. The persistence of masked deleterious alleles in hybrids is a potential concern, as these harmful genetic variants could become problematic if population sizes stay low or decline even further, or inbreeding increases. Such scenarios could undermine long-term recovery efforts. Thus, while naturally occurring hybridisation presents a promising opportunity to boost genetic resilience, it also highlights the need to maintain large, stable populations. Without sufficiently large population sizes, the expression of deleterious alleles could threaten future generations (van der Valk et al., 2019).

Recent demographic reconstructions further emphasized the severe impact of whaling, particularly on Antarctic blue whales, which suffered a pronounced genetic bottleneck aligning with the height of hunting activities (Branch et al., 2008). Although all blue whale lineages showed reduced genetic diversity, the Antarctic population experienced the steepest decline in effective population size (Ne) (Chapter 4). The relatively high levels of heterozygosity across all lineages suggest that enough genetic variation persists to support ongoing recovery efforts. Nevertheless, while heterozygosity is often favoured as an indicator of genetic health and resilience, it is less sensitive to the loss of specific alleles during bottlenecks. This means populations can maintain heterozygosity while still losing valuable allelic diversity, which is essential for long-term adaptability (Allendorf, 1986; Allendorf et al., 2008). However, the low levels of genetic load and negligible inbreeding in these populations support sufficient genetic variation for the potential recovery of all lineages.

As these findings illustrate, the road to full genetic recovery for blue whales is complex and fraught with challenges. However, with effective conservation strategies, there is hope. Maintaining large, stable populations is critical—not only to prevent the realisation of deleterious genetic variants but also to ensure that blue whales remain resilient in the face of future environmental changes. The continued protection and monitoring of these species are needed to preserve both their genetic health and their ability to adapt to a rapidly changing world.

5.4 Importance of Genomic Data for Species Conservation

Overexploitation of species has often been driven by economic incentives, where the rarity of a species increases its market value, making its extinction a profitable outcome (Diamond et al., 1997). This pattern has been observed in various industries, such as fishing, hunting, and logging, where high-value species are initially targeted (Dulvy et al., 2003; Grogan et al., 2008; Rowcliffe et al., 2003). As these species become rare, their economic worth escalates, further intensifying exploitation (Almeida et al., 2023). In multispecies exploitation systems, a process known as "opportunistic exploitation" can occur, where the decline of a highly valued species leads to increased harvesting of more common but less desirable species (Branch et al., 2013). This shift effectively subsidises the extinction of the valuable species, as they continue to be exploited opportunistically even at low densities (Thurner et al., 2021). This highlights the urgent need for regulatory measures to protect species that are at risk of extinction when exploitation can expand to include other species.

Integrating genomic data into conservation strategies provides a powerful tool for enhancing the resilience of species at risk, such as the blue whales. The use of whole-genome sequencing offers a more comprehensive understanding of genetic health compared to relying on a limited number of SNPs, as evidenced by this thesis. For instance, while SNP-based approaches and coalescent models (Chapter 3) were insufficient to detect the impact of recent bottlenecks, whole-genome analyses revealed a clear picture of population declines (Chapter 4). By identifying genomic regions under selection and tracking genetic diversity over time, researchers can better predict which populations are most at risk from environmental changes and human activities (Grummer et al., 2019; Ouborg et al., 2010; Theissinger et al., 2023). Moreover, understanding how historical and contemporary factors influence genetic connectivity and adaptive capacity is vital, particularly in light of accelerating climate change and the presence of positively selected genes linked to traits like preweaning lethality in baleen whales (Chapter 2). This emphasizes the evolutionary pressures affecting reproductive success and highlights the importance of maternal condition in shaping offspring survival. Consequently, conservation efforts must target the specific vulnerabilities of each population to ensure their long-term survival.

The findings of this thesis underscore the importance of maintaining genetic diversity and population connectivity in blue whales (Chapter 3 & 4) and other baleen whales (Chapter 2). As climate change accelerates, shifting ocean temperatures and prey distributions are likely to exacerbate the environmental pressures already faced by these species (Franks & Hoffmann, 2012). For example, rising sea temperatures are predicted to affect the distribution of krill, the primary prey of various Antarctic baleen whales (Atkinson et al., 2004). This concern is compounded by a fourfold increase in overfishing driven by the nutritional supplement and aquaculture industries, which could further jeopardise the whales' feeding efficiency and reproductive success (Savoca et al., 2024) in already endangered species (Braulik et al., 2023; Clapham et al., 1999; Thomas et al., 2016). Other subspecies and species like the Australasian pygmy blue whale or the Bryde’s whale, which occupies warmer tropical waters, may initially be less directly impacted by rising temperatures. However, the ongoing trend of ocean warming and shifting prey distributions could still lead to changes in range, migration pattern, and competition for resources in overlapping habitats between species (Chen et al., 2022; Van Weelden et al., 2021). This highlights the complexity of how different populations may react to climate change as certain species or subspecies may be more vulnerable than others due to their specific ecological needs and geographic ranges. Consequently, the need for population-specific conservation strategies that account for the unique ecological requirements of different whale populations is required.

In addition to climate change, anthropogenic threats such as ship strikes, noise pollution, and fisheries bycatch further compound the challenges faced by baleen whales, particularly as they migrate through heavily trafficked areas (Calambokidis et al., 2019; Erbe et al., 2018; Tulloch et al., 2020). These threats, coupled with the legacy of whaling, indicate the need for international conservation efforts to focus on protecting critical habitats, migration corridors, and feeding grounds to support population recovery and maintain gene flow (Chapter 4; Allen, 1980; Reeves, 2022; Theissinger et al., 2023). As blue whale populations recover from past exploitation, it will be crucial to support both demographic growth and genetic health, especially in smaller, more vulnerable populations like Antarctic and pygmy blue whales (Chapter 4). The findings of this thesis emphasise that effective conservation strategies must go beyond preventing targeted hunting or accidental mortality, which has mostly been the main management and conservation focus in the past century (Figure 5.1; Reeves, 2022). To ensure the long-term viability of, for example, blue whales, efforts must also be implemented on preserving gene flow between lineages, protecting critical habitats for food resources and breeding grounds (which still remain unknown for some of the subspecies), and closely monitoring genetic diversity over time. Conserving cetaceans is not a one-time task with actions that can be completed and set aside. Instead, conservation efforts must be continually overseen, monitored, and, whenever necessary, adapted to ensure their effectiveness over time, also known as ‘adaptive management’.

The genomic insights gathered from this research not only have critical implications for blue whales but also represent the challenges faced by other species of marine megafauna. By employing whole-genome sequencing and advanced genomic tools, conservation biologists can gain a deeper understanding of the evolutionary processes shaping species survival. These insights are crucial for informing conservation policies in a rapidly changing world, ensuring that conservation strategies effectively address the unique needs of different populations. As we continue to confront the complex challenges posed by climate change and human activities, prioritising genetic health and resilience will be paramount to securing a sustainable future for baleen whales and the marine ecosystems they inhabit.

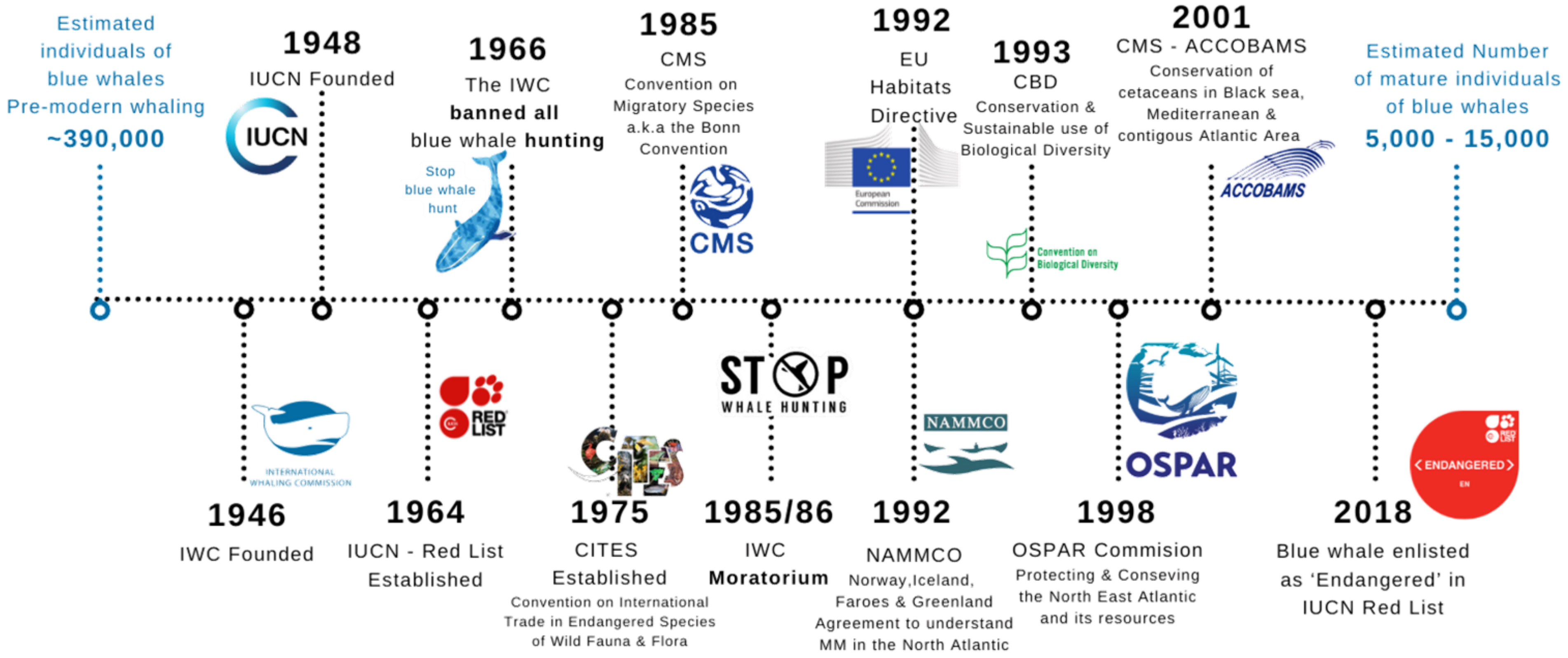


Figure 5.1. Some major global conservation and policy frameworks for whale protection, with an emphasis on blue whales from the 20th to 21st century, including estimated population changes over time (timeline adapted from: Branch et al., 2008; Caddell, 2023; CITES, 2000; Cooke, 2018; Hovden, 2023; IUCN, 2024; OSPAR Commission, 2020; Plouffe, 2021; Tønnessen & Johnsen, 1982).

Conclusion

This thesis explored the evolutionary genomics of the Balaenopteridae family, particularly focusing on blue whales (*Balaenoptera musculus*), to elucidate the molecular mechanisms driving species diversification and genomic responses to environmental and anthropogenic pressures. This research represents the first evaluation of genomic health in blue whales, offering insight into how this species has retained relatively high genetic diversity despite historical and contemporary pressures. By examining the evolutionary history and genomic adaptations within the Balaenopteridae family, this research deepens the understanding of how baleen whales have responded to environmental changes over time, particularly in relation to stress resistance, diving adaptations, and specialised reproductive strategies.

The findings emphasised the molecular mechanisms underpinning key adaptations such as gigantism, hypoxia tolerance, immune system efficacy and lunge-feeding techniques, which are crucial for the survival and diversification of blue whales and other balaenopterids. Ultimately, this research enriches the comprehension of the genomic basis of diversification in baleen whales and sheds light on the molecular changes linked to their adaptive radiation, thereby contributing to the broader understanding of cetacean evolution. Building on this foundation, this thesis also explored how past climatic events, like those during the Pleistocene, contributed to the genetic divergence of blue whale lineages, informing our understanding of how environmental fluctuations shape species' evolutionary trajectories.

From a conservation perspective, the evaluation of blue whale genomic health in the context of ongoing climate change and anthropogenic impacts underscores the importance of maintaining genetic diversity and connectivity across populations. The identification of positively selected genes related to immune function and stress response, low genetic load and high heterozygosity, suggests that this species may be resilient against future challenges, though certain lineages, such as pygmy blue whales, may remain vulnerable due to their reduced genetic variation.

The findings emphasize the need for population-specific conservation strategies that integrate genomic data to enhance the resilience of blue whales and other marine megafauna. Prioritising the protection of critical habitats, migration corridors, and genetic connectivity among populations will be paramount in supporting recovery efforts and mitigating the impacts of environmental changes. Future work should focus on integrating genomic insights into global conservation efforts to ensure the long-term survival of these species in a rapidly changing world.

The insights gained from this thesis not only enhance the understanding of blue whales and their evolutionary adaptations but also extend to other large marine megafauna. By employing advanced genomic tools and methodologies, these studies contribute to the broader field of evolutionary biology and conservation, emphasizing the need for continued research to navigate the complex challenges posed by environmental changes and human activities. As we strive to secure a sustainable future for baleen whales and the marine ecosystems they inhabit, prioritising genetic health and resilience will be crucial for their survival.

Supplementary Information

Appendix S1 – Chapter 2

The supporting tables are available from Zenodo: https://zenodo.org/records/13291373

Table S2.1. PAML results per species – Results for the branch-site tests for all the 10,159 cetartiodactyla orthologous genes on seventeen species separated per group; genes tested for each group (Mysticeti, Odontoceti and Land) are shown on a different sheet. Columns signify the following: the likelihood ratio test (LRT) used to compare different evolutionary models, specifically, LRT0 compares M2a vs M0, LRT1 compares M2a vs M2a1, and LRT2 compares M2a vs M2a_rel; w0 (ω Zero), representing the ratio of non-synonymous to synonymous substitutions (dN/dS) under the null model where the value for the lineage is constrained to be zero; wF (ω Foreground), indicating the dN/dS ratio for the foreground branch (the lineage of interest) where positive selection is hypothesized and the value can be greater than 1; wB (ω Background), showing the dN/dS ratio for the background branches (all other lineages in the phylogeny) with values ranging between 0 and 1; and Sig (Significance), which indicates whether the test result is statistically significant based on the LRT value and the models being tested, with significance determined by comparing the LRT value to a critical value specific to each model. When the three LRT values are significant then the gene is considered a candidate gene.

Table S2.2. List of genes where positive selection is estimated to have occurred using the PAML analysis for each species of balaenopterids and eschrichtid. All positive selection events have their corresponding Gene Ontology (GO) and Kegg Orthology (KO) terms retrieved from different sources using the BUSCO2GKO.pm in-house script available at https://github.com/Yuma248/MELFUnction/.

Table S2.3. PAML results bifurcation of branches in mysticetes – Results for the branch-site tests for the sequences present in the branches of nine species using three models in PAML (M0, M2a and M2a1); candidate genes of each splitting branch within the mysticeti and the outgroup (P. macrocephalus) are shown on a different sheet. Columns signify the following: the likelihood ratio test (LRT) used to compare different evolutionary models, specifically, LRT0 compares M2a vs M0, LRT1 compares M2a vs M2a1, and LRT2 compares M2a vs M2a_rel; w0 (ω Zero), representing the ratio of non-synonymous to synonymous substitutions (dN/dS) under the null model where the value for the lineage is constrained to be zero; wF (ω Foreground), indicating the dN/dS ratio for the foreground branch (the lineage of interest) where positive selection is hypothesized and the value can be greater than 1; wB (ω Background), showing the dN/dS ratio for the background branches (all other lineages in the phylogeny) with values ranging between 0 and 1; and Sig (Significance), which indicates whether the test result is statistically significant based on the LRT value and the models being tested, with significance determined by comparing the LRT value to a critical value specific to each model. When the three LRT values are significant then the gene is considered a candidate gene.

Table S2.4. List of genes where positive selection is estimated to have occurred using the PAML analysis for each bifurcation of the tree within mysticetes. Sheet1 has a figure with the tree and the corresponding numbers for each bifurcation of the other sheets. All positive selection events have their corresponding Gene Ontology (GO) and Kegg Orthology (KO) terms retrieved from different sources using the BUSCO2GKO.pm in-house script available at https://github.com/Yuma248/MELFUnction/.

Table S2.5. List of all genes previously reported in the literature taxa within the mysticeti species and ancestral branch of mysticeti (Cao et al., 2021; Ding et al., 2022; Keane et al., 2015; Kishida & Thewissen, 2012; McGowen, Tsagkogeorga, Williamson, et al., 2020; Nam et al., 2017; Seim et al., 2014; Sun et al., 2019; Tollis et al., 2019; Tsagkogeorga et al., 2015; Wang et al., 2015; Xu et al., 2012; Yim et al., 2014; Zhou et al., 2018) compared to all the balaenopterids and eschrichtid positive selected unique genes found in the analyses of this paper.

Table S2.6. List of enrichment analysis mainly focus on Mammalian Phenotype Terms with their corresponding number of genes matched per term and p-values using a Bonferroni correction (p-value<0.05) for each species and each bifurcation shown on different sheets. Highlighted in green are the duplicated terms along the species or bifurcations.

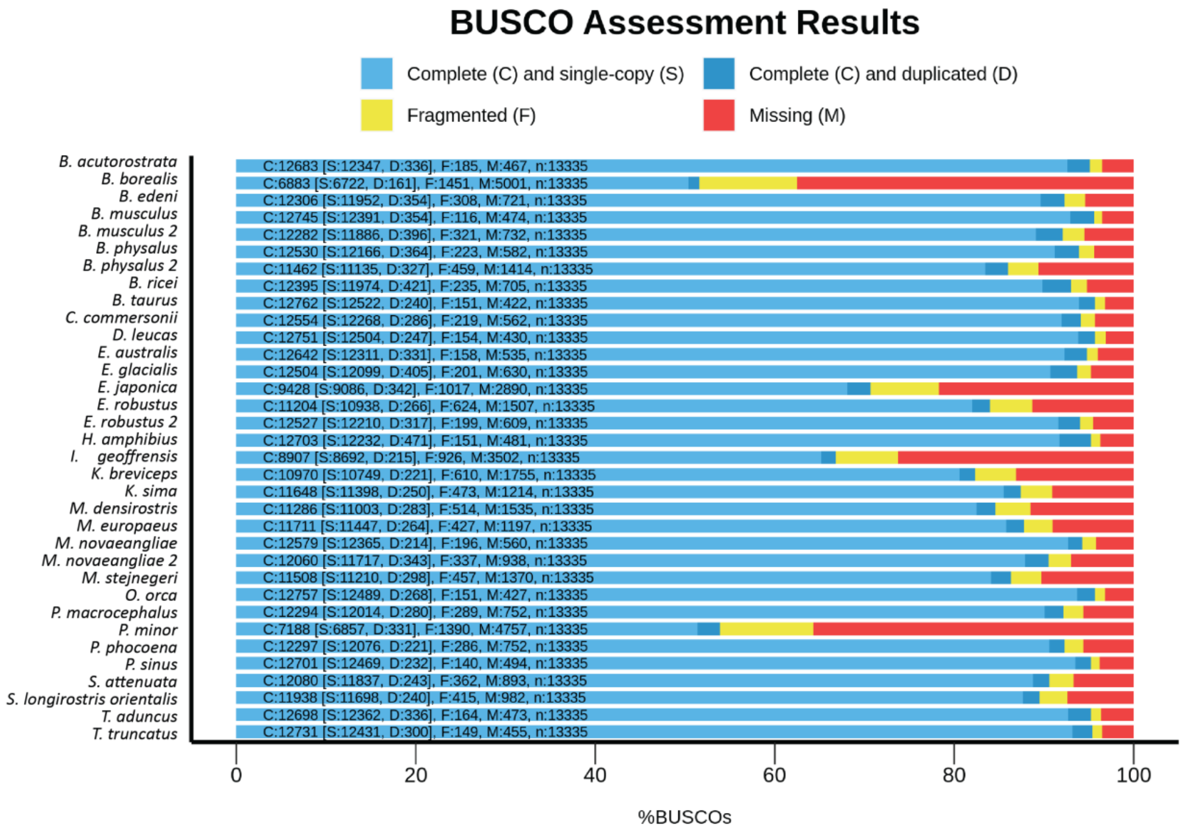


Figure S2.1. BUSCO assessment results for all the whole-genomes available online relevant to the study to evaluate completeness using 13,335 orthologous genes for the order Cetacea.

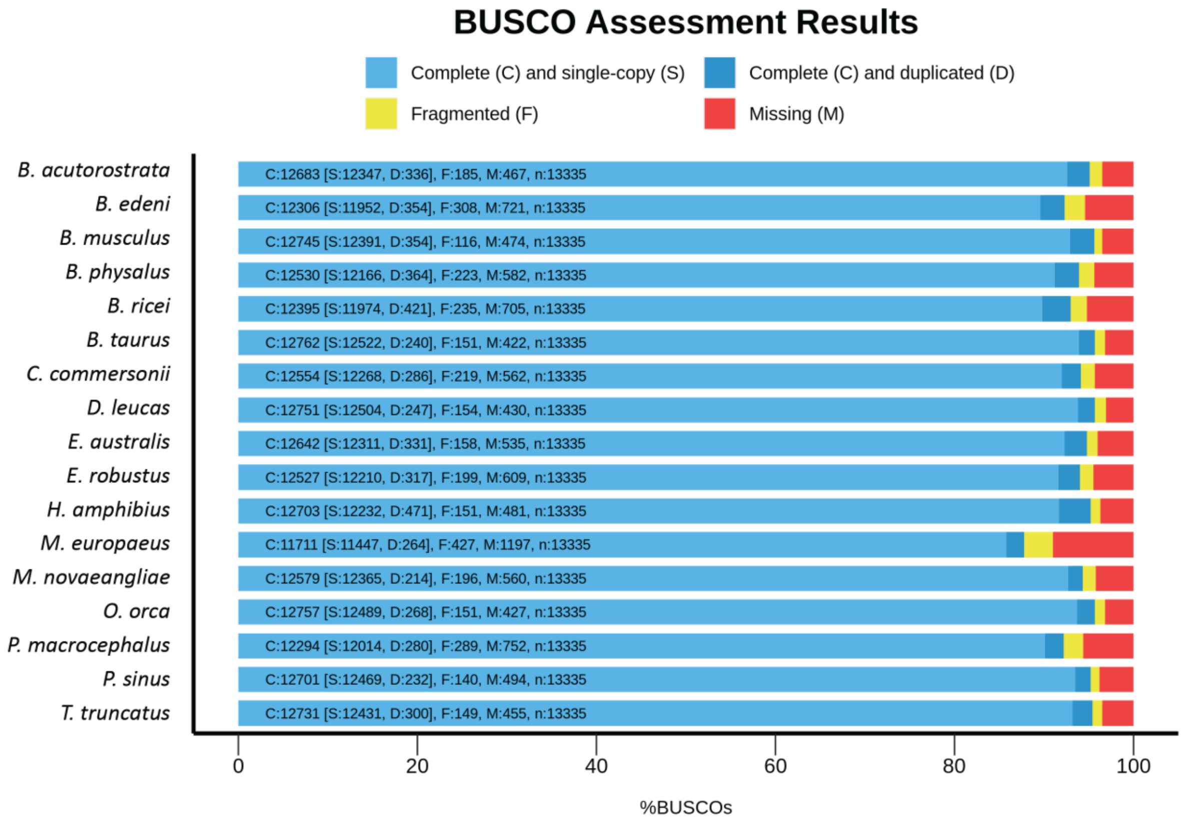


Figure S2.2. Based on BUSCO assessment results, seventeen species were chosen and reassessed guaranteeing each species had 85% or more completeness of the genomes using 13,335 orthologous genes for the order Cetacea.

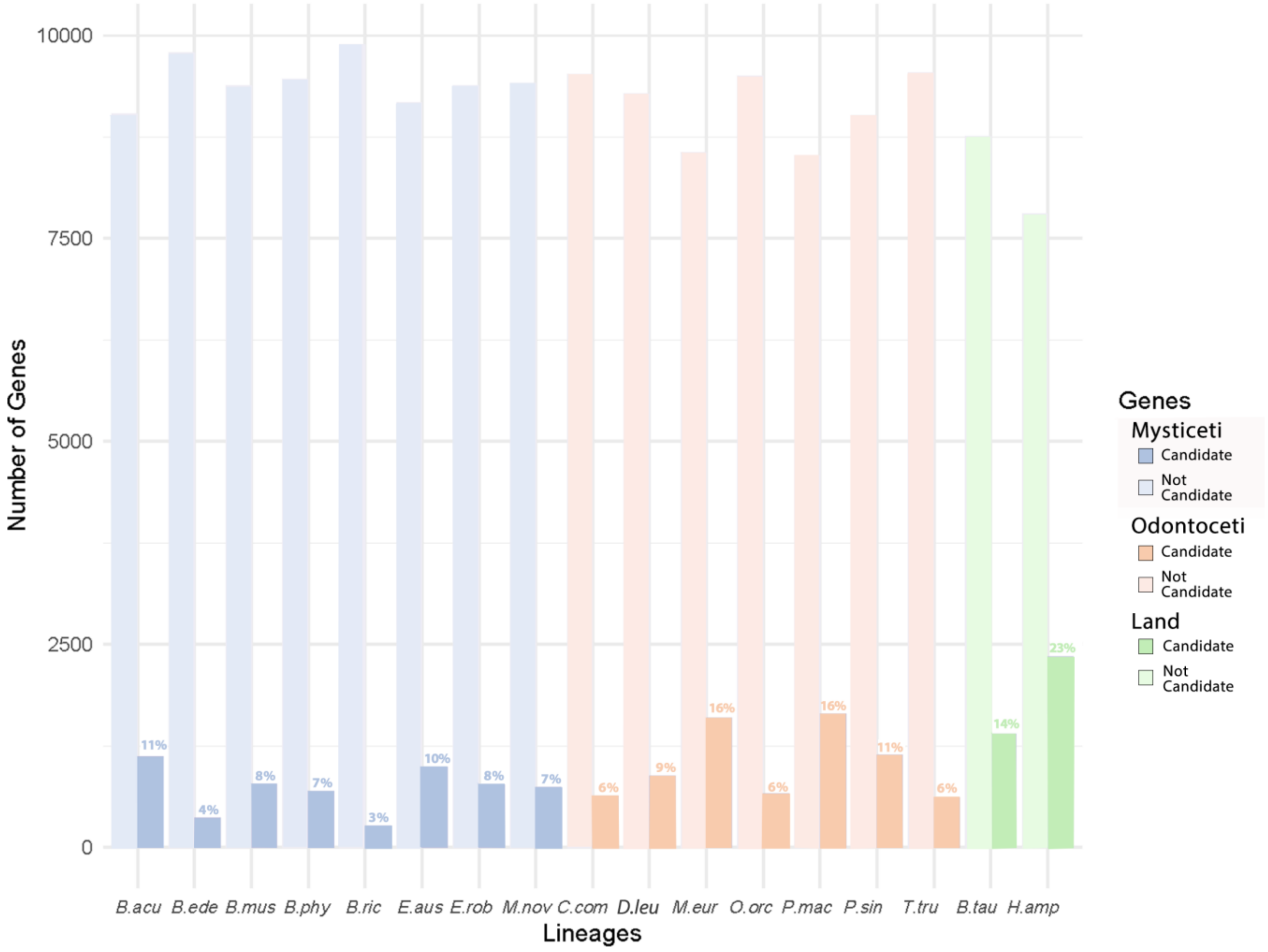


Figure S2.3. Number of candidate and not candidate genes for each chosen species based on the results from the analyses performed using the software PAML. The numbers above the 'Candidate' genes indicate the percentage of genes among the total tested (10,159 genes) that have been identified as being under positive selection.

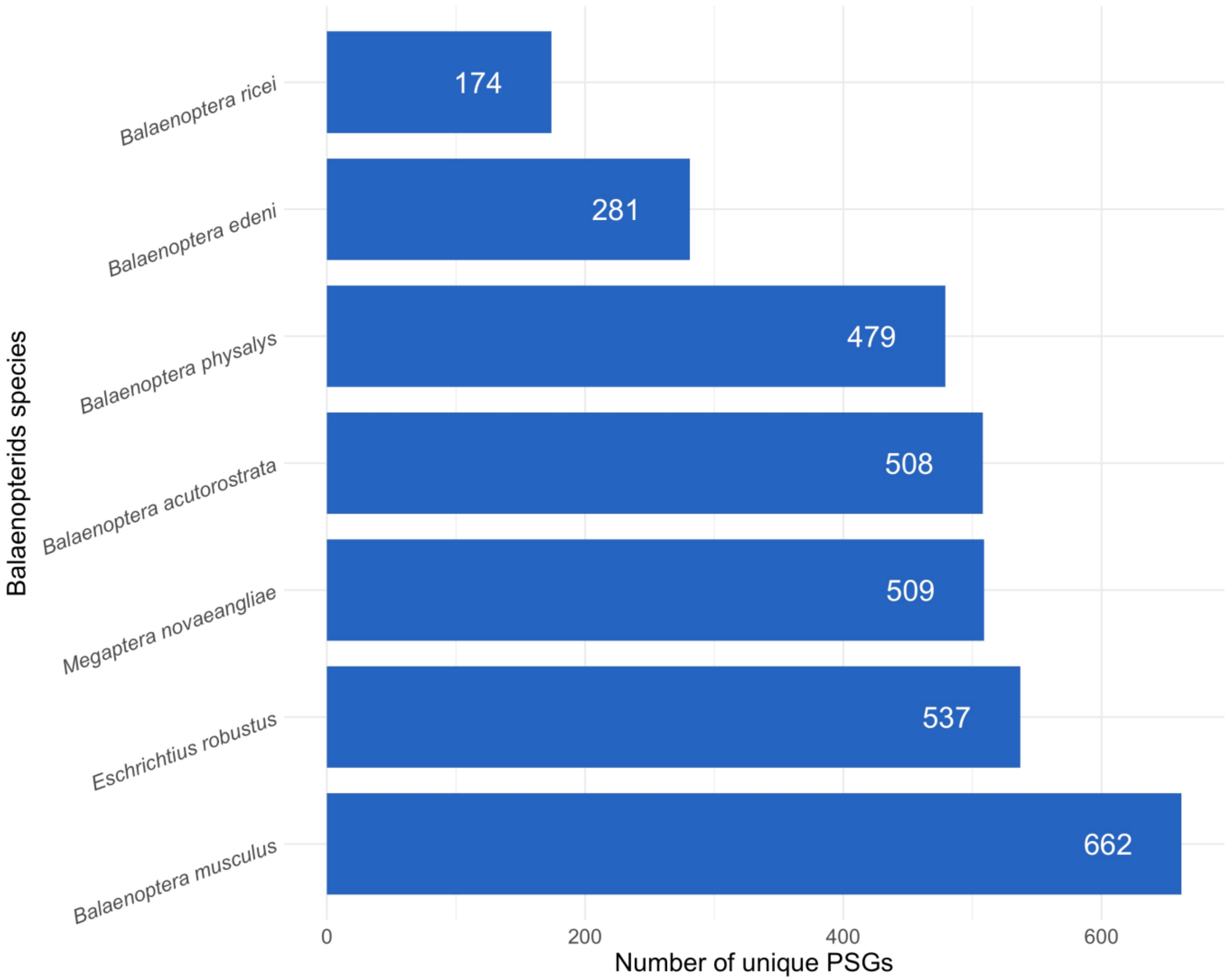


Figure S2.4. Number of genes identified as positive selected genes in the species within the Balaenopteridae and Eschrichtidae families for a total of 3,150 genes.

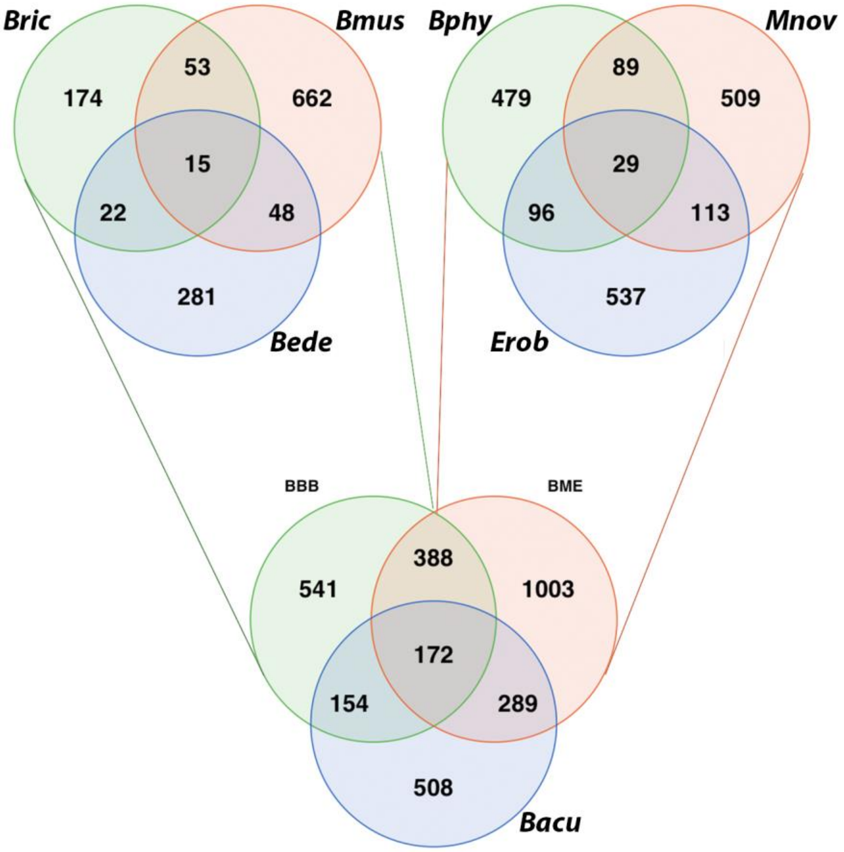
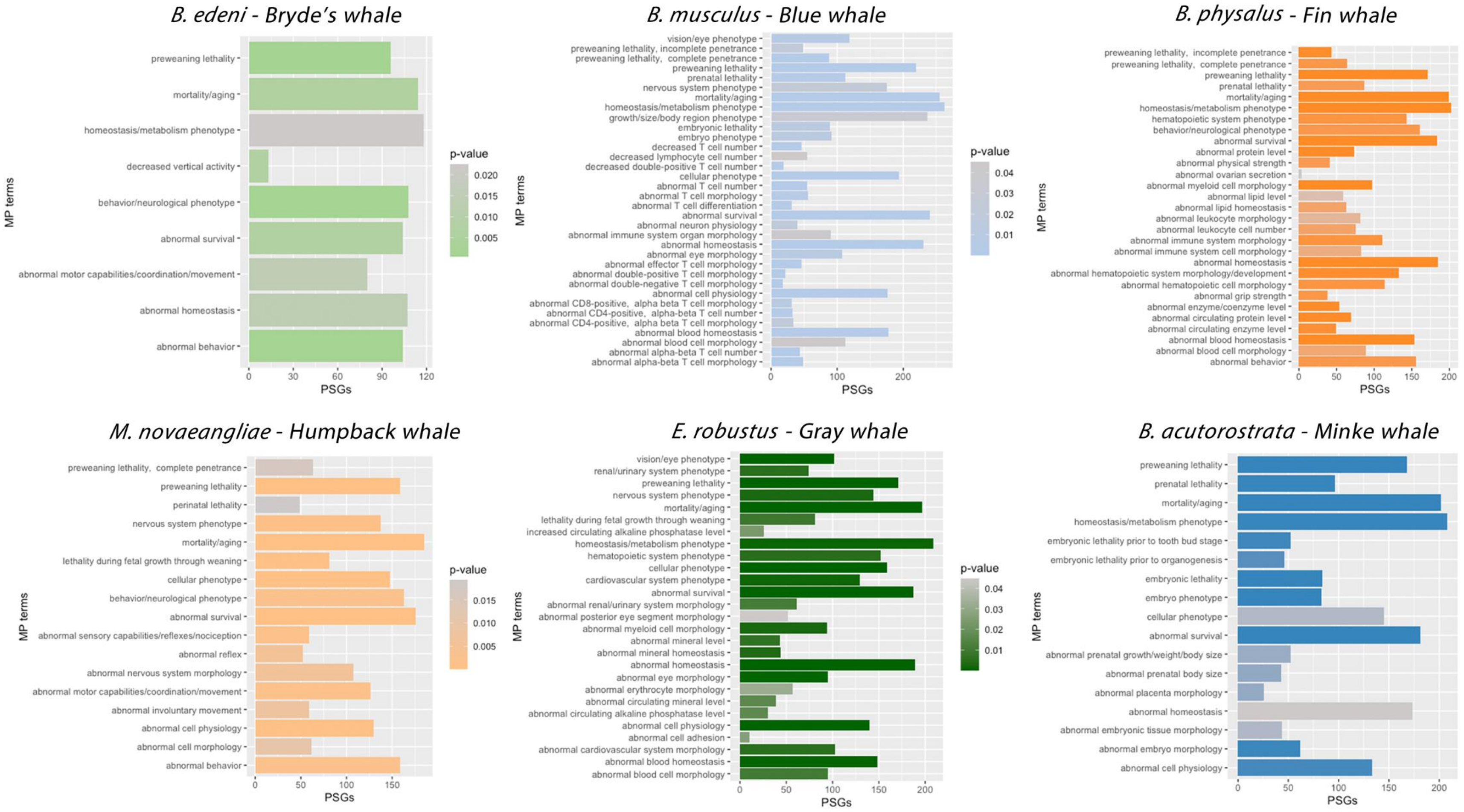


Figure S2.5. Orthologous gene clusters in the balaenopterid and eschrichtid lineages, found to be under positive selection. Shown as venn diagrams of unique and shared genes based on whole genome data from the different species evaluated: *Balaenoptera musculus (Bmus), Balaenoptera edeni (Bede), Balaenoptera ricei (Bric);* Together as BBB*. Megaptera novaeangliae (Mnov), Balaenoptera physalus (Bphy), Eschrichtius robustus (Erob);* Together as BME. And the sister clade species *Balaenoptera acutorostrata (Bacu)*.



B. edeni - *Bryde's whale*

B. musculus - *Blue whale*

B. physalus - *Fin whale*

M. novaeangliae - *Humpback whale*

E. robustus - *Gray whale*

B. acutorostrata - *Minke whale*

Figure S2.6. Bar plots showing on the x-axis the number of positive selected genes (PSGs) and in the y-axis the mammalian phenotype (MP) terms found to be enriched with the corresponding p-values using a Bonferroni correction for each species. A high-quality version of the figure is available on figshare https://figshare.com/s/a4c63a537aa4563eb8c7.

Appendix S2 – Chapter 3

Table S3.1. Coalescent model likelihoods based on palaeoceanographic events used as hypotheses for testing the diversification of the three major blue whale (*Balaenoptera musculus*) lineages identified, using FastSimCoal2.

Model	MaxEstLhood	AIC	deltaAIC	AkaikeWeight
AfterLGM - South	-53779.893	247675.560	8845.910	0
BeforeLGM - South	-53827.938	247896.815	9067.166	0
AfterLGM - Ind/WestPac	-51931.204	239162.032	332.383	6.66807E-73
BeforeLGM - Ind/WestPac	**-51859.028**	**238829.650**	**0.000**	**1**
AfterLGM - EastPac	-52592.036	242205.276	3375.627	0
BeforeLGM - EastPac	-52652.974	242485.906	3656.256	0
With Migration				
AfterLGM - South	-52487.791	241737.2102	10662.16172	0
BeforeLGM - South	-50322.508	231765.7135	690.6650038	1.0568E-150
AfterLGM - Ind/WestPac	-50780.919	233876.7742	2801.725674	0
BeforeLGM - Ind/WestPac	-50492.978	232550.7569	1475.708365	0
AfterLGM - EastPac	-51812.723	238628.4072	7553.358693	0
BeforeLGM - EastPac	**-50172.532**	**231075.0485**	**0**	**1**
Between Miocene & LGM - South	-52355.787	241129.3094	241129.3094	0
Between Miocene & LGM - EastPac	-51336.104	236433.4956	236433.4956	0
Miocene - South	-53512.695	246457.0676	15382.01906	0
Miocene - EastPac	-52093.588	239921.8383	8846.789817	0
With Migration + Bottleneck from whaling				
BeforeLGM - South	-56248.136	259062.2389	27987.1904	0
BeforeLGM - EastPac	-56342.226	259495.5394	28420.49087	0
Individual Lineages Ne				
South - Stable	-57237.947	263590.487	8682.085905	0
South - Growth	-57236.756	263585.0023	8676.601147	0
South - Decline	**-55352.656**	**254908.4011**	**0**	**1**
Ind/WestPac - Stable	-45673.068	210332.2511	513.3014793	1.7257E-112
Ind/WestPac - Growth	-45673.111	210332.4491	513.4995016	1.563E-112
Ind/WestPac - Decline	**-45561.606**	**209818.9496**	**0**	**1**
EastPac - Stable	-53249.511	245223.0605	4520.283084	0
EastPac - Growth	-53250.319	245226.7815	4524.004061	0
EastPac - Decline	**-52267.944**	**240702.7774**	**0**	**1**

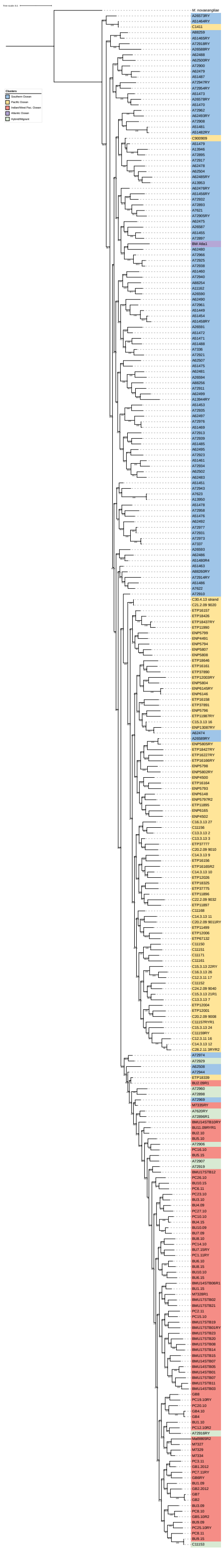


Figure S3.1. Maximum likelihood tree of blue whales (*Balaenoptera musculus*) using 16,661 SNPs with the software IQTree2. Each lineage is represented by a colour, the pink represents whales from the Indian/West Pacific Ocean lineage, the yellow represents whales from the Eastern Pacific Ocean lineage, the blue represents whales from the Southern Ocean lineage, and the purple represents the North Atlantic Ocean sample. The individuals in green within the lineage of the Indian/West Pacific are samples previously identified as first-generation hybrids between Southern Ocean and Indian/West Pacific Oceans lineages and migrants (Attard et al., 2012). The numbers on the branches correspond to the bootstrap values. The tree was rooted using the humpback whale (*Megaptera novaeangliae*) as the outgroup. Due to figure size, please refer to the high-quality version on figshare https://figshare.com/s/ddf4f74e7ec7f0507c34.

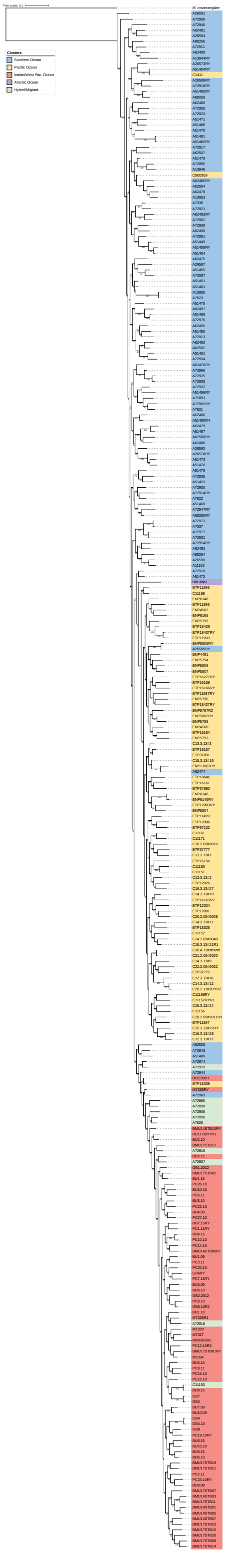


Figure S3.2. Complete Maximum likelihood phylogeny of blue whales (*Balaenoptera musculus*) using 16,661 SNPs within the software RAxML, with the bootstrap support values presented for each node. Each lineage is represented by a colour, the pink represents whales from the Indian/West Pacific Ocean lineage, the yellow represents whales from the Eastern Pacific Oceans lineage, the blue represents whales from the Southern Ocean lineage, and the purple represents the North Atlantic Ocean sample. The individuals in green within the lineage of the Indian/West Pacific are samples previously identified as first-generation hybrids between Southern Ocean and Indian/West Pacific Oceans lineages and migrants (Attard et al., 2012). The numbers on the branches correspond to the bootstrap values. The tree was rooted using the humpback whale (*Megaptera novaeangliae*) as the outgroup. Due to figure size, please refer to high-quality version on figshare https://figshare.com/s/ddf4f74e7ec7f0507c34.

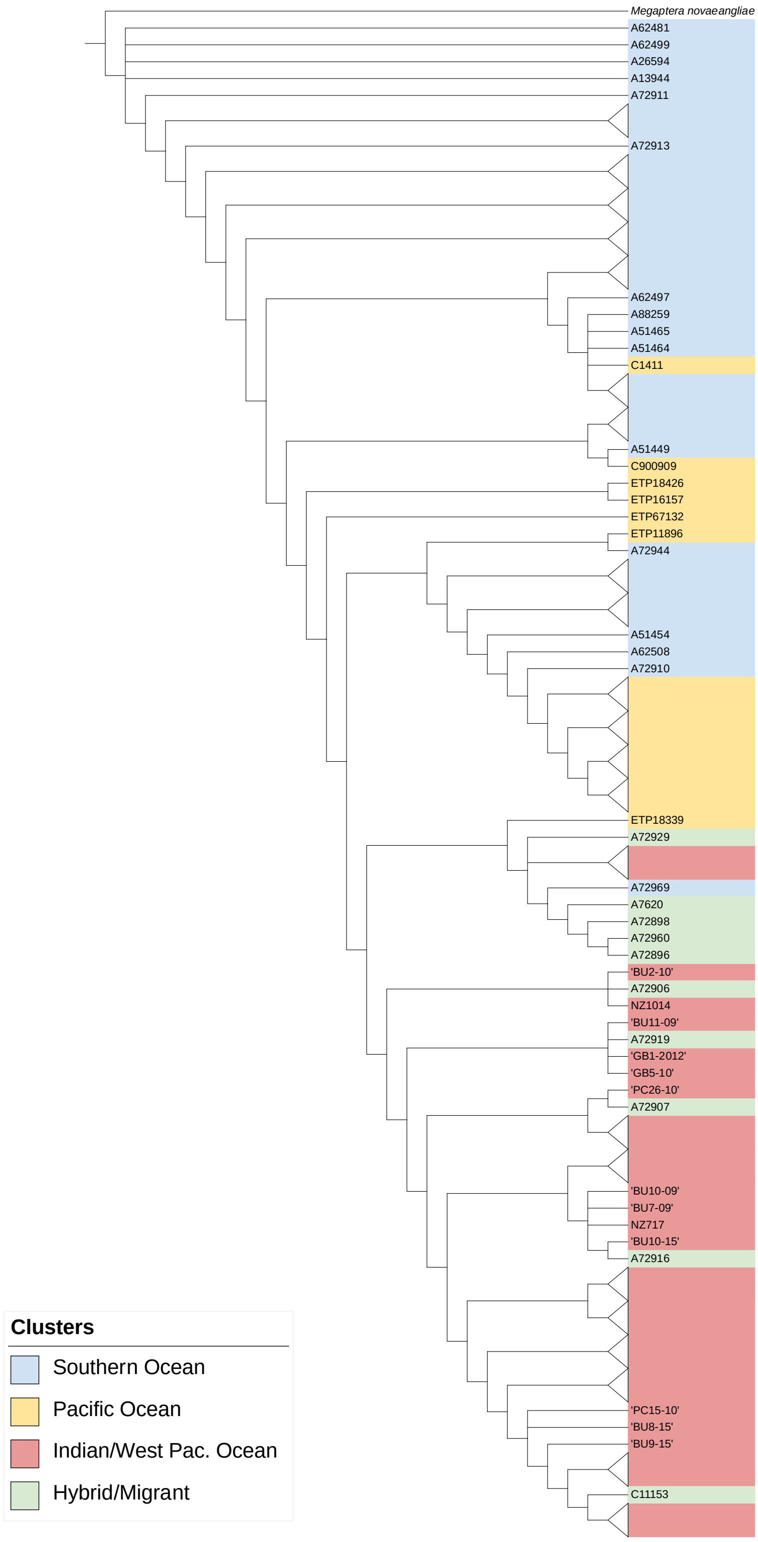


Figure S3.3. The SVDquartets tree of blue whales (*Balaenoptera musculus*) using 16,661 SNPs within the software PAUP*, using 100,000 random quartets and 100 bootstraps. Each lineage is represented by a colour, the pink represents whales from the Indian/West Pacific Ocean lineage, the yellow represents whales from the Eastern Pacific Oceans lineage, the blue represents whales from the Southern Ocean lineage, and the purple represents the North Atlantic Ocean sample. The tree was rooted using the humpback whale (*Megaptera novaeangliae*) as the outgroup. The individuals in green within the lineage of the Indian/West Pacific are samples previously identified as first-generation hybrids between Southern Ocean and Indian/West Pacific Oceans lineages and migrants (Attard et al., 2012). Due to figure size, please refer to high-quality version on figshare https://figshare.com/s/ddf4f74e7ec7f0507c34.

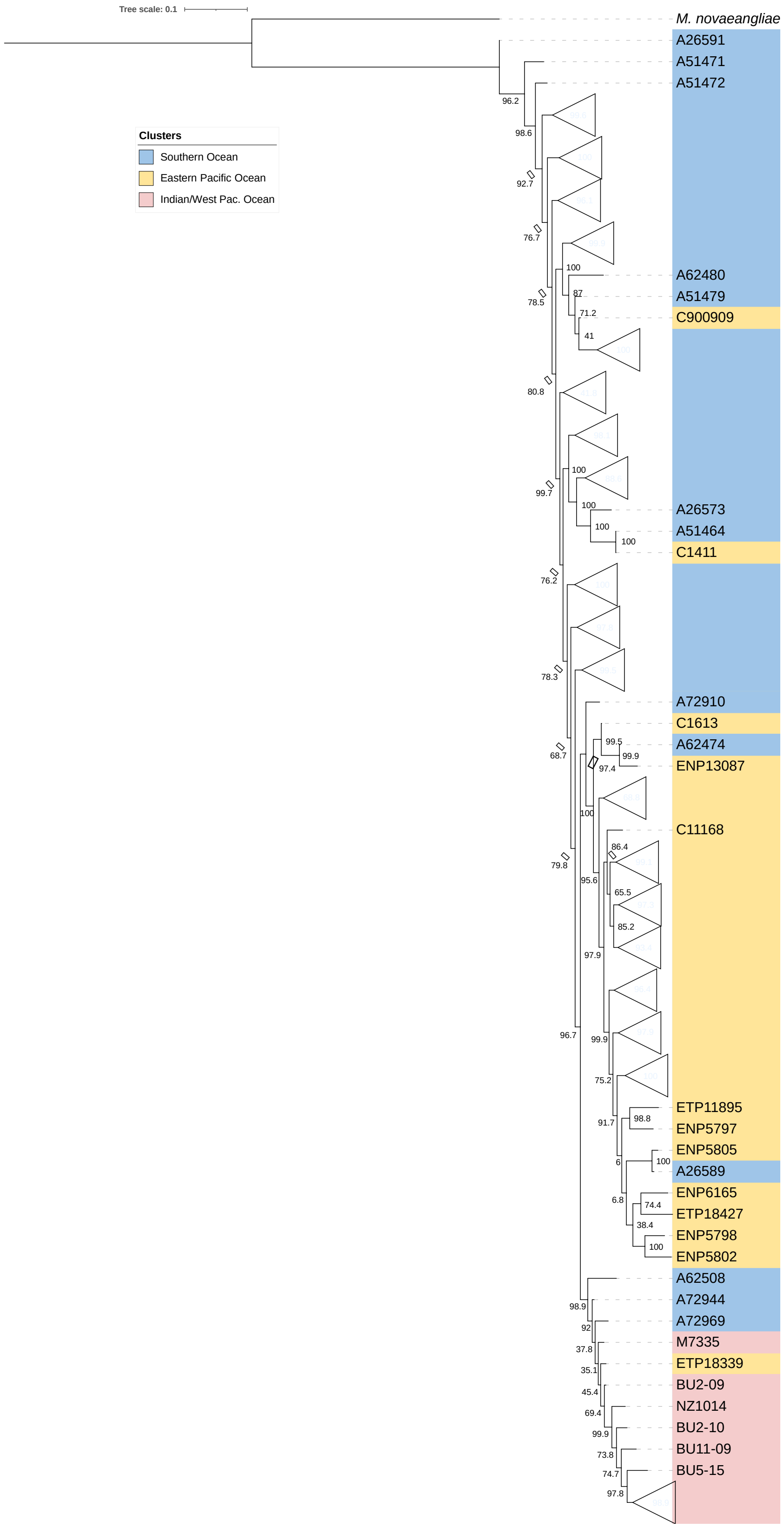


Figure S3.4. Maximum likelihood tree of blue whales (*Balaenoptera musculus*) using 16,661 SNPs with the software IQTree2 without the first-generation hybrids previously identified. Each lineage is represented by a colour, the pink represents whales from the Indian/West Pacific Ocean lineage, the yellow represents whales from the Eastern Pacific Ocean lineage and the blue represents whales from the Southern Ocean lineage. The triangles represent collapsed clades, grouping samples from the same lineage. The numbers on the branches and within the triangles correspond to the bootstrap values. The tree was rooted using the humpback whale (*Megaptera novaeangliae*) as the outgroup.

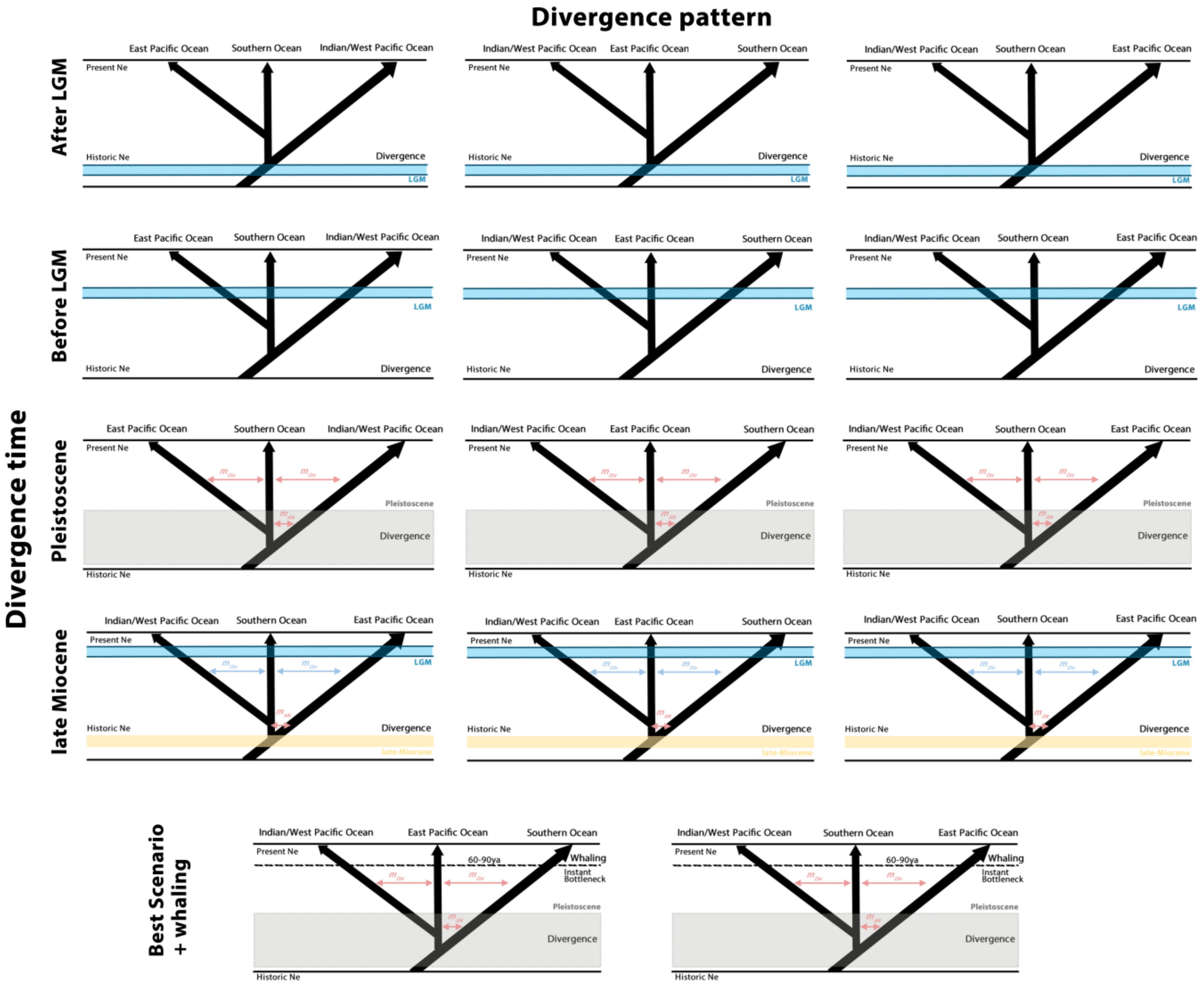


Figure S3.5. Representation of all the models tested for the diversification of blue whales (*Balaenoptera musculus*) using the coalescence method in fastsimcoal2. The models include continuous gene flow following population divergence with migration varying according to the model. In all models, the population diverge at T_{AN} for the ancestral divergence and T_{Div} for the secondary divergence.

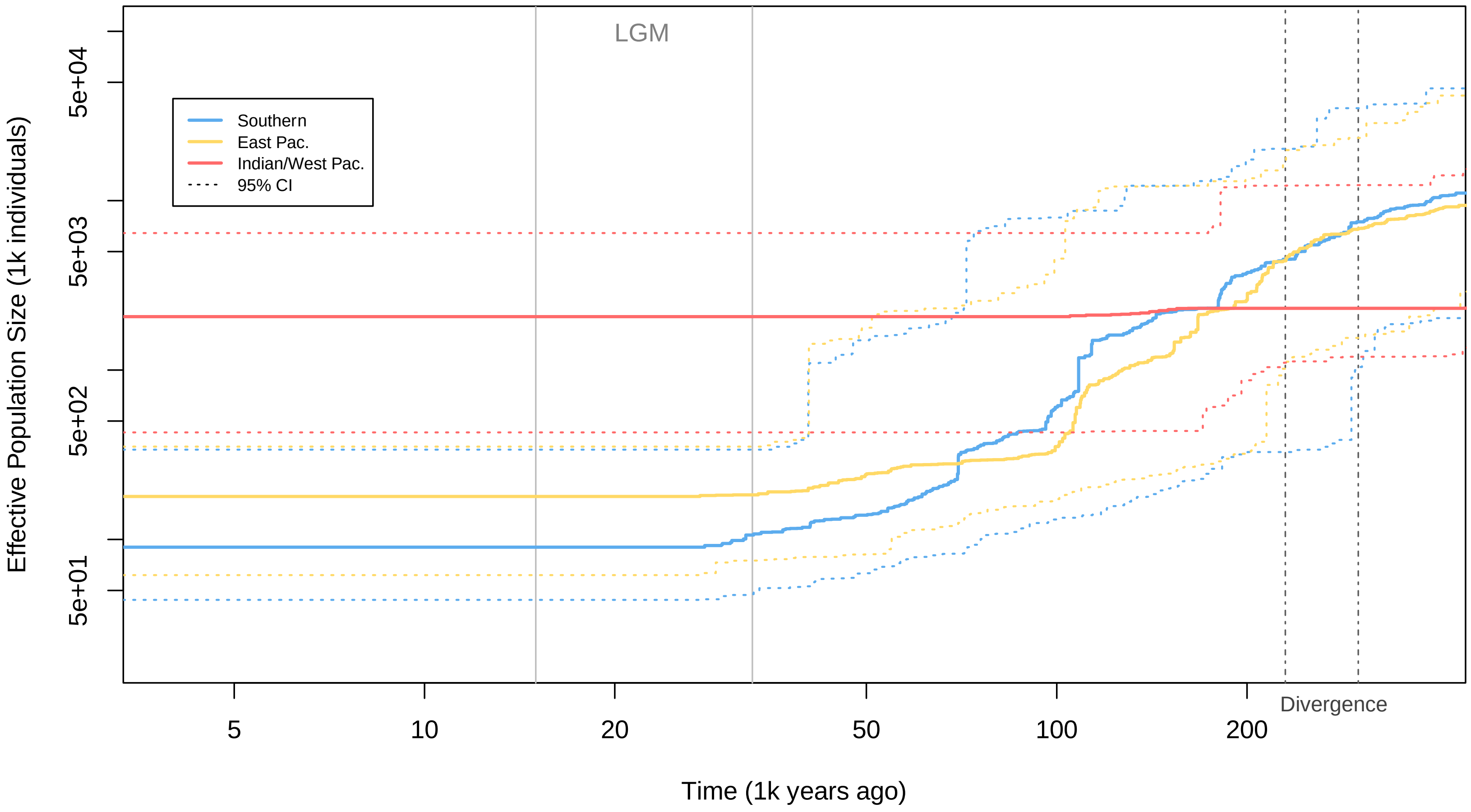


Figure S3.6. Effective population size (Ne) of blue whales (*Balaenoptera musculus*) estimated based on folded site frequency spectrum with a generation time of 10 million years ago, using the method of stairway plot (Liu & Fu, 2020). The filled line corresponds to the medians and the dotted lines correspond to the 2.5 and 97.5 percentiles, respectively. The pink line represents the Indian/West Pacific Oceans lineage, the yellow line represents the Eastern Pacific Ocean lineage, and the blue line represents the Southern Ocean lineage.

Demographic history analysis

Based on the folded SFS estimated from the genome-wide SNP data, the stairway plot software version 2.1.1 (Liu & Fu, 2020) was used to infer the temporal changes in θ for each population size. This method was employed, following the recommended 67% of sites for training and 200 bootstraps on the folded SFS. The optimal number of random breakpoints was selected based upon the recommended $(nseq)-2/4$, $(nseq)-2/2$, $3\,(nseq-2)/4$, and $nseq-2$, where $nseq$ was two times the sample size, as the number of random breakpoints. The same value of mutation rate and generation time selected for the coalescence analyses was assumed. The estimations were done for the period of 0.1 to 100000 kya.

Additionally, to validate the results produced by the stairway plot, we tested stable population size, exponential growth and exponential decline for each subspecies using fastsimcoal, for a total of nine models. The mutation rate, generation time and timing were the same as previously for the stairway plot. To imply population expansion, reduction or constant, the parameter of growth rate was varied following the fastsimcoal manual's recommendations. Each model was run 100 independent times (100000 simulations per run and 40 ECM cycles). We selected the run with the highest likelihood and using AIC, compared and chose the best performing models.

The three lineages (Southern, Eastern Pacific, and Indian/West Pacific Oceans) revealed very similar trends, with a continuous decline in the effective population size of blue whales since the mid-Pliocene (Fig. 5). In the Indian/West Pacific Oceans lineage, the first decline occurred from 2.5 Mya to 1.8 Mya before the present, coinciding with the Indo-Pacific "biogenic bloom" period at~4 Mya, which documented major changes in deep-sea ventilation and productivity (Gupta & Thomas, 1999). As a consequence, the atmospheric CO_2 drawdown accelerated and caused the climate system to destabilise by the late Pliocene, launching a period of intense cooling and ice accumulation (Filippelli, 1997). The second decline occurred 0.75 Mya to 0.45 Mya before the present, when the benthic assemblages underwent a significant change, accompanied by a sharp increase in glacial amplitudes (Gupta et al., 2006). By contrast, the Southern Ocean and Eastern Pacific Ocean lineages experienced a gradual decline from 5 Mya to 0.03 Mya, when the LGM began (Clark et al., 2009). These results corroborate our coalescence analysis, placing the divergence of the sub-species before the LGM and a continuous decline for each lineage. However, since stairway plots have previously been shown to be biased by noise on the SFS, they must be interpreted with caution (Lapierre et al., 2017; Patton et al., 2019).

Appendix S3 – Chapter 4

Table S4.1. Blue whale samples and the outgroup Rice's whale (*B. ricei*) with the average coverage of sequenced reads from whole genomes with its corresponding NCBI SRA ID or Assembly ID source.

Sample Name This Study	SRA ID or Assembly ID	Location	Coverage	Publication	Year Collected	Sex	Busco Completeness Coverage
Antarctica							
Ant1		Antarctica	30x		1999	M	94.7%
Ant1H		Antarctica	30x	Hybrid	2007	M	94.8%
Ant2		Antarctica	5x		2001	M	85.7%
Ant2H		Antarctica	7x	Hybrid	2007	F	90.7%
Ant3		Antarctica	7x		2004	F	91.2%
Ant3H		Antarctica	5x	Hybrid	2007	F	87.2%
Ant4		Antarctica	7x		2006	M	90.7%
Ant4H		Antarctica	5x	Hybrid	2007	M	88.6%
Ant5		Antarctica	7x		2006	M	91.6%
Ant5H		Antarctica	7x	Hybrid	1993	M	90.7%
Ant6		Antarctica	7x		2006	M	91.0%
Ant7		Antarctica	5x		2007	M	86.0%
Ant8		Antarctica	7x		2007	M	91.6%
Ant9		Antarctica	7x		2007	M	91.4%
OldAnt	SRR14357005	Antarctica	6X	Jossey et al. (2024)	1938	F	93.7%
Pygmy							
AusBU1		Bonney Upwelling	30x		2015	F	94.8%
AusGB2		Geographe Bay	7x		2012	F	89.4%
AusGB3		Geographe Bay	5x		2009	M	88.9%
AusPC4		Perth Canyon	7x		2011	M	89.8%
AusPC5		Perth Canyon	5x		2010	F	88.4%
NZ1		NZ	30x		2017	M	94.7%
NZ2		NZ	5x		2014	F	84.2%
NZ3		NZ	7x		2017	F	92.4%
NZ4		NZ	7x		2017	F	91.6%
NZ5		NZ	5x		2017	M	83.7%
Atlantic							
Atl1	SRR14357010	Nova Scotia, Canada	28X	Jossey et al. (2024)	2019	M	95.0%
Atl2	SRR14357029	Svalbard, Norway	21X	Jossey et al. (2024)	2016	F	95.0%
Atl3	SRR14467046	Newfoundland, Canada:	99X	Jossey et al. (2024)	2014	F	95.7%
Atl5	SRR14357011	Newfoundland, Canada	19X	Jossey et al. (2024)	2017	F	95.8%
Atl6	SRR14357022	Svalbard, Norway	19X	Jossey et al. (2024)	2017	M	95.8%

Atl7	SRR14357025	Svalbard, Norway	18X	Jossey et al. (2024)	2017	F	95.8%
Atl8	SRR14357030	Svalbard, Norway	19X	Jossey et al. (2024)	2016	M	95.8%
Atl9	SRR14357033	Svalbard, Norway	18X	Jossey et al. (2024)	2014	F	95.8%
Atl10	SRR14357035	Newfoundland, Canada	33X	Jossey et al. (2024)	2014	F	95.8%
Pacific							
Pac	SRR10251433	California, U.S.A.	Reference/ Chr-length	Bukhman et al. (2024)	2020	M	95.1%
Outgroup							
Rice's whale	Balaenoptera_ricei_HiC	n/a	Chr-length	DNA Zoo	n/a	?	93.0%
Rice's whale*	GCF_028023285.1	Gulf of Mexico	Chr-length	Vertebrate Genomes Project	2023	?	n/a

* While producing this manuscript a more complete chromosome-length genome of the Rice´s whale became available, which was used to call the SNPs for the genetic load analysis.

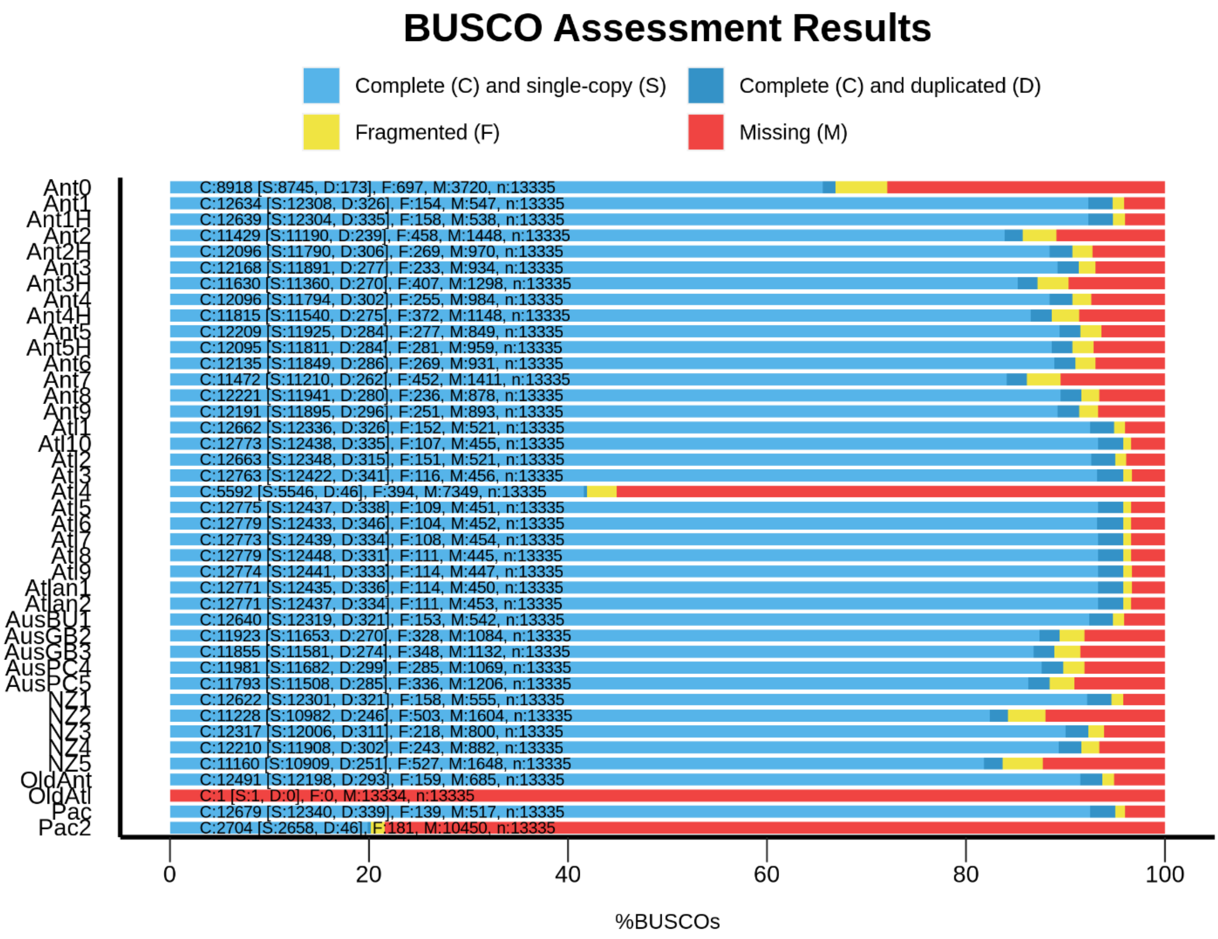


Figure S4.1. BUSCO assessment results for all the whole-genomes sequenced in this study and those available online relevant to the study to evaluate completeness using 13,335 orthologous genes for the order Cetacea.

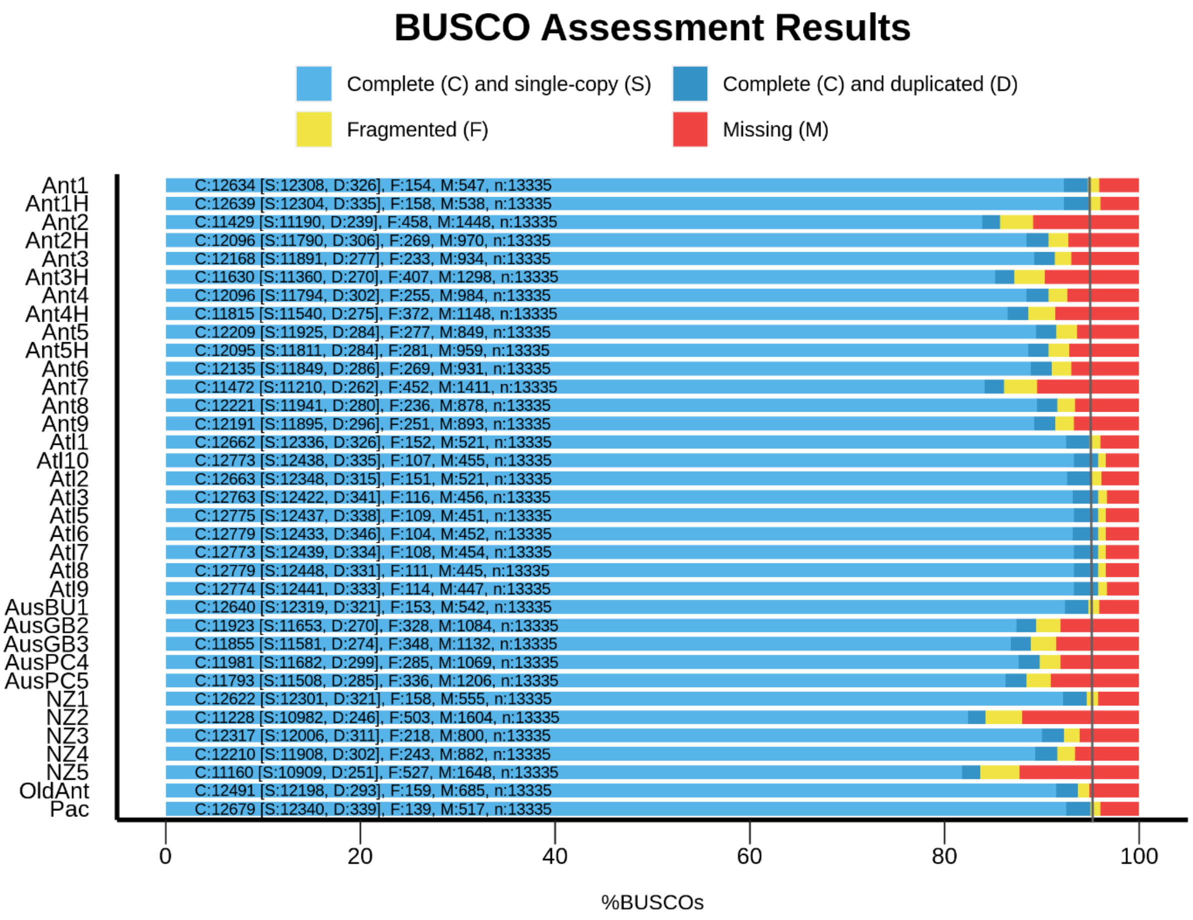
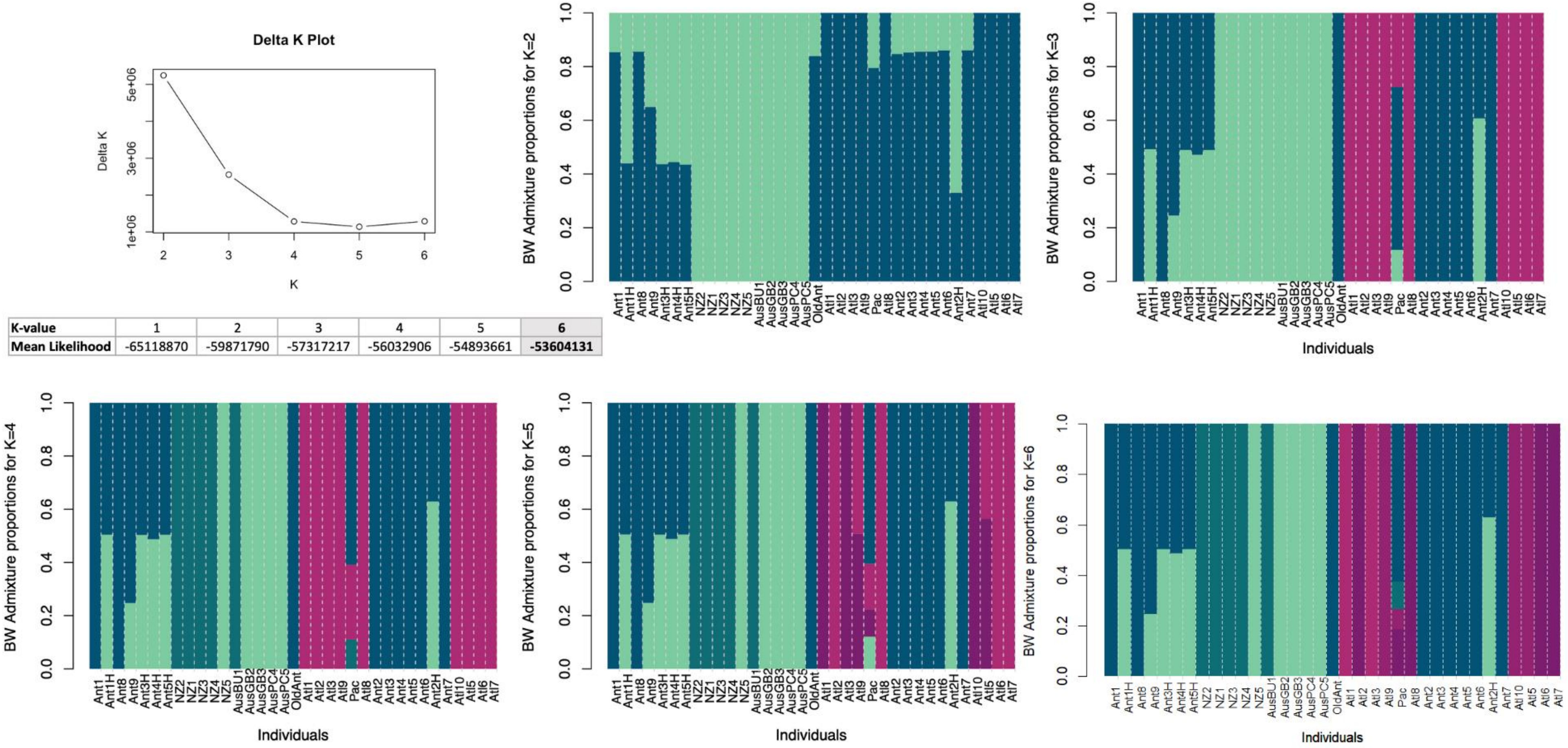


Figure S4.2. Based on BUSCO assessment results, 35 individuals were chosen and reassessed guaranteeing each individual had 80% or more completeness of the genomes using 13,335 orthologous genes for the order Cetacea.



K-value	1	2	3	4	5	6
Mean Likelihood	-65118870	-59871790	-57317217	-56032906	-54893661	**-53604131**

Figure S4.3. Clustering results of blue whale lineages from Antarctic, Atlantic, Pygmy, Pacific and Hybrids, based on 1,520,428 unlinked SNPs using NGSAdmix for admixture analysis, with k values ranging from 1 to 6. The accompanying delta K plot indicates the most likely number of genetic clusters (best k value) to be 2 while the mean likelihood indicates the most likely number to be 6. The colour coding of the clusters is consistent with the scheme used in Figure 1, providing a visual representation of the genetic structure and admixture patterns across the populations.

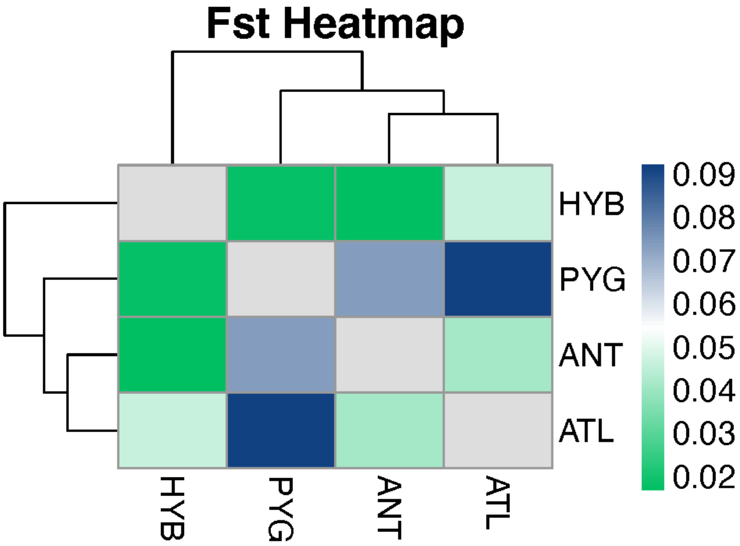


Figure S4.4. Heatmap of pairwise Fst values illustrating genetic differentiation between hybrid (HYB), pygmy (PYG), Antarctic (ANT), and Atlantic (ATL) blue whale populations. Higher Fst values indicated in dark blue and lower indicated in darker green, represent genetic divergence. The Pacific lineage was excluded from this analysis due to only one individual representing this group, limiting meaningful comparison.

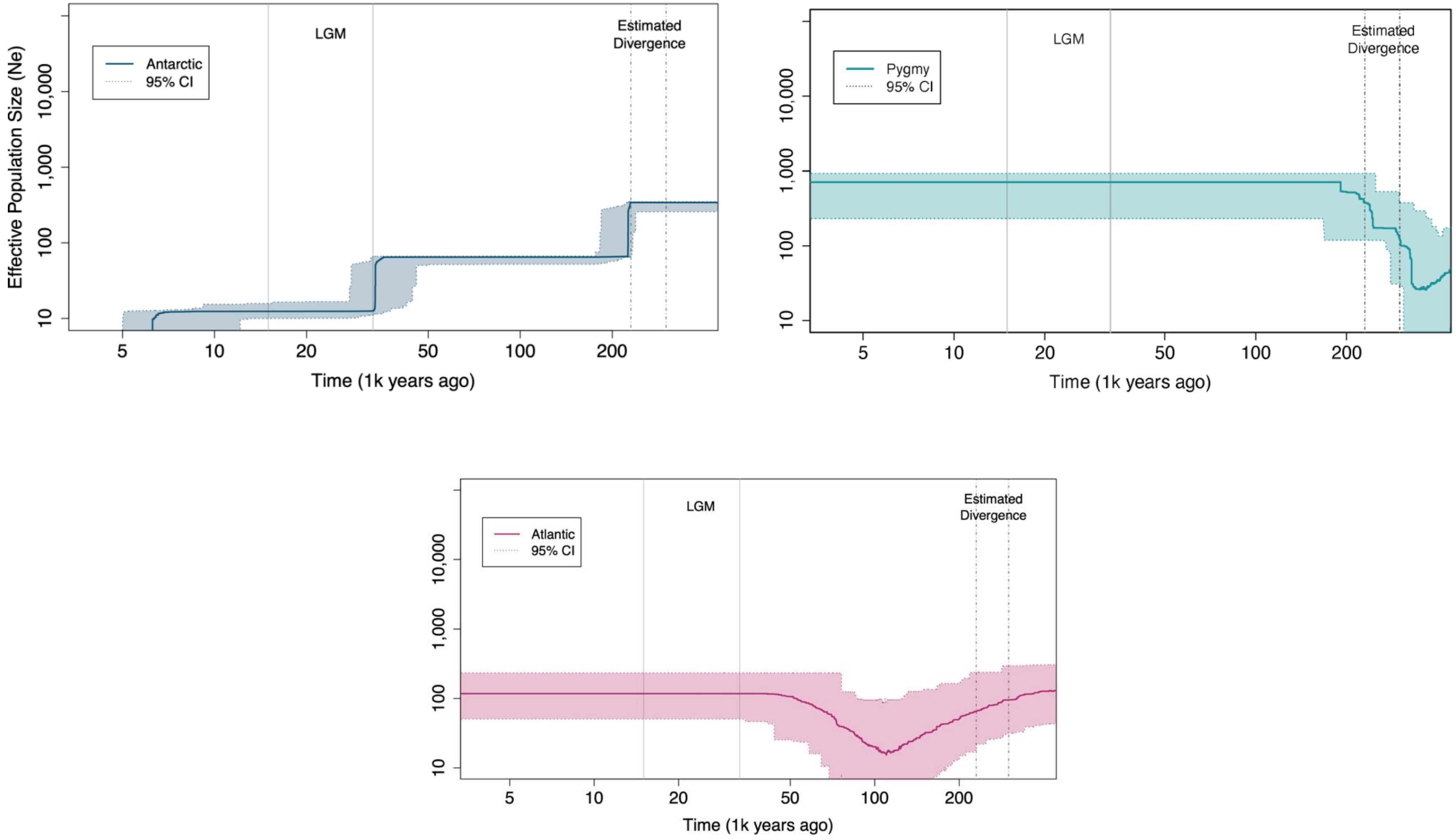


Figure S4.5. Stairway plots illustrating the changes in effective population size (Ne) over time for Antarctic, Pygmy, and Atlantic blue whale populations. The y-axis represents effective population size (Ne), while the x-axis shows time in thousands of years. Each plot includes a 95% confidence interval, highlighting the uncertainty around population size estimates and the estimated time of divergence (~200-300,000).

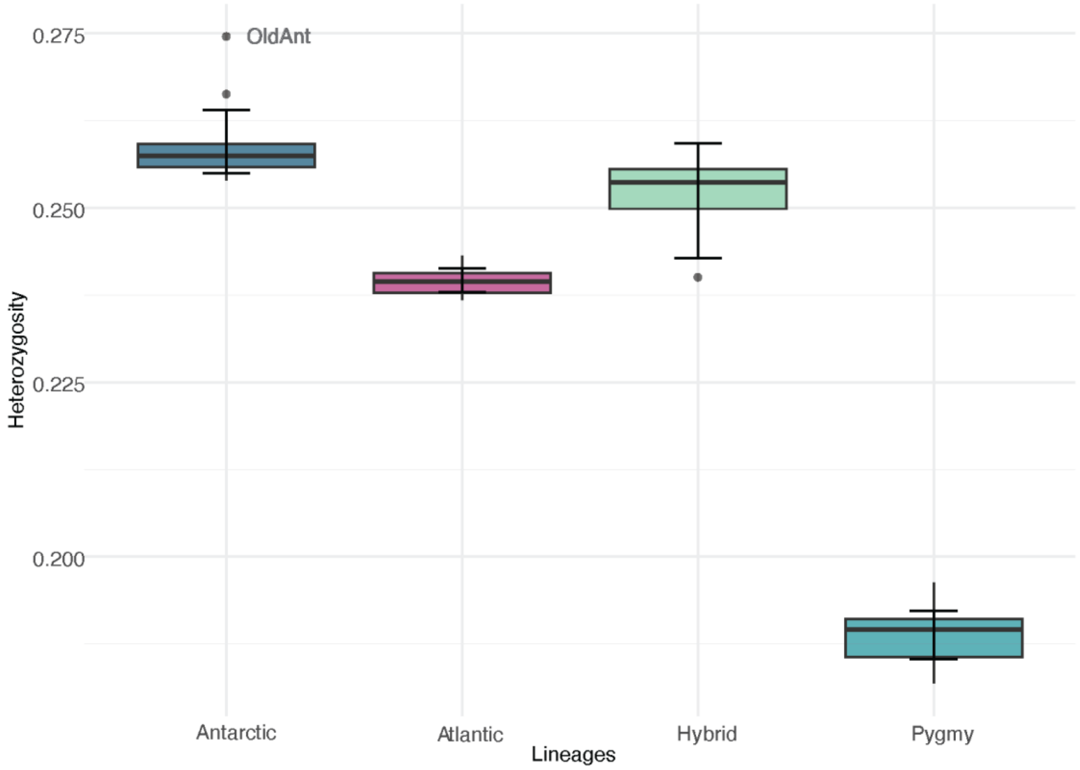


Figure S4.6. Boxplot showing the levels of heterozygosity for each blue whale lineages: Antarctic (ANT), Atlantic (ATL), Hybrids (HYB) and Pygmy (PYG) using the 1,520,428 unlinked SNPs. The outlier in the Antarctic lineage represents the OldAnt sample collected in 1938.

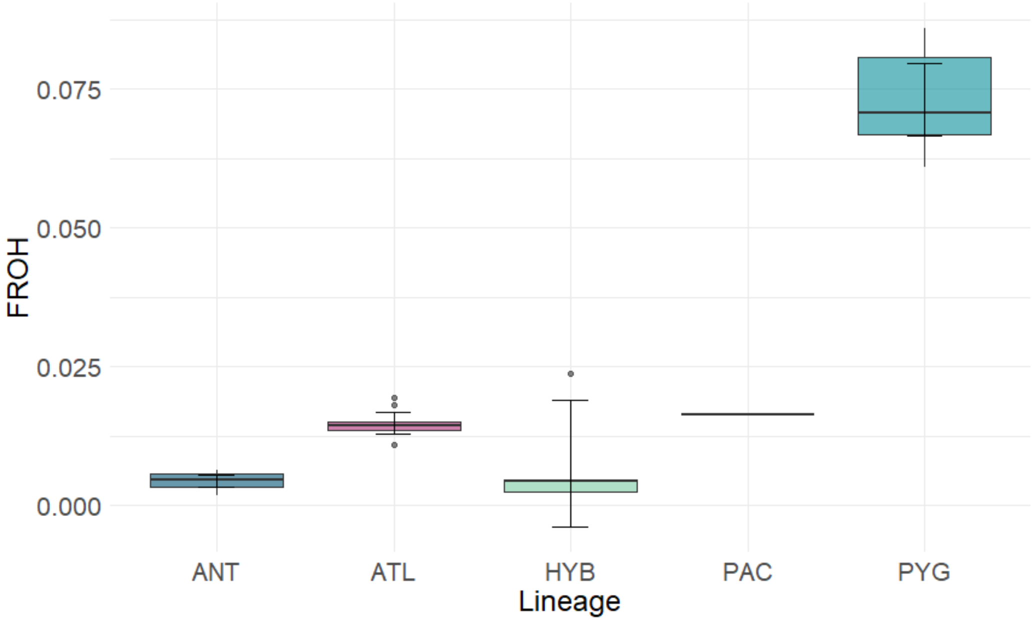


Figure S4.7. Boxplot showing the FROH values (y-axis) for each blue whale lineage, with each box coloured according to the corresponding lineage (Antarctic, Atlantic, Hybrid, and Pygmy). The Pacific lineage is not coloured in the boxplot as it is represented by only one individual. FROH represents the proportion of the genome in runs of homozygosity, which is used to estimate inbreeding levels. The low FROH values across lineages indicate minimal inbreeding, suggesting that these populations have maintained genetic diversity despite historical population declines.

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