Comparative genomics of bottlenose dolphins (genus *Tursiops*)

by

Kimberley Claire Batley

BSc (Mar Biol) (Hons)

Thesis
Submitted to Flinders University
for the degree of

Doctor of Philosophy
College of Science and Engineering
1st September 2021

CONTENTS

List of Figures

List of Tables

Summary

With ongoing climate change, new selective pressures are expected to threaten global biodiversity. Many populations and species will have to shift their distributional range or adapt to less favourable habitats. Warming oceans, the emergence of novel diseases, and increased human activities, particularly in coastal regions, greatly threaten the persistence of marine mammals worldwide. Genetic diversity is important for populations and species to persist and is influenced by complex interactions between selection and drift, migration, and mutation, which in turn is influenced by demographic factors. Marine mammals most at risk of population declines are those with relatively small population sizes, low genetic diversity, and reduced gene flow, as observed in several dolphin species. Bottlenose dolphins (genus *Tursiops*, family Delphinidae) have a widespread distribution and show high levels of morphological, ecological, and molecular variation between inshore and offshore populations. It is generally thought that offshore dolphins repeatedly colonised inshore habitats when they became available during interglacial periods, and that divergent evolutionary and selective pressures acting upon different ecotypes likely resulted in the formation of species and subspecies within the genus. These species and subspecies often differ in their vulnerability; for example, inshore populations generally have low genetic diversity, small populations sizes and exhibit high site fidelity to coastal areas with high human use. To better understand the biology of bottlenose dolphins and to advance their conservation management it is important to assess genomic diversity, adaptive potential and eco-evolutionary processes impacting their populations and species.

This study utilised datasets from 98 whole genomes (88 generated here and 10 sourced from online databases) to investigate the evolution and adaptation of bottlenose dolphins. The emphasis was on Southern Hemisphere lineages (16 localities were sampled across three ocean basins) but comparisons with Northern Hemisphere lineages were also carried out. Firstly, a quality reference genome for the southern Australian bottlenose dolphin (SABD) was generated and assembled. The SABD was previously described as the Burrunan dolphin, *T. australis*, a separate species to the Indo-Pacific bottlenose dolphin, *T. aduncus*, and common bottlenose dolphin *T. truncatus*. However, recent studies have suggested the Burrunan dolphin is more likely to represent a subspecies of *T. aduncus*. This genome provided a key resource to investigate the evolution of this taxon and its relationships to other inshore and offshore bottlenose dolphin lineages. Reconstruction of a maximum likelihood phylogenomic tree, based on 500 single-copy and complete genes from the vertebrate orthologous database supported SABD within a monophyletic *T. aduncus* clade, and as sister to the *T. aduncus* lineage from eastern Australia. Species and lineage-specific signatures of positive selection were then tested by comparing the ratio of substitution rates between branches and sites. Genes with similar gene functions were positively selected among species, suggesting that similar gene functions may be hotspots of shared positive selection among delphinid species, and may be associated with aquatic

phenotypes. Comparison of genes positively selected within the bottlenose dolphin lineages revealed 13 of the same genes were under positive selection in multiple inshore lineages, providing evidence of parallel evolution in these lineages. These findings suggest that comparable selective pressures of the inshore environment may be driving parallel evolution in genes relating to DNA damage, repair and apoptotic processes, immune responses and eye development, and informs about the evolutionary mechanisms driving adaptation and genomic divergence of bottlenose dolphins.

Secondly, the concept of parallel evolution driven by niche divergence was further explored by comparing the relationship between genomic diversity, runs of homozygosity (ROH) and demographic histories. A strong relationship between ecotype (inshore and offshore), genomic diversity and runs of homozygosity (ROH) was observed. The inshore lineages display considerably lower diversity than offshore populations, and a greater proportion of their genome covered by ROH. In the Southwest Atlantic Ocean (Brazil) the inshore subspecies, the Lahille's bottlenose dolphin (*Tursiops truncatus gephyreus*), recorded the lowest levels of genomic diversity for any *Tursiops* lineage and population, and similar to values reported for some of the most vulnerable and endangered mammals (e.g., cheetah (*Acinonyx jubatus*), snow leopard (*Panthera uncia*), Tasmanian devil (*Sarcophilus harrisii*)). Reconstruction of demographic histories using a hybrid method that leverages both the Sequentially Markovian Coalescent (SMC) and Site-Frequency-Spectrum highlighted parallel demographic histories within ecotypes. The inshore lineages generally experienced bottlenecks during the last glacial maximum (LGM), while the offshore lineages expanded during this period. The two inshore *T. truncatus* (*T. t. gephyreus* and Gulf of Mexico) followed similar patterns to the offshore lineages, which may reflect increased connectivity during periods of limited habitat availability. All lineages exhibited relatively stable population sizes throughout the past 1,500 years, until recently, when inshore lineages began to expand. At the same time *T. t. gephyreus* began to decline and may be the cause of the extremely low diversity, which is further inferred by the many small ROH observed in this inshore lineage. The results of this chapter highlight the role of niche divergence in the evolution of bottlenose dolphins and provide support for natural selection facilitating parallel adaptation of populations to similar environments.

Disease outbreaks have emerged as a major threat to cetacean populations worldwide, particularly for species that exhibit high social connectivity and gregarious behaviour, and for populations that are immunologically naive, small, and threatened. Cetacean morbillivirus (CeMV) has been a contributing factor in the death of tens of thousands of dolphins worldwide but has only recently been involved in the death of bottlenose dolphins throughout Australian waters. Given the low genetic diversity observed for inshore bottlenose dolphins and their exposure to a growing amount of pressure in coastal environments, it is important to understand the susceptibility of populations and the genomic basis of

immune responses to CeMV. Using whole genomes from survivor and non-survivor SABDs from a population that suffered an unusual mortality event linked to CeMV, association-based methods based on 10 million Single Nucleotide Polymorphisms (SNPs) revealed evidence of selection at 15,769 SNPs. Annotation of these SNPs disclosed 295 protein coding genes, which included 50 genes with functions relating to the innate and adaptive immune systems, and to cytokine signalling pathways. Prediction of the candidate SNPs and their functional effect identified missense mutations within the immune genes *CD300LF*, *NFATC2* and *NFKBIZ,* which may be implicated in the regulation and expression of interleukins and T cells. Candidate genes also included those known to be involved in immune responses to other morbilliviruses, such as measles in humans and the phocine distemper virus in pinnipeds (e.g., *IL4α*), while a lack of diversity was observed in some immune genes known to be important in combatting viruses in mammals (e.g., Toll-like receptors). These results highlight the importance of cytokines, T cells and interleukins in fighting CeMV infection, and adds to our understanding of major marine mammal immune responses.

This study generated the first reference genome for the SABD, providing a much-needed resource to understand the evolution of bottlenose dolphins. In addition, it generated a whole genome dataset that was used to clarify the phylogenomic relationship of bottlenose dolphins, and to identify species- and lineage-specific genes and pathways under positive selection in bottlenose dolphins. That dataset was also used to elucidate the influence of niche divergence and demographic history on present day genetic diversity and on putative parallel adaptation of *Tursiops* spp. The work also disclosed candidate immune genes putatively involved in CeMV susceptibility and resistance in dolphins. This thesis makes an original contribution to advance our knowledge on eco-evolutionary patterns and adaptive potential of bottlenose dolphins and it generates information that can be integrated into policy and action plans to promote sound conservation management strategies. The latter is particularly timely for bottlenose dolphin lineages that exhibit low diversity, small population sizes and are most vulnerable to a growing amount of environmental and anthropogenic stressors.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and 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.

Kimberley Batley

Acknowledgements

I would like to thank my supervisors A/Prof Luciana Möller and Prof. Luciano Beheregaray. I will be forever grateful for the opportunity to live out my childhood dream of researching dolphins. Thank you for sharing your knowledge and passion with me and for always believing in me. I'd like to thank Yuma, for whom without this thesis would not have been possible. Thank you for always remaining patient and believing in me, we made a good team!

Thank you to all members of CEBEL and MELFU, I have made some friendships that will last a lifetime. Bec, when I started this journey, I never thought that I'd come out with a best mate. Thank you for always listening to me, supporting me, and for agreeing that every day is a "treat yourself" kind of day. Isabella, you are the clumsy, noisy friend that I never knew I needed. Thank you for always encouraging and supporting me. You turned bad days into good days, and always made me feel like I could achieve anything, I wish you every bit of luck for your PhD and with your whole genomes. Thank you, Andrea, for being a very entertaining office buddy, for putting up with my moods and for always being there for a chat when I needed it.

I would like to thank my incredible friends, whom without their distractions and banter I may have gone insane. A special thanks to my Jets family. Seeing you four times a week was the best distraction and is what kept me pushing for the four years. Tez, you will never know how important you were for me throughout these four years. You are a the most selfless person I have met, and I thank you for always listening and for just being weird.

Finally, I'd like to thank my family. Your unwavering love, support and encouragement is what drives me to be the best version of myself. Mum, you are the strongest person I know. Without your strength I would not have completed this thesis. Dad, thank you for teaching me the importance of remaining calm and relaxed, and always having the time to listen, no matter where you are in the world. To my best mate and brother, Nathan, I would never have accomplished what I have if I didn't have you to look up to. Lastly, thank you Tom. You are my rock. You were by my side every step of the way, and I can't thank you enough for always listening and supporting me through my many endeavors and obsessions.

This PhD would not have been possible without all your support!

Ethics Statement

All samples used in this thesis were collected in accordance with the relevant guidelines and regulations of the country in which the work was conducted.

In South Australia, fieldwork was conducted under permits from the Department for Environment and Water (DEW), permits #K25761-6, #E25889 and #E26171 and under Ministerial Exemption from Primary Industries Resources South Australia (PIRSA), exemption #9902648, #9902404, #9902714, #9902601. Animal ethics approval was obtained from the Flinders University Animal Welfare Committee, project #E375, #E310 and #E326.

In Eastern Australia, biopsy samples were collected under licenses from the Department of Environment and Climate Change, License Number: S10763 and the Marine Parks Authority, Permit Number: PSGLMP 2008 / 003. Animal ethics approval was obtained from the Macquarie University Animal Ethics Committee (AEC Reference Number: 2007 / 013).

Biopsy samples from the Southwest Atlantic Ocean were collected under regional permits (Brazil: SISBIO 24429-1 issued to PAC Flores, SISBIO 24407-2 issued to PF Fruet) and transferred to Australia under CITES permits 11BR007432/DF and 2011-AU-647980.

Chapter 1: General Introduction

1.1 Biodiversity loss

Biodiversity is becoming increasingly recognised as one of the key measures of ecosystem health with species, genetic, ecosystem and functional diversity all being vital for ecosystem processes, stability, and persistence (Mace et al. 2012). However, biodiversity loss at all four levels has become a major conservation concern with human impacts threatening species, populations and ecosystem processes globally at an alarming rate (Ceballos et al. 2015). Human-induced extinctions correspond well with the expansion of modern humans out of Africa, with a continued rapid growth of the human population putting great stress on the natural environment and its biodiversity (McKee et al. 2004, Tilman et al. 2017). By 3,000 years ago human induced impacts had led to the extinction of over half the terrestrial mammalian megafauna species, and 15% of avian species (Barnosky et al. 2011, Tilman et al. 2017). The human population has grown to over 25 times larger than it was 3 kya (Tilman et al. 2017), with resulting overexploitation, habitat destruction, introduction of invasive species, spreading of pathogens and parasites, pollution, and climate change among some of the key drivers of the biodiversity loss seen today (reviewed in Mazor et al. 2018).

Habitat loss, degradation and fragmentation is widely considered the world's leading cause of biodiversity loss (Johnstone et al. 2014), as is the overexploitation of species for commercial and recreational purposes. Overexploitation has contributed to the decline of 72% of species that are classified as near threatened on the International Union for Conservation of Nature (IUCN) Red list (Maxwell et al. 2016). In addition, climate warming and related extreme weather events are already negatively impacting wildlife populations, and in the absence of effective management, an increasing number of species are expected to be at risk of decline and extinction (Parmesan 2006, Thomas et al. 2013, Morán-Ordóñez et al. 2017). Organisms generally adapt to local environmental conditions, such as average temperatures, and are able to acclimatise to temperatures around the average (Hoegh-Guldberg and Bruno 2010). Beyond these averages, however, such as during extreme weather events, organisms face challenges to adapt (Hoegh-Guldberg and Bruno 2010) and are likely to either shift their home range, or not survive. Environmental change, habitat fragmentation and pollution are also contributing to rapid changes in pathogen biology, with some pathogens emerging, and others re-emerging in wild populations (e.g. Altizer et al. 2003, Morens et al. 2004, Acevedo-Whitehouse and Cunningham 2006, Karlsson et al. 2014, Cunningham et al. 2017). By triggering large morbidity and mortality events, these infectious agents have the ability to alter the function and structure of ecosystems, influence host genetic diversity and constrain population growth or lead to declines (Van Bressem et al. 1999, Altizer et al. 2003, Ward and Lafferty 2004). Indeed, examples of novel pathogens causing widespread population declines in wildlife populations are becoming more common (Fenner 2000, Altizer et al. 2003, Lazenby et al. 2018). For example, emergence of the white-nose syndrome has devastated bat populations across northern America, leading to a decline of at least 75% in some

populations, and threatening regional extinction of the little brown bat (*Myotis lucifugus*) (Blehert et al. 2009, Frick et al. 2010). The influence of these stressors on wildlife populations are expected to be exacerbated under growing environmental stress, with populations and species predicted to face greater and stronger selective pressures into the future (Foden et al. 2013).

Currently, one quarter of all mammal species listed on the IUCN Red List are threatened to extinction (Schipper et al. 2008). The current rate of extinction is substantial and thought to be similar to those estimated for the five global mass extinction events of the past 500 million years (Barnosky et al. 2011, Ceballos et al. 2015). However, even in species that are not currently threatened, population-level vulnerability exists, with monitored vertebrate populations estimated to have declined on average by 68% over the past five decades (WWF 2020, Bradshaw et al. 2021). These continuous declines may eventually lead to species-level extinctions, additional biodiversity loss, and impact on local ecosystem processes (Ceballos and Ehrlich 2002, Ceballos et al. 2015).

1.2 Importance of maintaining marine biodiversity

The declining trend of global biodiversity is causing widespread changes to ecosystem functions, structure, and processes (Chapin et al. 2000, Hooper et al. 2005), including in marine ecosystems. One third of the world's human population reside in coastal regions and rely heavily on marine ecosystems for many essential goods, services, and cultural benefits (Worm et al. 2006, Barbier 2017). Marine ecosystems provide goods in the way of food, plant and animal resources; services such as recreation, tourism and protection (reviewed in Barbier 2017); and cultural benefits through interactions, cultural practices and products (Fish et al. 2016). Maintaining biodiversity is also important for ecosystem functioning, including nutrient storage and cycling, sustaining biological and genetic diversity, the regulation of trophic level dynamics and the shaping of community structure (Tavares et al. 2019). In the marine environment, megafauna are expected to contribute the most to supporting ecosystems due to their body size and mass, with species removal expected to have widespread impacts on ecosystem functioning and structure (Tavares et al. 2019). For example, overfishing of large predatory shark species in the United States led to an increase in abundance of their prey items, small elasmobranchs (rays, skates and small sharks) and the subsequent decline of scallop populations (Myers et al. 2007). This had large ramifications for the scallop fishery, ending a century-long industry (Myers et al. 2007). Ecosystem resilience may also be impacted by top level predators, as is the case of the bumphead parrotfish (*Bolbometopon muricatum*) that promotes reef resilience by removing structurally weak table corals through predation, which would otherwise weaken the structure of the coral reef system (reviewed in Estes et al. 2016b). These examples highlight the direct and indirect impacts of the removal of species or populations on entire ecosystems, as well as the importance of conserving biodiversity for ecosystem functioning. The rate at which the marine environment is changing places great stress on populations and species globally, and particularly on those with a reduced capacity to adapt. Therefore,

in the absence of large-scale and effective conservation and management plans, the rate of population and species level extinctions and their impact on ecosystem services will continue to accelerate.

1.3 Marine mammals and their function, current status, and threats

Marine mammals are often termed "ecosystem sentinels" due to their key roles in maintaining ecosystem structure and functions, their potential to provide insight into overall ecosystem health, and as indicators of anthropogenic impacts (Ross 2000, Wells et al. 2004, Hazen et al. 2019). They control important functions such as nutrient recycling, and in shaping food webs and community structure (Tavares et al. 2019). Their population and species declines may have permanent consequences for ecosystem functioning and services. For example, some marine mammals may enhance primary productivity in biological hotspots through the vertical mixing and horizontal transfer of nutrients from deep ocean sources that may later be consumed by other species (Roman and McCarthy 2010, Roman et al. 2014, Doughty et al. 2016). The relative contribution of nutrient recycling by marine mammals is still unknown, however, this process is thought to have been reduced with human-induced declines of great whale populations (*Balaenopteridae*, *Physeteridae*, *Balaenidae*, *Eschrichtiidae*) (Roman and McCarthy 2010, Doughty et al. 2016). Reductions in marine mammal populations have also been linked to changes in the structure of food webs (Estes and Palmisano 1974, Springer et al. 2003, Estes et al. 2016a). A classic example is the decimation of a North Pacific sea otter (*Enhydra lutris*) population due to hunting and potentially increased predation from killer whales (*Orcinus orca*) (Springer et al. 2003). This led to an increase in sea urchin density, and a subsequent decline in kelp density (Estes and Palmisano 1974). Further, the demise of the now extinct Steller's sea cow (*Hydrodamalis gigas*) that lived in, and fed on kelp forests, is thought to have been indirectly linked to loss of kelp forest habitat (Estes et al. 2016a). In addition to their important ecological roles, marine mammals also provide great societal and economic benefits through wildlife tourism. Wildlife tourism can provide tourists with improved physiological health and conservation awareness. In 2008 it was estimated that whale watching (including dolphins and porpoises) contributed US$2.1 billion to the global economy (O'Connor et al. 2009). Yet, despite the ecological, societal and economic importance of marine mammals, they are disproportionately less well studied than terrestrial mammals (Schipper et al. 2008), with 17% of marine mammal species being data deficient (IUCN 2020). Of the species that have adequate data available, 36% are estimated to be threatened (Schipper et al. 2008), but more recent estimates may be closer to 32%, with almost 8% being near threatened (IUCN 2020). Further, at least 50% of data sufficient species have declining population trends (IUCN 2020), suggesting that marine mammal populations may continue to decline, unless appropriate recovery plans and conservation efforts are implemented (Schipper et al. 2008).

Marine mammals are highly vulnerable to anthropogenically induced climate change and human activities due to their population biology and life history traits (Fair and Becker 2000, Silber et al. 2017).

Species that are vulnerable to climate change include those with restricted ranges, slow reproductive rates, delayed age at maturity and specialised diets, all of which are traits characteristic of some marine mammal species (Fair and Becker 2000, Pacifici et al. 2015, Silber et al. 2017). Although there are limited physical barriers in the oceans that limit marine mammal dispersal, some species show high site-fidelity to coastal regions of high use, have limited connectivity to adjacent populations and low genetic diversity (Möller et al. 2007, Charlton-Robb et al. 2014, Fruet et al. 2014, Zanardo et al. 2016b, Pratt et al. 2018). Due to these traits, some populations are particularly vulnerable to human impacts, with unintentional fishing and harvesting of aquatic resources, development and disturbance of habitats, climate change and extreme weather events, and pollution and disease among some of the key threats at a global level (Davidson et al. 2012). Direct fisheries interactions pose a significant threat to marine mammal populations worldwide, with fisheries bycatch estimated to cause the death of at least 650,000 individuals annually (Read 2008, Davidson et al. 2012). This was a major factor in the first human-caused cetacean extinction, the baiji (*Lipotes vexillifer*), and is also the cause of the rapid decline of the most critically endangered marine mammal today, the vaquita (*Phocoena sinus*) (Turvey et al. 2007, Read 2008, Rojas-Bracho and Reeves 2013, Jaramillo-Legorreta et al. 2019). In addition, some marine mammals are particularly sensitive in their behavioural responses to habitat invasion by humans, with changes in behaviour (and sometimes mortality) often observed in response to habitat destruction and intrusion, and to acoustic disturbance (Tyack et al. 2011, Rycyk et al. 2018). The frequency of mass stranding events of the elusive beaked whales (genus *Ziphius*) has been on the rise since the 1960's, corresponding well with the development of naval mid-frequency active sonar (Bernaldo de Quiros et al. 2019). Humans also threaten populations worldwide through the release of pollutants into the marine environment, with polychlorinated biphenyls causing substantial harm to wildlife populations (Roos et al. 2012). Exposure to these compounds is expected to lead to a collapse of 50% of killer whale (*Orcinus orca*) populations over the next 100 years (Desforges et al. 2018), while contaminants in beluga whales (*Delphinapterus leucas*) and California sea lions (*Zalophus californianus*) have been linked to cancer occurrence (Martineau et al. 2002, Randhawa et al. 2015, Baines et al. 2021).

Human accelerated climate change and resulting extreme weather events also threaten community dynamics, distribution, and persistence of marine mammal populations (Schumann et al. 2013, Albouy et al. 2020). The primary response of individuals, populations and species to a changing climate is to track their favoured ecological niche by shifting their range and distribution (Chen et al. 2011). For example, a poleward expansion of the generally tropical and subtropical Bryde's whale (*Balaenoptera brydei*) has been observed, likely driven by climate and oceanographic influences on prey availability (Kerosky et al. 2012). Extreme weather events have also been linked to compromised physiological responses of marine mammals, with sea surface temperature anomalies in the north-eastern Pacific Ocean reducing the immunocompetence of California sea lion pups (Banuet-Martinez et al. 2017). Changes in disease dynamics are also likely outcomes of climate change and threaten marine mammal

populations worldwide, with the number of incidences and intensity of infectious disease outbreaks appearing to have increased over the past 30 years (Gulland and Hall 2007, Hoegh-Guldberg and Bruno 2010, Sanderson and Alexander 2020). Changes in the prevalence, severity, transmission, and abundance of pathogens have been linked to the expansion of pathogens and host ranges, and increased host susceptibility induced by environmental stressors (Drew Harvell et al. 2002, Williams et al. 2002, Hoegh-Guldberg and Bruno 2010). For example, after major flooding events in New South Wales, Australia, novel poxvirus infections were observed in estuarine populations of Indo-Pacific bottlenose dolphins (*Tursiops aduncus*), highlighting the influence of environmental and physiological stressors on the manifestation of some diseases (Van Bressem et al. 2009b, Fury and Reif 2012). Recent modelling of climatic factors and mass mortality events associated with infectious diseases highlighted an upward trend in the occurrence of disease outbreaks and sea surface temperatures, with 61% of outbreaks occurring during periods of elevated sea surface temperatures (Sanderson and Alexander 2020). Marine mammals worldwide are faced with rapidly changing and stressful environments, and those that can adapt to these changes will be better equipped to persist into the future.

Currently, at least five marine mammal species are globally extinct (Magera et al. 2013, IUCN 2020), and many more are threatened or near threatened. However, with scientifically informed and targeted conservation efforts, the maintenance and/or recovery of populations and species is possible. Over 40% of marine mammals on the IUCN Red List have stable or increasing population trends (Magera et al. 2013, IUCN 2020). These include species that are, or were highly threatened due to prior commercial harvesting and pre-modern and modern hunting, such as the sei whale (*Balaenoptera borealis*), blue whale (*Balaenoptera musculus*), fin whale (*Balaenoptera physalus*), humpback whale (*Megaptera novaeangliae*), and the Mediterranean monk seal (*Monachus monachus*) (IUCN 2020). While humpback whales are currently the only of these species that has been de-listed to least concern, these trends highlight the potential of populations to positively respond to a shift from resource exploitation and human-induced impacts towards conservation efforts (Schipper et al. 2008). Given the importance of marine mammals in marine ecosystems and in societal and economic functioning, and their vulnerability to human impacts, population and species declines will have widespread impacts on ecosystem structure and function. It is therefore important that information from multiple disciplines are integrated to make predictions about how marine mammal populations are likely to respond to changing environments, and to ensure their protection and long-term viability.

1.4 Integrating genomics into species conservation efforts

Conservation strategies to maintain biodiversity and ecosystem functions are being developed by integrating a wide range of scientific disciplines (Shafer et al. 2015b). The ultimate goal of these efforts is to produce information that can be used to maintain the long-term viability of species and populations by mitigating species and population threats, and to clarify aspects about species biology, ecology,

conservation status, and genetics (Brandies et al. 2019). A fundamental aspect of a population's viability is its genetic diversity (Reed and Frankham 2003), that is, the amount of variability in the genome that helps mitigate the deleterious effects of inbreeding and provides potential for populations to withstand or adapt to environmental changes (Brandies et al. 2019). Population size and connectivity are important factors that influence genetic diversity, with inbreeding and increased genetic drift (i.e., changes in allele frequency due to chance) often leading to a loss of genetic variability in small and/or isolated populations (Reed and Frankham 2003, Höglund 2009, McMahon et al. 2014). Therefore, genetic factors, including past and present fluctuations in genetic diversity, as well as population structure, genetic connectivity, and inbreeding (Shafer et al. 2015b) are of particular importance in disentangling the adaptive potential of wild populations.

The application of genetic techniques in conservation biology have provided great insight into population viability (Steiner et al. 2013), including how genetic drift influences genetic diversity, levels of inbreeding, and the amount of gene flow within and between populations (Ouborg et al. 2010). Advancing technologies and decreasing costs are allowing larger genomic datasets to be generated, including for non-model species, improving resolution to address unresolved conservation questions. Genomic datasets can help researchers understand how genetic variation differs across regions of the genome and within a species, and the importance of parts of the genome in evolutionary processes such as speciation, local adaptation, and adaptability to changing environments (McMahon et al. 2014). For example, some parts of the genome are invariant, while other gene regions, such as the major histocompatibility complex (MHC) involved in immune defence in vertebrates, are often highly variable (Acevedo-Whitehouse and Cunningham 2006). This, however, varies between species and populations, begging the question of how genomic regions and functions are important in species survival (McMahon et al. 2014). In addition, genomic datasets contribute to improving estimates of past and present demography, understanding diseases and host susceptibility, the genetic basis of inbreeding, phylogenomics and hybridisation (Fitzpatrick et al. 2011, Miller et al. 2012, Steiner et al. 2013, McMahon et al. 2014), all of which are important for population persistence.

Despite the IUCN recognising the importance of conserving genetic diversity (Reed and Frankham 2003, Garner et al. 2020), genetic factors are not commonly used in species conservation strategies and assessments (Laikre 2010, Garner et al. 2020), and the application of genomics in species conservation remains relatively limited (Shafer et al. 2015b, Brandies et al. 2019). Few species actually have genetic and/or genomic data available, even when genetic action is listed in their species recovery plan (reviewed in Brandies et al. 2019). In the terrestrial realm, several examples exist of how advancing genomic capabilities have facilitated the conservation and management of threatened species, such as the Tasmanian devil (Brandies et al. 2019). The emergence of a transmissible cancer, the devil facial tumour disease (DTFD), led to a decline of up to 80% of the population, resulting in the Tasmanian

devil becoming threatened (Lazenby et al. 2018). Genetic techniques based on a small number of markers, such as microsatellites, initially highlighted the lack of genetic diversity in immune genes, as well as more broadly across the species (Jones et al. 2003, Cheng and Belov 2011). Genomic data, including reduced representation genome sequencing (RRS), reference genome assembly and whole genome resequencing, then greatly improved resolution of genetic inferences for the species. These resources allowed the detection of population substructure, relatedness between founders of an insurance population, the role of DFTD in swamping local adaptation, and regions of the genome likely linked to a resistant phenotype (Epstein et al. 2016, Hendricks et al. 2017, Wright et al. 2017, Fraik et al. 2020, Wright et al. 2020). In particular, by identifying DFTD-associated regions of the genome, it was possible to identify individuals or populations that may be more resistant to infection, providing targets for the development of potential treatments, and genetic markers to screen other populations (Wright et al. 2015, Epstein et al. 2016, Wright et al. 2017, Margres et al. 2018). These large datasets have also enabled more robust estimates of demographic histories (Patton et al. 2019) and are currently being used to investigate inbreeding depression and adaptation to captivity (Brandies et al. 2019). This in-depth research has provided managers with advice on translocations of Tasmanian devils to enhance functional diversity, inform the management of the insurance population, and aid in the development of vaccines (Hogg et al. 2017, Pye et al. 2018, Brandies et al. 2019, Hogg et al. 2020). This study system highlights how genomics can be used as a tool to address a broad range of questions to inform and enhance conservation and management strategies of a threatened species, and to help understand their potential to persist in a changing environment. With the current declining trend of biodiversity and an increasing number of threats to many species, it is imperative that learnings from conservation genomics are applied more broadly to species and populations that are vulnerable to human impacts and of conservation concern.

1.5 Genomic advancements and applications to marine mammal systems

Genomic studies in the marine environment are lagging behind those in terrestrial environments (Kelley et al. 2016, Grummer et al. 2019). This is despite the ocean comprising 70% of the Earth's habitats, and its diversity from coastal and estuarine to pelagic and abyssal zones that harbour the majority of the Earth's biomass. This issue is mostly due to the difficulties in obtaining samples from elusive species, the technical constraints often associated with working in marine environments, and the restricted possibility for experimental manipulations (Ribeiro et al. 2017). However, advancing computational methodology and sequencing technologies in the last decade, as well as decreasing costs, have enabled greater opportunities for genomic studies of non-model organisms (Larsen and Matocq 2019), including marine mammals. A review of trends in marine mammal genomic studies showed that prior to 2008 less than five studies in this area were published per year (all utilising mitogenomes), but following sequencing trends, the number of genomic publications, types of data generated and questions being

addressed have changed considerably (Cammen et al. 2016). Sequencing advances have facilitated improved capabilities and opportunities to generate genomic data, including using RRS, whole genome sequencing and resequencing datasets to address a wider range of biological questions and conservation concerns. This has resulted in a rapid growth in the number of publications each year, including for a greater number of marine mammal species. These types of genomic data have provided greater insight into genomic variability and selection across the genome, demographic history, phylogenomics, disease susceptibility and adaptation. Together, these developments have contributed to an improved understanding of population and species evolution, vulnerability and adaptive potential, and provide the next step forward to implementing targeted management frameworks to improve conservation efforts.

Population genetic studies have traditionally used a small number of neutral markers to provide insights into population structure, connectivity, migration, phylogenetics, relatedness and inbreeding (e.g. Krutzen et al. 2003, Attard et al. 2010, Frere et al. 2010, Pratt et al. 2018). However, RRS allows a greater number of neutral and adaptive markers that are distributed across the genome to be genotyped, improving the resolution of traditional analyses, and creating new avenues to study adaptation (Andrews et al. 2016, Cammen et al. 2016, Attard et al. 2018b). This type of data had not been published for a marine mammal prior to 2014 (reviewed in Cammen et al. 2016), but since then numerous studies have made use of RRS to resolve and better understand population structure and dynamics, such as population size, demographic history, relatedness and inbreeding (Moura et al. 2014b, Shafer et al. 2015a, Lah et al. 2016, Attard et al. 2018a, Attard et al. 2018b, Cammen et al. 2018, Genoves et al. 2020, Peart et al. 2020, Barceló et al. 2021), phylogenomics (Viricel et al. 2014, Moura et al. 2015, Foote and Morin 2016, Moura et al. 2020), genomic variation and disease susceptibility and resistance (Hoffman et al. 2014, Cammen et al. 2015a, Batley et al. 2019), and adaptation to ecological niches (Moura et al. 2014b). For example, Lah et al. (2016) found that RRS data improved the resolution to delineate population differentiation in the highly mobile harbour porpoise (*Phocoena phocoena*). Other studies have used genomics to extend inferences drawn from traditional markers, such as to understand adaptive variation and adaptation to ecological niches. Attard et al. (2018b) drew important conservation implications from a lack of population structure and adaptive variation between pygmy blue whales (*B. musculus brevicauda*) of two Australian feeding aggregations (Attard et al. 2010, Attard et al. 2018b). The lack of neutral and limited adaptive divergence may be driven by blue whales travelling large distances between feeding areas and to breeding grounds and needing to adapt to a range of environmental conditions and feeding strategies (Attard et al. 2018b). Other species show some local adaptation to different ecotypes or selective pressures. For instance, variation in regions of the killer whale genome were associated with feeding specialisations and habitat use (Moura et al. 2014b), while common bottlenose dolphins (*Tursiops truncatus*) seem to exhibit particular genomic variations associated with resistance to harmful algal blooms (Cammen et al. 2015a). While the number of published RRS datasets are still relatively limited for marine mammals, they provide great potential in

delineating fine-scale population structure, local adaptation and selective pressures, and insights into how populations and species may respond to ongoing climate change. It is expected that as more genomic resources become available for marine mammals, such as reference genomes, epigenomes, and transcriptomes, RRS approaches, including RADseq, Single Nucleotide Polymorphism (SNP) arrays and target sequence capture (reviewed in Cammen et al. 2016) will be utilised more often.

1.6 Marine mammal reference genomes

High-quality reference genomes are necessary for the study of functional, comparative and population genomics within and between species, with knowledge gained from these studies essential for biodiversity conservation (Rhie et al. 2020). Over the past decade, significant initiatives have been underway to generate high-quality reference genomes for almost all living biota, including for eukaryotes (Earth BioGenome Project), and for vertebrates (e.g. Vertebrate Genomes Project, Rhie et al. (2020) and the Genome 10K Project, Koepfli et al. (2015)). Local efforts, such as the Oz Mammals Genomics initiative (OMG), aims to sequence the genomes of Australian mammals (Potter and Eldridge 2017). Many other initiatives exist, including those that aim to improve the assemblies of those already published (e.g. DNAZoo, Dudchenko et al. (2017)), as well as taxa specific initiatives such as the Cetacean Genomes Project (in collaboration with the Vertebrate Genomes Project) that aims to sequence high-quality genomes for all cetacean species (Morin et al. 2020a). The first reference genome for a marine mammal was that of the common bottlenose dolphin, which was made available on NCBI's online genome database in 2012 at 2.59x coverage and containing over 240,000 scaffolds (Table S1.1). This genome has received the most attention of all marine mammals, with considerable efforts to further improve its genome quality. For example, the coverage of the genome was further increased as part of a comparative analysis of 29 mammalian genomes to better characterise signatures of evolutionary constraint in mammals and humans (Lindblad-Toh et al. 2011). A further nine papers have since probed the genome to understand the macroevolutionary transition of mammals to an aquatic lifestyle, and to search for regions and genes under positive selection in cetaceans (McGowen et al. 2012, Shen et al. 2012, Nery et al. 2013, Sun et al. 2013, Yim et al. 2014, Foote et al. 2015, Keane et al. 2015, Park et al. 2015, Warren et al. 2017). Since this first marine mammal genome was made available, there has been a rapid increase in the number of assemblies and genomes generated and submitted to public genome databases (Figure 1.1). At the time of writing, there were 119 genome assemblies available for marine mammals (sirenians = 5, marine fissipeds = 7, pinnipeds = 30, cetaceans = 77) on NCBI or DNAZoo, of which 55 species and sub-species (sirenians = 3, marine fissipeds = 3, pinnipeds = 13, cetaceans = 36) have an available genome. These assemblies' range in their coverage, completeness, and assembly level (Table S1.1), but they all provide essential resources for investigating and advancing knowledge on genomic features and species conservation. For example, researchers have been able to better understand inbreeding, genomic diversity and demography (Vijay et al. 2018, Beichman et al. 2019,

Fan et al. 2019, Westbury et al. 2019, Hooper et al. 2020, Morin et al. 2020b), adaptation (Keane et al. 2015, Kishida et al. 2015, Autenrieth et al. 2018, Park et al. 2018, Beichman et al. 2019, Fan et al. 2019), phylogenomics and hybridisation (Árnason et al. 2018, Foote et al. 2019, Lammers et al. 2019) in marine mammal systems, even using highly fragmented assemblies (Morin et al. 2020a).

Reference genomes also provide important insights into the evolution of mammals in general. Brüniche-Olsen et al. (2018) compared the genomes of 78 mammalian species (including six marine mammal species) to assess the relationship between genomic diversity, runs of homozygosity (inbreeding) and conservation status and population biological traits, such as population size, latitudinal distribution, body mass and trophic level. That study found that population traits have a significant effect on genomic diversity and inbreeding, but found no relationship between genomic diversity and conservation status (Brüniche-Olsen et al. 2018). Genomic variation has long been recognised as an important factor in species diversity, health, and in evolutionary processes (Reed and Frankham 2003, Garner et al. 2020), yet that study suggested that conservation status is a poor indicator of genomic diversity and argued that genomic diversity should be incorporated into species conservation status assessments.

In addition, reference genomes provide information about the demographic history of species and the role that this plays in shaping patterns of genomic diversity that may underpin population persistence. Analysis of the highest quality and most complete marine mammal genome to date, the vaquita genome, identified the lowest genomic diversity for any mammal (Morin et al. 2020b). This finding was thought to result from long-term small population size rather than a recent population bottleneck (Morin et al. 2020b). This species is critically endangered, and these results suggest that recovery of the vaquita is likely not hindered by genetic factors. Reference genomes have also enabled the search for genomic signatures that may be vital to species adaptation or survival, such as genes linked to cancer and aging in long-lived species (Keane et al. 2015, Tejada-Martinez et al. 2021). Genome assembly and analysis of the longest-lived mammal, the bowhead whale (*Balaena mysticetus*) revealed a loss and gain of genes that may be associated with cancer and age-related disease resistance, and genes associated with DNA and cell-cycle repair, providing insights into the evolutionary mechanisms that may confer longevity in some species (Keane et al. 2015). Finally, while reference genomes provide the next necessary step to understand key evolutionary processes, they also provide important resources for improving mapping and to call SNPs from RRS data (Maroso et al. 2018), as well as in generating SNP panels that may prove important for genotyping individuals and populations at functionally important genes (e.g. Brandies et al. 2019). Reference genomes and assemblies are currently being generated at an exceptional rate (Figure 1.1), and it is expected that reference genomes for at least a further 13 cetacean species will be released in 2021 (Beling 2021). It is evident that in the past few years, marine mammals have attracted a lot of interest from researchers that has enabled numerous insights into the evolution and adaptation of marine mammals.

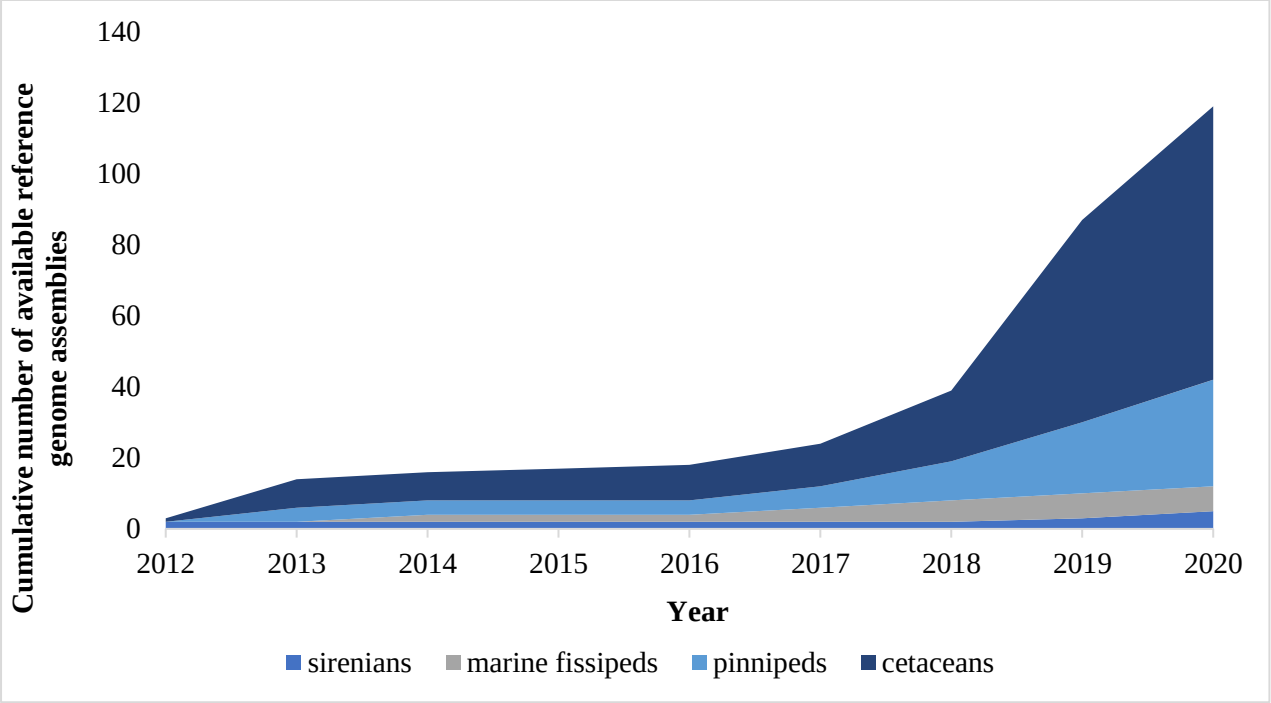

Figure 1.1: Trends in the number of reference genome assemblies becoming available since 2012 for the four taxonomic groups of marine mammals (sirenians: manatees and dugongs; marine fissipeds: polar bears and sea otters; pinnipeds: seals, sea lions and walruses; cetaceans: whales, dolphins and porpoises).

1.7 Whole genome resequencing studies of marine mammal populations

Reference genomes are often sequenced at high depth (usually upwards of 100x, Cammen et al. (2016)), combine multiple library techniques (short and long-insert libraries), and generally require a great deal of expertise, cost, computational resources and time to generate (Brandies et al. 2019). Counteracting this however, reference genomes have made it possible to sequence reads from genomes at a lower coverage (≥ 2x) that can then be aligned to pre-existing and high-quality reference genomes, allowing a greater number of individuals from multiple populations to be sequenced at a lower cost (Cammen et al. 2016). Like RRS, this enables the study of genomic variation between individuals within and between populations, but across the entire genome. This allows the exploration of variation in phenotypes, disease susceptibility, and adaptation to environmental conditions and habitats, which are all important for understanding a species adaptive potential. Despite the possibilities that whole genome resequencing data provides, few studies have utilised whole genome resequencing data in marine mammal systems to date. To the best of my knowledge, the few studies that have so far utilised this type of data have investigated speciation, demographic history, population differentiation, adaptation to ecological niches, and inbreeding in polar bears (*Ursus maritimus*) (Liu et al. 2014), killer whales (Foote et al. 2016, Foote et al. 2019, Hooper et al. 2020) and bottlenose dolphins (Vijay et al. 2018, Louis et al. 2020). Whole genome population genomic studies offer the ability to understand how

populations and species have independently evolved through selection and adaptation to novel habitats and environmental conditions, even for species that have recently diverged. For example, a comparison of polar and brown bear (*Ursus arctos*) genomes from three populations revealed that the two species diverged less than 500 kya (Liu et al. 2014). The work also found that regions in the polar bear genome are under greater positive selection, particularly in genes that may be associated with the reorganisation of the cardiovascular system (Liu et al. 2014). Similarly, Louis et al. (2020) explored the divergence and ecotypic evolution of inshore and offshore pairs of common bottlenose dolphins from two ocean basins in the Northern Hemisphere. The evolution of the inshore ecotype between ocean basins (North Pacific and North Atlantic oceans) were relatively independent, but similar between regions in the same ocean basin (northeastern and northwestern Atlantic), and genes with behavioural and ecological functions were found to be under parallel evolution (Louis et al. 2020). These comparative genomic approaches provide new possibilities to explore the genomic mechanisms that allow species to extend their ranges and adapt to novel environments and selective pressures.

1.8 The evolution of bottlenose dolphins

Bottlenose dolphins have a widespread distribution occupying almost all ocean basins, except for polar regions (Figure 1.2). The rapid radiation of the sub-family Delphininae, including bottlenose dolphins, has resulted in incomplete lineage sorting and even possible hybridisation (Amaral et al. 2012). Consequently, bottlenose dolphin taxonomic relationships are heavily debated. Currently, only two species of bottlenose dolphins are accepted; the Indo-Pacific bottlenose dolphin, *T. aduncus,* and the common bottlenose dolphin, *T. truncatus* (Committee on Taxonomy of the Society for Marine Mammalogy 2020). Despite these species often occupying overlapping habitats (depending on the ocean region), they face disparate selective pressures across their range that has likely led to the pronounced level of morphological, molecular, and ecological variation observed within the genus (Moura et al. 2013, Wickert et al. 2016, Gridley et al. 2018, Pratt et al. 2018, Moura et al. 2020). For example, the common bottlenose dolphin occupies inshore, nearshore, and offshore regions, with a high level of divergence often observed between inshore and offshore populations (Hoelzel et al. 1998, Louis et al. 2014a, Louis et al. 2014b, Fruet et al. 2017, Nykanen et al. 2018, Costa et al. 2019). This divergence has led to the acceptance of three subspecies; the Black Sea bottlenose dolphin (*T. t. ponticus*), common bottlenose dolphin (*T. t. truncatus*) and the Lahille's bottlenose dolphin (*T. t. gephyreus*) (Viaud-Martinez et al. 2008, Costa et al. 2016, Wickert et al. 2016, Fruet et al. 2017). The latter inhabits estuaries and inshore waters from southern Brazil to Argentina (Figure 1.2) and has been classified as vulnerable due to small and declining population trends (Fruet et al. 2014, Fruet et al. 2017, Vermeulen et al. 2019). In the Indo-Pacific region, however, the inshore form is generally classified as the Indo-Pacific bottlenose dolphin (Figure 1.2). These dolphins are almost entirely inshore, often residing in highly urbanised coastal regions, and generally live in small populations with limited

connectivity to adjacent populations (Möller et al. 2007, Wiszniewski et al. 2009, Charlton-Robb et al. 2014, Zanardo et al. 2016b, Pratt et al. 2018). In southern Australia, a third species, the Burrunan dolphin (*T. australis*) has been suggested based on genetic data (Charlton-Robb et al. 2011a), but is not generally accepted by the marine mammal scientific community due to insufficient morphological support (Jedensjö et al. 2017, Jedensjö et al. 2020). Greater genomic resolution suggests this may be a subspecies of the Indo-Pacific bottlenose dolphin (Jedensjö et al. 2017, Jedensjö et al. 2020, Moura et al. 2020, Pratt 2020), and will be referred to here as the southern Australian bottlenose dolphin (SABD). With the current debate surrounding the taxonomy of bottlenose dolphins, it is important to investigate the evolutionary history of bottlenose dolphins and speciation mechanisms.

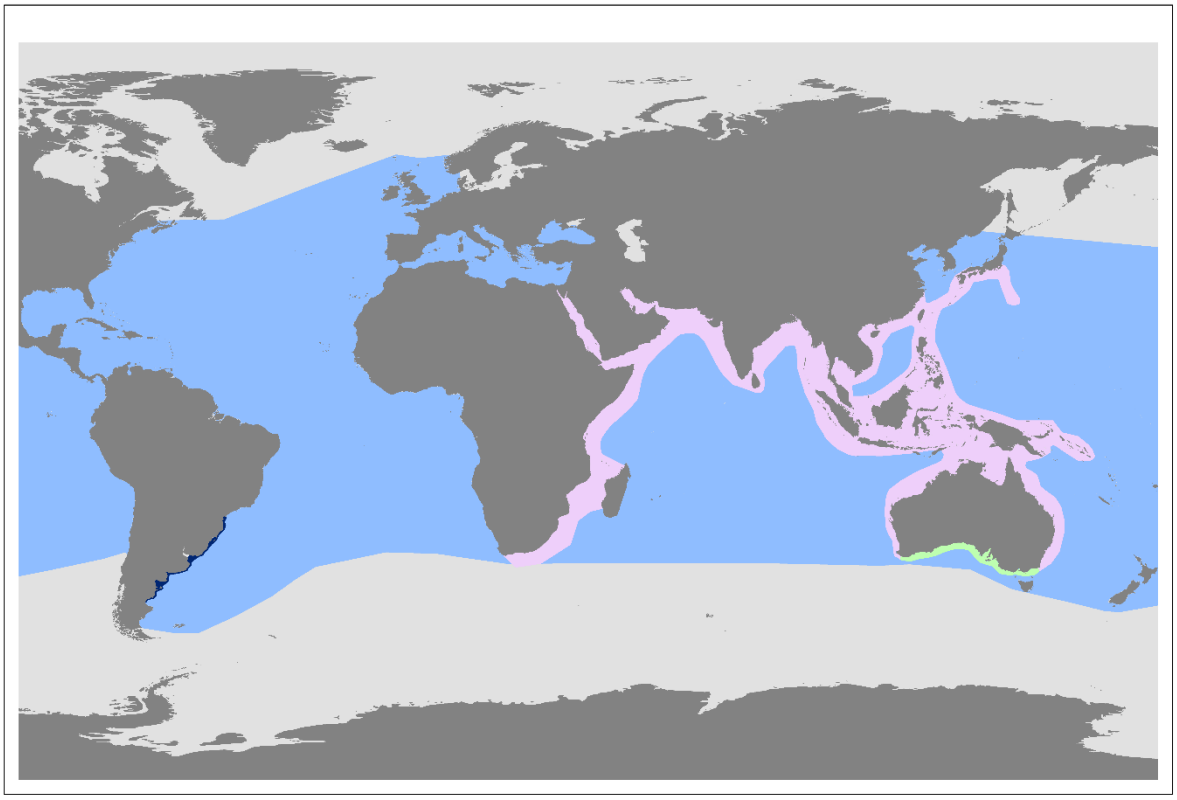

Figure 1.2: The estimated global distribution of bottlenose dolphin species and subspecies in this study, including the two currently accepted species: the common bottlenose dolphin (*T. truncatus*) (light blue), the Indo-Pacific bottlenose dolphin (*T. aduncus*) (pink): the accepted subspecies, Lahille's bottlenose dolphin (*T. t. gephyreus*) (dark blue); and the potential subspecies, the southern Australian bottlenose dolphin (*T. aduncus*) (green). The map is redrawn from the IUCN red list marine mammal shape file; last accessed Feb 16, 2019 at https://www.iucnredlist.org/resources/spatial-data-download, with adaptation from the expected global distribution of *T. aduncus* from the Encyclopedia of Marine Mammals.

In the marine environment, inshore and offshore habitats tend to vary quite considerably in their oceanographic features, with environmental heterogeneity (e.g. differences in sea surface temperatures, salinity and the distribution of prey) linked to the genetic structuring of bottlenose dolphins (Natoli et al. 2005, Bilgmann et al. 2007b, Díaz-Gamboa et al. 2018), and the morphological and ecological

diversity observed across the genus (Louis et al. 2014a, Louis et al. 2014b, Costa et al. 2019). For example, both ecotypes of bottlenose dolphins generally differ in their body and fin size, skull characteristics, skin colouration and pattern, and in their feeding ecology (Jedensjö et al. 2017, Díaz-Gamboa et al. 2018, Genoves et al. 2020, Jedensjö et al. 2020). These differences are not however always uniform across all inshore-offshore pairs, with some comparisons showing reversed traits, or even weakened contrasts between the two (Louis et al. 2014a, Pratt 2020). In Australia and the northwestern Atlantic Ocean, the offshore lineage is generally larger than their inshore counterpart (Charlton-Robb et al. 2011a, Louis et al. 2014a). In the South Atlantic Ocean, the inshore lineage is generally larger than the offshore (Costa et al. 2016, Díaz-Gamboa et al. 2018). In the northeastern Atlantic Ocean only weak differences in body size can be observed (Louis et al. 2014b). These disparities are thought to be due to variation in the level of differentiation between oceanographic features, and the time of divergence between inshore and offshore lineages (Louis et al. 2014a). Advancing technologies have now enabled the ability to infer the influence of environmental conditions on adaptation and the subsequent differentiation between inshore and offshore lineages. For example, evidence of parallel adaptation to inshore habitats in the North Atlantic revealed the importance of genes involved in cognitive functioning and feeding (Louis et al. 2020). In the Southern Hemisphere, Pratt (2020) found similar patterns of parallel adaptation, but in genes involved in major bodily systems (e.g. cardiovascular, sensory, musculoskeletal, gastrointestinal, energy production, nervous and osmoregulatory systems), which may be in response to differences in depth, prey abundance, and distribution. Expanding on this work, whole genome resequencing improves the ability to understand the role of selective pressures on accelerating selection in protein coding genes across the genome, and that may be of functional importance and evolving under natural selection.

From a historical perspective, climatic and geological events have also been linked to the genetic subdivision of bottlenose dolphins (Moura et al. 2013, Louis et al. 2014a). For example, offshore common bottlenose dolphins are suggested to have colonised newly available inshore habitats that opened with the melting of sea ice after the Last Glacial Maximum (Louis et al. 2014a, Louis et al. 2014b, Nykanen et al. 2019, Louis et al. 2020). In the Northern Hemisphere, there is evidence of founder events associated with colonisation of post-glacial habitats, with subsequent divergence between inshore and offshore populations (Nykanen et al. 2019, Louis et al. 2020). This is further supported by the finding of lower genetic diversity in inshore lineages than in offshore ones (Hoelzel et al. 1998, Natoli et al. 2004, Louis et al. 2014a, Lowther-Thieleking et al. 2015). In the North Pacific however, climatic changes are proposed to have caused population bottlenecks (rather than founder events) of inshore lineages (Vijay et al. 2018). In the Southern Hemisphere, little is known about the demographic history of bottlenose dolphins, including divergence times between species and subspecies, and how this has shaped patterns of genetic diversity and inbreeding. It has been hypothesised that the Lahille's bottlenose dolphin diverged from the offshore common bottlenose dolphin after the Last Glacial

Maximum, with divergent selection and reproductive isolation leading to the divergence of the two lineages (Fruet et al. 2017, Pratt 2020). Regarding the SABD, limited knowledge on their historical population size or divergence time exists, however, phylogenomic analysis suggest that genomic divergence between the SABD and other Indo-Pacific bottlenose dolphins may have occurred earlier than the divergence between the Lahille's and the common bottlenose dolphin in the Southwest Atlantic Ocean (Pratt 2020). Time of divergence, historical demography, and their role in shaping patterns of genomic diversity and inbreeding in bottlenose dolphins remain to be empirically tested in the Southern Hemisphere. Investigating these factors will provide greater insight into the evolution of bottlenose dolphins, their response to past climatic events, and their potential to persist with ongoing climate change.

Infectious diseases are another important selective force that play an important role in driving genome evolution. Regarding cetaceans, Morbillivirus has emerged as one of the most significant viral threats and conservation concerns worldwide. Bottlenose dolphins appear particularly vulnerable to cetacean morbillivirus (CeMV), with the virus being the contributing factor of numerous unusual mortality events in inshore and offshore populations (Van Bressem et al. 2014, Ohishi et al. 2019). It has been suggested that climate change and the resulting rise in sea surface temperatures will lead to an increase in the frequency of disease-related unusual mortality events (Sanderson and Alexander 2020). Therefore, it is important that variation in immune responses to CeMV infection is investigated to gain knowledge on the role of host genetic factors in disease susceptibility and immune responses of dolphins to CeMV infection. This will prove vital in identifying populations and species that are particularly susceptible to succumbing to CeMV or that may be immunologically naive.

1.9 Thesis aims

Despite efforts to sequence reference genomes for cetacean species, a limited number of studies have included a single genome from species from the Southern Hemisphere (Foote et al. 2016, Foote et al. 2019, Hooper et al. 2020, Moura et al. 2020). To the best of my knowledge, no study so far has used a whole genome population genomics approach for cetaceans from this hemisphere. This leaves a knowledge gap regarding the evolution and adaptation of species, particularly for bottlenose dolphins that exhibit considerable variation within the genus. The overall aim of this thesis is to understand the evolution and adaptation of bottlenose dolphin species and subspecies in the Southern Hemisphere. This was addressed by first generating a reference genome for the endemic southern Australian bottlenose dolphin (SABD), which will provide a valuable tool for understanding the mechanisms for which this potential subspecies has evolved and adapted. Whole genome resequencing data from biopsy samples of *Tursiops* from multiple species, subspecies, and lineages across the Southern Hemisphere, as well as common dolphins (*D. delphis*), were then generated and complemented with available genomes of

Tursiops from the Northern Hemisphere. With this, phylogenomic relationships within the bottlenose dolphin genus and signatures of positive selection were explored, providing insights into the role of selective pressures on the adaptive divergence of dolphins to the different marine environments they inhabit. Secondly, this work assessed the relationships between ecotype (inshore and offshore), genomic diversity and demographic history to better understand the genomic consequences of repeated niche divergence. Finally, an association-based framework was implemented to investigate the role of genetic variants and genes in the resistance and susceptibility of bottlenose dolphins to a highly infectious virus, CeMV, which has killed thousands of dolphins worldwide.

Chapter 2: A novel reference genome and positively selected genes in bottlenose dolphins

2.1 Contributions

Kimberley Batley – conception of study design, data analysis and interpretation, writing of manuscript.

Luciana Moller – primary supervisor – conception of study and guidance in design and interpretation, collection of samples from eastern and southern Australia, and drafting of manuscript.

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

Jonathan Sandoval-Castillo – guidance and assistance in bioinformatics and data analysis.

Nikki Zanardo – collection of biopsy samples from live, free-ranging dolphins from South Australia.

Pedro Fruet – collection of samples from Brazil.

2.2 Abstract

Understanding the mechanisms driving phenotypic adaptation, genomic divergence and speciation is a fundamental goal of comparative genomic studies. This study addresses the evolution of bottlenose dolphins (genus *Tursiops*) using whole genomes of nine bottlenose dolphin lineages from across the globe and one common dolphin lineage (*Delphinus delphis*) from Australia. First, a high-quality reference genome for a putative subspecies, the southern Australian bottlenose dolphin (SABD), was sequenced, assembled and used as a tool to re-evaluate its classification. The genome size was 2.3 Gb and assembled into 23 chromosome-length scaffolds with an N50 of 21 Mb. Reconstruction of a phylogenomic tree based on 500 vertebrate orthologous genes supported SABD within a monophyletic clade of Indo-Pacific bottlenose dolphins and sister to eastern Australian *T. aduncus*. It also confirmed that Lahille's bottlenose dolphin (*T. t. gephyreus*) from Brazil is most closely related to the offshore *T. truncatus* from the Southwest Atlantic Ocean. Species and lineage-specific signatures of positive selection revealed that similar gene functions were selected across the three species used in the study (*T. truncatus*, *T. aduncus* and *D. delphis*). This suggests that similar gene functions may be hotspots of shared positive selection across delphinid species. Likewise, developmental biology and wound healing genes were positively selected across bottlenose dolphin lineages, highlighting the potential importance of these genes within the genus *Tursiops*. The finding of the same genes positively selected in multiple inshore lineages suggests comparable selective pressures across inshore habitats are likely driving parallel evolution in the inshore lineages. This research provides impetus for future studies on the evolution, conservation, and taxonomic status of *Tursiops*, and those informing on the mechanisms driving divergence and adaptations in dolphins

2.3 Introduction

Studying the mechanisms by which natural selection moulds evolution and contributes to adaptive phenotypes is a fundamental goal of comparative genomics (Kunstner et al. 2010). This expanding field has shed light on both the macro- and microevolution of phenotypic traits in wildlife systems. For example, by identifying genes with signatures of accelerated adaptation, knowledge has been gained on the evolution of traits associated with wound healing and combatting age-related conditions in long-lived mammals (Keane et al. 2015, Marra et al. 2019, Quesada et al. 2019), immune responses and host-pathogen interactions (Kosiol et al. 2008, Shultz and Sackton 2019), and the colonisation of extreme environments (e.g. high-altitude, and desert regions) (Davies et al. 2018).

Cetaceans (whales and dolphins) provide a unique system to study both macro- and microevolutionary changes. The movement of terrestrial mammals to a completely aquatic environment, and the subsequent evolution of cetaceans during the Eocene represents one of the most successful and well-studied transitions into a drastically different niche (McGowen et al. 2014, Cammen et al. 2016). To

succeed in the marine environment several phenotypic adaptations ensued, including the reduction of hindlimbs, development of underwater hearing, the posterior movement of the nostrils and detachment of the pelvis from the spine, among others (Uhen 2010, McGowen et al. 2020). After the transition to sea, cetaceans diversified into two major groups: toothed (Odontoceti) and baleen (Mysticeti) whales, with unique and highly specialised adaptations within each group (McGowen et al. 2020). The remarkable success of the transition to sea and the resulting adaptations, combined with advancements in next generation sequencing and analytical approaches, has generated great interest in detecting molecular signatures of aquatic and ecological adaptation that may contribute to this diversity and corresponding evolutionary changes (Nery et al. 2013). For example, studies have revealed regions and genes under positive selection in modern cetaceans with functions that relate to hypoxia tolerance, changes in locomotor morphology, communication strategies and systems, and improved vision in low light environments (McGowen et al. 2012, McGowen et al. 2014, Yim et al. 2014, Foote et al. 2015, Tian et al. 2017, Huelsmann et al. 2019, Hindle 2020). In addition, inactivation of protein-coding genes with functions relating to chemosensory abilities, such as olfactory, vomeronasal and gustatory systems have been observed (McGowen et al. 2014, Kishida et al. 2015). Together, these studies unveil the role of selective pressures on genotypes that are better suited to the marine environment. Concomitantly with the availability of novel ecological niches in the marine environment, these adaptations have enabled the range expansion, colonisation, and diversification of cetaceans across all oceans and into disparate habitats.

Delphinidae is the largest cetacean family, comprising of more than 37 species including the killer whale (*Orcinus orca*), bottlenose dolphins (genus *Tursiops*) and common dolphins (genus *Delphinus*). This family arose through a rapid radiation during the mid-late Miocene (~11-15 mya) (McGowen et al. 2009, McGowen 2011, Amaral et al. 2012, Perrin et al. 2013), with divergent selective pressures associated with disparate and novel ecological niches and subsequent adaptive divergence leading to extensive variation within this family. The cetacean system also provides great potential to understand microevolutionary changes associated with environmental differences. Bottlenose dolphins (subfamily Delphininae) have a widespread distribution, yet despite being highly mobile in an environment with a lack of hard physical barriers, there is evidence of adaptive divergence to different niches, resulting in remarkable ecological, morphological and molecular variation (Moura et al. 2013, Wickert et al. 2016, Gridley et al. 2018, Pratt et al. 2018, Moura et al. 2020). Currently, only two separate species are recognised: the common bottlenose dolphin (*T. truncatus*) and the Indo-Pacific bottlenose dolphin (*T. aduncus*) (Committee on Taxonomy of the Society for Marine Mammalogy 2020). The common bottlenose dolphin has a cosmopolitan distribution in both inshore, nearshore, and offshore waters, with habitat release during post-glacial cycles creating ecological opportunities for diversification between inshore and offshore ecotypes (Hewitt 2000, Louis et al. 2014a). The resulting divergence observed between the two ecotypes has led to the classification of three subspecies, including the Lahille's

bottlenose dolphin (*T. t. gephyreus*) from the Southwest Atlantic Ocean (SWAO). This subspecies occupies coastal and estuarine waters from southern Brazil to Argentina, and sometimes occur in sympatry with the offshore *T. t. truncatus* (Fruet et al. 2017). In the Indian and western Pacific Oceans, however, the inshore form is generally classified as *T. aduncus* and they almost entirely occupy coastal and estuarine waters (Bilgmann et al. 2007b, Möller et al. 2007, Zanardo et al. 2016b). Indo-Pacific bottlenose dolphins generally exhibit fine-scale population structure, high site-fidelity, and small population sizes with low genetic diversity (Hoelzel et al. 1998, Natoli et al. 2004, Möller et al. 2007, Louis et al. 2014a, Lowther-Thieleking et al. 2015), with some support for further subdivision within the species (Gray et al. 2018). Evidence of genetic divergence between *T. aduncus* from the eastern and southern coast of Australia has led to the suggestion of a third species endemic to southern Australian waters, the Burrunan dolphin, *T. australis* (Charlton-Robb et al. 2011a), but a lack of morphological evidence and recent genetic studies suggest that this most likely represents a subspecies of *T. aduncus* (Moura et al. 2020, Pratt 2020), which is therefore referred to here as the southern Australian bottlenose dolphin (SABD).

Inshore and offshore environments tend to differ quite considerably in their oceanographic features. For example, changes in salinity, sea surface temperatures, and the distribution of prey species are thought to influence population genetic differentiation in bottlenose dolphins (Natoli et al. 2005, Bilgmann et al. 2007b, Díaz-Gamboa et al. 2018), and may also drive phenotypic divergence observed between inshore and offshore lineages (Louis et al. 2014a). In most cases, inshore populations live in small social groups with high site fidelity, and low genetic diversity (Wells et al. 1987, Möller 2012), whereas offshore populations generally live in larger groups, with greater dispersal and genetic diversity (Silva et al. 2008, Bearzi et al. 2009, Fruet et al. 2017). The two forms also differ in their body size and fin size and shape, skull characteristics, skin colouration and patterns, and in their feeding ecology (Jedensjö et al. 2017, Díaz-Gamboa et al. 2018, Genoves et al. 2020, Jedensjö et al. 2020). These differences are not, however, always uniform across their distribution, with some lineages exhibiting unique or reversed traits, or weaker contrasts between the two forms (Louis et al. 2014a). In some regions, coastal forms are smaller than their offshore counterparts, but this pattern is reversed in the SWAO (Costa et al. 2016) and in the southeast Atlantic Ocean (Díaz-Gamboa et al. 2018), and almost absent in the northeastern Atlantic Ocean (Louis et al. 2014a). Differences in the level of contrast in phenotypic traits are thought to be due to varying differentiation in environmental conditions, time of divergence between the inshore and offshore populations, and differences in selective pressures (Louis et al. 2014a). Therefore, it is important that genes under accelerated positive selection between inshore and offshore lineages, as well as between ocean basins, are identified to further understand drivers of adaptation and speciation.

While inshore and offshore ecotypes exist worldwide with clear but inconsistent variation observed between them, most studies to date have investigated the macroevolution of cetaceans during the transition to an aquatic lifestyle, and few studies have investigated the microevolution of ecotypes and lineages, including within *Tursiops*. Advancing technologies and analyses have enabled the progression in understanding ecotype formation and environmental adaptation in bottlenose dolphins. For example, in the Northern Hemisphere and at the whole genome level, Louis et al. (2020) found evidence of parallel adaptation to coastal habitats within ocean regions, with genes involved in cognitive processes and metabolism found to be important in adapting to inshore environments. Comparatively, in the Southern Hemisphere, but using reduced-representation genome sequencing data, Pratt (2020) found similar patterns of parallel evolution in inshore lineages and between ocean regions, and implicated several major bodily systems that may be involved in the success in this environment. This study expands on the work by Pratt (2020) by sequencing the whole genomes of bottlenose dolphins and a closely related species from the Delphininae subfamily (the common dolphin, *Delphinus delphis*) to clarify phylogenetic relationships within *Tursiops* and to identify protein-coding genes evolving under positive selection at the species and lineage levels. Specifically, we first provide an important tool for understanding the evolution of the proposed subspecies of *T. aduncus*, the SABD, by sequencing and assembling a reference genome for this taxon. Second, by sequencing and assembling the genomes of five inshore and offshore lineages of *T. aduncus*, *T. truncatus* and one lineage of *D. delphis*, and complementing these data with available genomes from *Tursiops* from the Northern Hemisphere, species and lineage-specific signatures of positive selection were able to be investigated. It is hypothesised that species-specific signatures of positive selection will relate to the disparate selective pressures associated with occupying different marine habitats (i.e., inshore, nearshore, offshore), and that comparable selective pressures across inshore habitats will drive parallel evolution in the inshore lineages. To the best of our knowledge, this is the first study to investigate signatures of positive selection between all recognised species/subspecies and lineages of bottlenose dolphins in the Southern Hemisphere, and the first to compare ecotype evolution of these dolphins across ocean basins in the Southern and Northern Hemispheres.

2.4 Methods

2.4.1. Samples, sequencing, and data collection

Free ranging bottlenose dolphins (Indo-Pacific bottlenose, *T. aduncus* and common bottlenose dolphins, *T. t. truncatus*) and common dolphins (*D. delphis*) were biopsy sampled from 16 locations in waters of two countries (Australia and Brazil) (Figure 2.1; Table 2.1). Samples were collected between 1999 and 2015 using either a handheld biopsy pole (Bilgmann et al. 2007a) or a remote biopsy gun (Krützen et

al. 2002). A total of 29 samples across five *Tursiops* lineages and a single *D. delphis* lineage from Australia were selected for whole genome sequencing (Table 2.1). Genomic DNA was extracted from skin tissue following the salting out method described in Sunnucks and Hales (1996). In brief, a small piece of skin tissue (~2 x 2 mm) was cut into smaller fragments that were then rinsed with H_2O and dried to remove the preservation buffer. The skin tissue was then placed in TNES buffer, Proteinase K and RNase, and either incubated while shaking at 55°C for 3 hours (hr), or at 37°C overnight. The solution was removed from the incubator and 5 mol NaCl was mixed into the solution, placed into a -80°C freezer for 5 minutes (min) before being centrifuged for a further 5 min. The liquid was then carefully removed and put into a new, clean tube (excluding the pellet), and repeated to ensure high quality DNA was obtained. The DNA was then precipitated by washing the DNA with 100% ethanol, before placing the solution in a -80°C freezer for 10 min and centrifuging. For cleaner DNA, the pellet was washed with 70% ethanol, and centrifuged between each wash. Finally, the DNA was dried for at least 5 min on an incubator at 37°C to remove all remaining ethanol and was then resuspended in warm distilled water. The quality of DNA extractions was initially verified using a ND-1000 spectrophotometer (NanoDrop, Thermo Scientific), and quantity assessed using a fluorometer (Qubit, Life Technologies). The quality of DNA was further visually determined on 2% agarose gels (produced in-house). All extractions that passed quality controls based on standards set by the sequencing agencies (Australian Genome Research Facility, AGRF, or Novogene) were sent for library preparation and whole genome resequencing.

To ensure the sample selected to represent the SABD reference genome was of the highest quality, the DNA was further cleaned using magnetic beads (Agencourt AMPure XP) at a low concentration ratio of x.25µl of DNA stock volume. This was done to bind larger DNA fragments and remove small molecular weight fragments (Quail et al. 2009). In short, the magnetic beads were mixed into the sample and incubated on a magnetic stand at room temperature, prior to removing the supernatant liquid containing small DNA fragments. Freshly diluted 80% ethanol was added to the remaining beads, without disturbing them and then repeated. The ethanol was removed and dried, before disturbing the magnetic beads attached to larger DNA fragments to release them. Finally, the supernatant liquid containing the large fragments of DNA were extracted from the beads and the quantity and quality were re-assessed as described above.

Libraries were prepared by the sequencing agency using the NEBNext Ultra II DNA library prep kit and either paired-end sequenced on two lanes of Illumina NovaSeq 6000 at AGRF, or paired-end sequenced on a single lane of the same platform at Novogene. The cleaned SABD sample was sequenced at a higher depth of coverage (~28x) to form the reference genome assembly, while all remaining samples (n = 88) were sequenced between 5 and 11x coverage.

2.4.2. Complementary data

The novel data for *Tursiops* and *D. delphis* lineages from the Southern Hemisphere generated in this thesis were complemented with whole genomes from *Tursiops* that were either supplied by Moura et al. (2020) or that are publicly available on the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI). Specifically, a single *T. aduncus* genome from the South African lineage, which represents the supposed holotype lineage of *T. aduncus* was provided by Moura et al. (2020). An additional three *T. aduncus* and four *T. t. truncatus* from the Northwest Pacific (Yim et al. 2014, Vijay et al. 2018), and a *T. t. truncatus* genome from the Gulf of Mexico (Foote et al. 2015) were also downloaded from the SRA. In addition, the killer whale (*Orcinus orca*) reference genome (GCA_000331955.2) (Foote et al. 2015) was downloaded from the NCBI GenBank to be used as an outgroup in the phylogenomic analysis. Locations of all samples used in this chapter are displayed in Figure 2.1 and Table 2.1.

Samples were grouped into inshore, nearshore, and offshore ecotypes based on prior knowledge of the populations and lineages sampled, except for the Gulf of Mexico *T. t. truncatus* which lacked metadata but was believed to represent an inshore animal (Foote, pers. comm.). The inshore ecotype represents dolphins that show a high degree of site fidelity to shallow coastal and estuarine waters and embayment's, while the offshore represents dolphins that inhabit less protected waters and exhibit greater connectivity (Möller et al. 2007). *D. delphis* are generally highly mobile and inhabit coastal and open oceans (Perrin 2009, Zanardo et al. 2016a), however some populations in eastern and southern Australia exhibit site fidelity in shallow, urbanised areas and may represent a 'nearshore' ecotype for comparative purposes (Kemper et al. 2008). Details of all samples included in this study and their respective ecotypes can be found in Table 2.1.

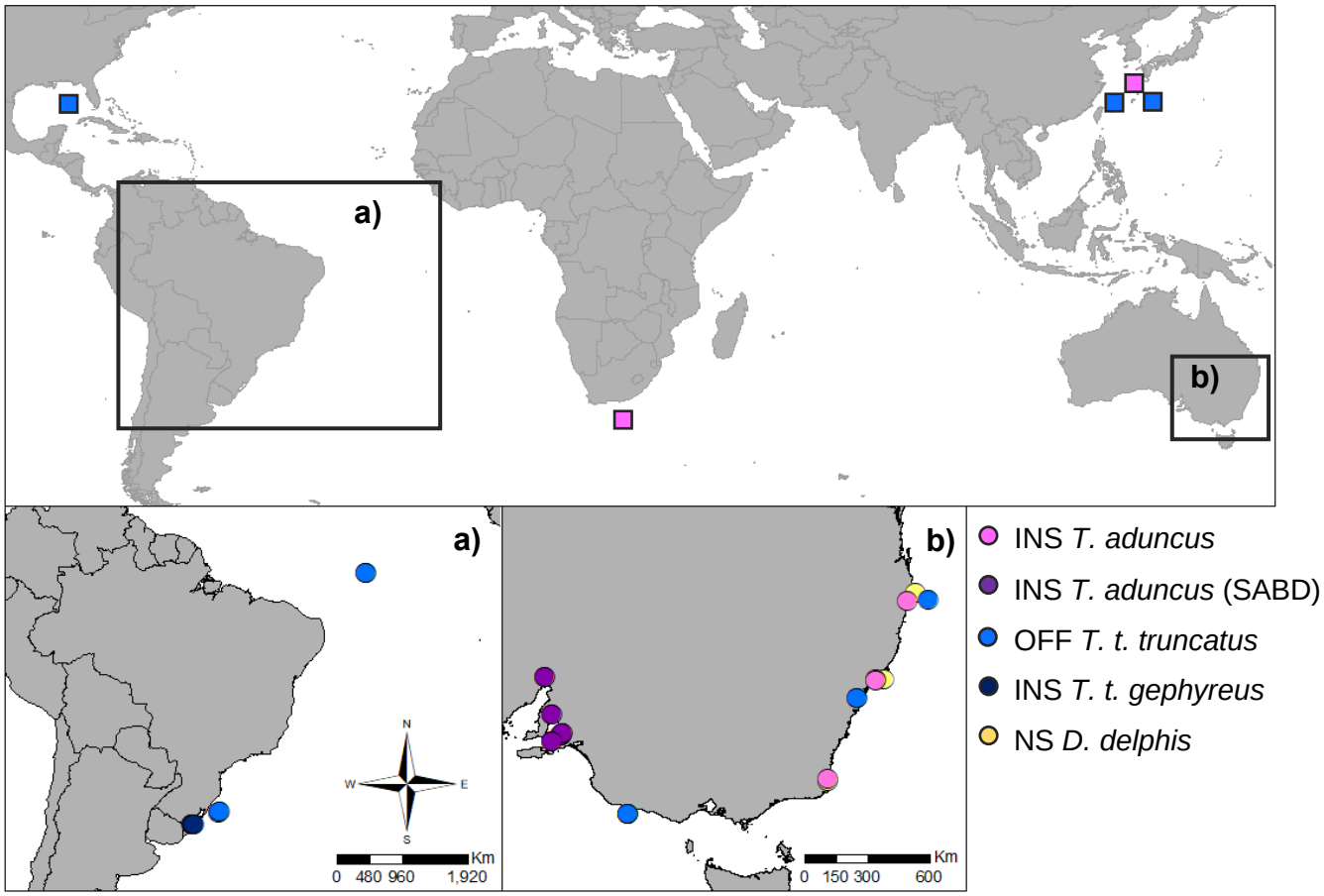

Figure 2.1: Sampling locations of inshore, nearshore, and offshore dolphin lineages included in the analysis of niche divergence and parallel evolution. All *T. t. truncatus* lineages represent offshore dolphins, excluding the single genome from the Gulf of Mexico. Circles indicate data generated in this study, while squares indicate genomes downloaded from the SRA (Yim et al. 2014, Foote et al. 2015, Vijay et al. 2018) or provided by Moura et al. (2020).

Table 2.1: Dolphin genomes generated in this thesis or downloaded from the Sequence Read Archive (SRA) or NCBI Genbank that were used for the inshore-offshore comparative analysis of Chapters 2 and 3.

Species	Sample	Sex	Sampling Location	Ecotype	Year sampled	Genome-wide coverage	SRA accession	Reference
T. aduncus	AD12A	F	Adelaide, South Australia	Inshore	2014	28.1	SRR13817261	**Data generated in this project**
	AD47	M	Adelaide, South Australia		2014	8.8	SRR13817253	
	CJ149	F	Cape Jervis, South Australia		2015	7.6	SRR13817237	
	PA33	M	Port Augusta, South Australia		2005	8.4	SRR13817234	
	PW88	M	Port Wakefield, South Australia		2015	8.8	n/a	
	TFB012	F	Twofold Bay, eastern Australia			6.3	n/a	
	PS16	M	Port Stephens, eastern Australia		1999	8.8	n/a	
	PS86	F	Port Stephens, eastern Australia		2000	7.9	n/a	
	YA19	F	Yamba, eastern Australia		2007	9.3	n/a	
	SthAfrica	M	South Africa			20.1	n/a	Moura et al. (2020)
	SRR5357656	F	Jeju Island, South Korea			32.0	SRX2653495	
	SRR5357655	M	Jeju Island, South Korea			25.0	SRX2653496	
	SRR5357657	M	Jeju Island, South Korea			22	SRX2653497	Vijay et al. (2018)
T. truncatus	SRR2148843	F	Penglai, Shandong, China	Offshore		10	SRX1136398	
	SRR2148844	M	Penglai, Shandong, China			12	SRX1136399	
	SRR2148845	F	Nanjing, Jiangsu, China			12	SRX1136400	
	SRR940825	M	Near Taiji-cho, Wakayamaken, Japan			43.0	SRX326370	Yim et al. (2014)
	SRX200685	F	Off Isle au Pitre, Mississippi Sound, Louisiana	Inshore		34.0	SRX200685	Foote et al. (2015)

	CN04	F	Cape Nelson (Portland), southern Australia			8.7	n/a	
	CN06	F	Cape Nelson (Portland), southern Australia			10.7	n/a	
	CN01	M	Cape Nelson (Portland), southern Australia			8.3	n/a	
	TD12	M	Southern Brazil	Offshore	2010	8.6	n/a	**Data generated in this project**
	TD13	F	Southern Brazil		2010	7.6	n/a	
	AP01	F	St. Paul's and St. Peter's Archipelago, Brazil		2006	8.3	n/a	
	AP08	M	St. Paul's and St. Peter's Archipelago, Brazil		2007	8.8	n/a	
	SY04	M	Sydney, eastern Australia			8.9	n/a	
	SY74	F	Sydney, eastern Australia			9.8	n/a	
	YA01	M	Yamba, eastern Australia			10.7	n/a	
T. t. gephyreus	LP31	F	Patos' Lagoon, Brazil	Inshore	2009	10.0	n/a	
	LP40	M	Patos' Lagoon, Brazil		2009	10.5	n/a	
	LP43	M	Patos' Lagoon, Brazil		2009	7.8	n/a	
	LP52	F	Patos' Lagoon, Brazil		2010	8.8	n/a	
D. delphis	GSV410	M	Adelaide, South Australia	Nearshore	2014	6.1	n/a	
	GSV412	M	Adelaide, South Australia		2014	5.4	n/a	
	GSV417	F	Adelaide, South Australia		2014	5.6	n/a	
	DD413	F	Broughton Island, eastern Australia		2003	10.3	n/a	
	BY03	F	Byron bay, eastern Australia		2005	8.6	n/a	
	TB14	F	Twofold bay, eastern Australia			9.1	n/a	
O. orca	Oorc	F				200.0	GCA_000331955.2	Foote et al. (2015)

**The O. orca genome was only used in Chapter 2: The evolution of the bottlenose dolphin*

2.4.3. T. aduncus (SABD) reference genome assembly and quality checks

The SABD reference genome was *de novo* assembled following the protocol described in Lischer and Shimizu (2017). This recently adapted assembly approach enables the use of a closely related species to guide the genome assembly (Lischer and Shimizu 2017), in our case a subspecies of *Tursiops*. Briefly, reads were quality trimmed, with sequencing adapters and PCR primers removed using Trimmomatic v0.32 (Bolger et al. 2014). Reads were then mapped to the *T. aduncus* genome (South Korea, Northwest Pacific lineage) (GCA_003227395.1) using Bowtie2 v2.4.3.2 (Langmead and Salzberg 2012). Base-wise sequencing coverage was calculated with BEDTOOLS v2.27.1 (Quinlan and Hall 2010), and blocks with continuous and homogeneous coverage were identified and annotated, before being merged into superblocks with a total length of 12 kb. Superblocks exceeding the total length were divided into overlapping superblocks of 100 kb with 300 bp overlap. Mapped and unmapped reads were then identified using SAMTOOLS v1.9 (Li et al. 2009). The read dataset for each superblock was assembled de novo using IDBA-UD, and the contigs from all superblocks were merged and aligned back to the reference genome using Minimap2 v2.12 (Li 2018). Unmapped pairs were then extracted and assembled *de novo* with IDBA-UD and added to the final assembly. Finally, scaffolds were generated by mapping the super contigs to the *T. truncatus* genome (GCA_003314715.1) using minimap2 and medusa v1.6 (Bosi et al. 2015).

The SABD reference genome was then further assembled into chromosome-length scaffolds, following a reference-assisted assembly method employed in the Ragout 2 package (Kolmogorov et al. 2018), and using the newly available chromosome-length assembly South Korean (Northwest Pacific) lineage of *T. aduncus* (Dudchenko et al. 2017, Dudchenko et al. 2018, Vijay et al. 2018). First, collinear blocks were reconstructed with SibeliaZ (Minkin and Medvedev 2019) and then the final assembly was built following a genome rearrangement approach using Ragout 2. Combined, this approach enables the assembly of large and complex mammalian genomes by allowing the inclusion of multiple reference genomes during the assembly process.

The X scaffold was identified by searching for X-linked genes across the chromosomes of the SABD reference genome. To do this, each gene name was searched for within the annotated reference genome, with 23 X-linked genes found on a single chromosome (Table S2.1). Given the reference genome is from a female individual, the Y chromosome was not sequenced and therefore would have been removed from the male genomes during the mapping process. The depth of coverage per scaffold was then calculated using SAMTOOLS and compared between males and females (dolphins were sexed prior using a polymerase chain reaction described in Banks et al. (1995)). Using this method of coverage, the X chromosome was further validated, with the male X scaffold showing a reduction of ~35% coverage, while females showed minimal reduction in coverage (~1%). This scaffold was removed for all downstream analyses.

The quality of the SABD reference genome was then assessed using QUAST v5.0.2 (Gurevich et al. 2013) against the *T. aduncus* chromosome-length genome assembly (Dudchenko et al. 2017, Dudchenko et al. 2018, Vijay et al. 2018). This was done to assess the quality and continuity of the SABD scaffolds against the chromosome-length scaffolds. Next, the completeness of the assembly was evaluated with Benchmarking Universal Single-Copy Orthologs (BUSCO) v3.1.0 (Simao et al. 2015, Waterhouse et al. 2017) by investigating the expected gene content from single-copy orthologs from the mammalia_odb9 database (4,104 genes).

2.4.4. Genome annotation

Annotation of the genome was performed using the GAWN v0.3.2. pipeline (Normandeau 2020), with some modifications. This pipeline reduces computational effort and time by creating an evidence-based annotation using the transcriptome from a closely related species, in this case the annotated *T. t. truncatus* (GCF_001922835.1). The genome mapping tool GMAP was first used to index the SABD reference genome before identifying and annotating the genes using the *T. t. truncatus* transcriptome. Since this transcriptome has several overlapping transcripts that are gene isoforms, the open read frames of the SABD genome-based transcriptome were merged, and the longest isoform of each transcript was selected. The resulting genome-based transcriptome was then used to create the final genome annotation, which was then aligned to the Swissprot Odontoceti protein database using Blastx. A total of 15,953 unique coding genes were annotated to the SABD reference genome.

2.4.5. Sequence alignment, SNP calling and filtering

The individual raw resequencing data was pre-processed following the pipeline adapted from GATK best practices [described in Batley et al. (2021), Chapter 4]. In short, adapters were removed, and poor-quality sequencing reads were trimmed using Trimmomatic v0.38. Using Bowtie2 v2.2.7, the remaining individual read pairs were then mapped to the *T. aduncus* (SABD) reference genome generated in this study, before marking duplicate reads, and locally realigning indels with SAMTOOLS (Li 2011). Finally, replicate reads from different sequencing lanes were merged into final individual BAM files using SAMTOOLS.

A high-quality Single Nucleotide Polymorphisms (SNPs) file for each lineage was required to generate a fasta file per lineage for downstream analysis (section 2.5.7). Therefore, SNPs were called from the SABD reference genome and filtered to include only high-quality SNPs following the methods described in Batley et al. (2021) (Chapter 4), with one change. In brief, BCFTOOLS (Li 2011) was used to call SNPs for all genomes (i.e. all lineages combined) from the reference SABD genome, before filtering the combined raw SNP dataset using VCFTOOLS and VCFFILTER (Danecek et al. 2011). SNP filtering steps included removing SNPs that were genotyped in less than 99% of individuals and had a Minor Allele Frequency (MAF) < 3%. Indels were then removed and only SNPs with a quality

and depth ratio of 2%, mapping quality >30, and mean depth <12 were retained, with the resulting dataset including 17,445,093 SNPs. To generate the SNP datasets per lineage, individuals from each lineage were then extracted with no filtering parameters. Each lineage specific SNP dataset was then zipped and indexed, before generating a consensus file per lineage using the 'consensus' tool in BCFTOOLS and the SABD reference genome. Each individual per lineage was used to generate the consensus fasta file to ensure that variation within the lineage was captured.

2.4.6. Phylogenomics

Complete and single-copy gene families from the vertebrata_odb10 database (3,354 genes in 67 species) were used to construct a phylogenomic tree for the SABD and other sequenced dolphin genomes. First, the BUSCO v5 pipeline was used for gene predictor training and to assess gene set completeness in all sequenced lineages against the vertebrate orthologous database (Waterhouse et al. 2017, Seppey et al. 2019). Specifically, BUSCO uses hidden Markov models (HMMs) within HMMER (Eddy 2011) for sequence comparisons, and AUGUSTUS (Keller et al. 2011) for gene predictions. Default parameters in PRANK (Löytynoja 2014) were then used to create multiple sequence alignments from the BUSCO identified single copy, and complete gene sets. As PRANK implements a phylogeny-aware alignment algorithm, a phylogenomic tree was first generated using PhyML v3.0 (Guindon et al. 2010). The aligned sequences of 500 single-copy and complete genes were then used to reconstruct a maximum likelihood phylogenomic tree using IQ-TREE (Nguyen et al. 2015). The tree was run on eight bottlenose dolphin lineages (excluding *T. aduncus* from South Korea due to computational demand), with *D. delphis* and *O. orca* used as outgroups. *D. delphis* was included based on their relatively close evolutionary relationship with *Tursiops* (Moura et al. 2020), while *O. orca* represents a more distantly lineage within the family Delphinidae. The IQ-TREE was run with the WAG+G+F model for amino acids with 1000 bootstraps (UFBoot), visualised in FigTree v1.4.4 (Rambaut 2014), and rooted with *O. orca*.

2.4.7. Identifying signatures of positive selection in the bottlenose dolphin genome

To assess variation in selective pressures between species, subspecies and lineages, a protein coding database was generated for six cetacean genomes available in OrthoDB v10 (Kriventseva et al. 2019): the common bottlenose dolphin, sperm whale (*Physeter macrocephalus*), killer whale (*Orcinus orca*), beluga whale (*Delphinapterus leucas*), minke whale (*Balaenoptera acutorostrata*) and Baiji (*Lipotes vexillifer*). The generated set of 12,112 genes included only those that were present in 80% of the species and that were single copy orthologs. Following the method described above, single copy and complete genes were identified for each sequenced lineage using BUSCO, and multiple sequence alignments were generated using PRANK. From the aligned genes, only those that were present in 90% of lineages

were kept, with a robust dataset of 9,464 single copy and complete genes selected for analysis of positive selection.

To test for evidence of positive selection on branches of the bottlenose dolphin tree and on branches outside the ingroup (i.e. *D. delphis* and *O. orca*), the codeml package of PAML v4.9 was implemented (Yang 2007). This was done separately at the species (*T. aduncus*, *T. truncatus*, *D. delphis* and *O. orca*) and lineage (bottlenose dolphin branches only) levels to test whether changes in genes are associated with divergent environmental niches. Each of the 9,464 genes were tested for signatures of positive selection using the branch-site model A, which allows the ratio of non-synonymous to synonymous substitutions (d*N*/d*S*, denoted as ω) to vary among both sites and branches (Yang 2007). The three models applied under the branch-site model A were M0, M1 and M2a. M0 assumes that the ω ratio does not vary among sites or lineages, while M1 assumes there are two classes of sites, one that does not vary between sites, but does between branches. Finally, M2 tests for selection by assuming three classes of sites – one with a value of 0, one with a value of 1, and one with a non-fixed value. Models M1 and M2a were tested for significance of difference of fit using a likelihood-ratio test (LRT). A gene was under positive selection if the model was significant against the null (LRT > 2.71). Gene functions of positively selected genes (PSGs) were then explored using gene ontology (GO) terms from UniProtKB (UniProt 2019), while gene pathways were assessed using human ENSEMBL identifiers and Reactome (Fabregat et al. 2018).

2.5 Results

2.5.1. T. aduncus (SABD) reference genome assembly and annotation

We assembled 2.37 Gb of the southern Australian bottlenose dolphin (SABD) genome, which is similar in size to the *Tursiops* genomes published online (*T. truncatus* GCA_011762595.1, *T. aduncus* GCA_011057625.1). Mapping the SABD genome against the *T. aduncus* chromosome-length assembly resulted in an improved genome assembly comprising of 23 chromosome-length scaffolds, with 99.62% of the initial genome assembly anchored onto these scaffolds. The remaining 0.38% (9.04 Mb) of the assembly consists of 6,713 short scaffolds, and combined, the final assembly has an N50 of 121 Mb. BUSCO analysis revealed a relatively high genome quality and level of completeness, with 93.6% of the mammalian BUSCO gene set complete in the SABD assembly. Following the GAWN pipeline, 21,161 genes and pseudogenes were detected, of which 15,953 protein-coding genes overlapped with the Odontoceti orthologous protein database (Table 2.2).

Table 2.2: Southern Australian bottlenose dolphin (SABD) assembly metrics.

Quality metric	
Total assembly size	2,378,373,335
Number of scaffolds	6,736
Largest scaffold size	159,031,325
N50	121,177,409
Mapping efficiency (*T. aduncus*)	95.47%
# chromosomes and plasmids	23 chromosome-length
Annotated genes	15,953
BUSCO completeness	93.6%

2.5.2. Phylogenomics

Reconstruction of the phylogenomic tree based on the 500 vertebrate orthologous genes shows that *Tursiops* forms a monophyletic group separate from *D. delphis*, and all lineages of *T. aduncus* and *T. truncatus* are respectively located in two well supported monophyletic clades (Figure 2.2). Within the *T. truncatus* clade, the accepted inshore subspecies *T. t. gephyreus* showed a sister relationship to the offshore *T. t. truncatus*, supporting its current classification (Committee on Taxonomy of the Society for Marine Mammalogy 2020). SABD is nested within *T. aduncus* and exhibits a sister relationship to the eastern Australian lineage (see Table S2.2 for ML distance matrix).

2.5.3. Signatures of positive selection

Of the 9,464 single copy and complete cetacean orthologous genes, a total of 42 unique genes were found to be under positive selection within the three Delphininae species studied (*T. aduncus*, *T. truncatus* and *D. delphis*) (Figure 2.2). Over 2,500 genes were found to be under positive selection in the *O. orca* genome, however, given the focus is on the evolution of the bottlenose dolphin genome, these results will not be discussed further. The greatest number of genes under positive selection was found in the outgroup with 21 genes under selection exclusively in *D. delphis*, followed by *T. truncatus* (10 genes) and *T. aduncus* (9 genes) (Figure 2.2). In general, gene functions (e.g., GO terms and pathways) overlapped between species but overlapping genes were less frequent. Four genes were found to be under selection within more than one species (*D. delphis* and either *T. truncatus* or *T. aduncus*) (Figure 2.2). Based on GO terms, *LRPAP1* and *NDUFAF6* may have a broad role in the metabolism pathway, while *MYH7B* is a slow twitch myosin and has functions relating to the formation of the cardiac muscle (Foote et al. 2015) (Table S2.3). Genes with functions relating to disease or the immune system functioning, gene expression (transcription), signal transduction, and metabolism were

positively selected in all species (Figure 2.2). Genes that may relate to developmental biology or vision and sensory perception were also found to be under selection in the three delphinid species investigated (Figure 2.2 and Table S2.3 for all PSGs and GO terms).

Between species, genes with functions involved in cell cycling and apoptosis (*TUBGCP6*, *ORC5*, *BIRC7* and *TERT*) were found to be under selection within *T. aduncus* and *D. delphis* (*NEK3*, *RELT*), while genes relating to wound healing (*T. truncatus: EGFR, WNT4, NF1; D. delphis: EVPL*) were positively selected within *D. delphis* and *T. truncatus* (Figure 2.2).

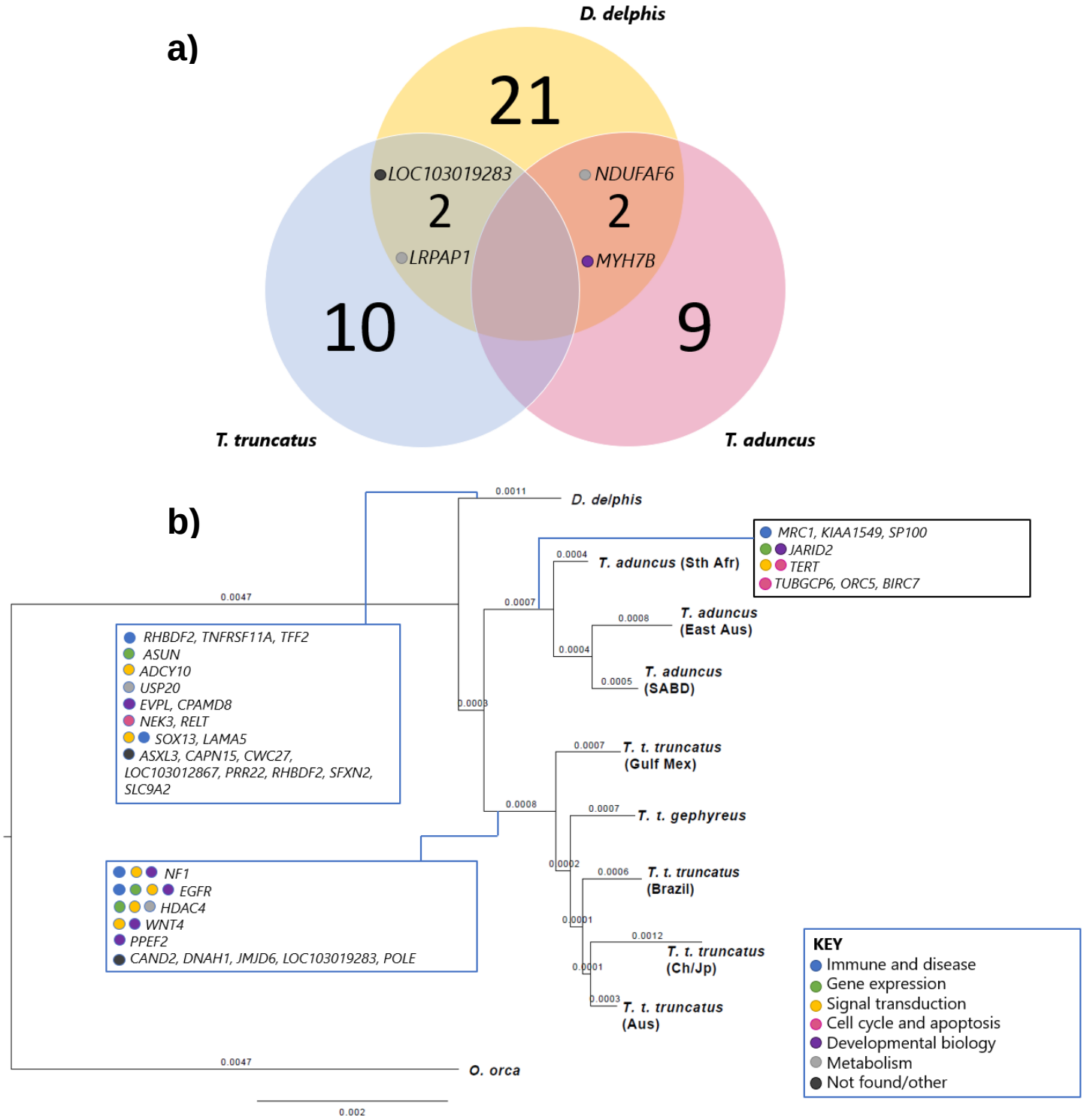

Figure 2.2: Maximum likelihood tree based on 500 complete vertebrate orthologous genes, depicting phylogenomic relationships of *Tursiops* lineages (*D. delphis* used as outgroup), and genes identified as putatively under positive selection; a) the number of unique and overlapping genes positively selected at the species level, and b) positively selected genes unique to each species and their functions.

Across all bottlenose dolphin lineages, between six and 17 genes were found to be under positive selection depending on the lineage (Figure 2.2), and a total of 78 unique genes were identified across the nine lineages. In general, a greater number of genes were under positive selection within lineages that occupy inshore habitats, and likewise, the same gene was generally found to be under selection in multiple inshore lineages, rather than in their offshore counterparts. For example, of the 17 genes positively selected in multiple lineages, 13 were positively selected between multiple inshore lineages, while four and no genes were under selection between mixed (inshore and offshore) and multiple offshore lineages, respectively (Table 2.3). In particular, the gene *DFFA* has functions relating to apoptosis and programmed cell death and it was found to be under positive selection within four of the inshore lineages ((*T. aduncus* (East Aust), *T. aduncus* (Sth Africa), *T. t. gephyreus* and *T. truncatus* (Gulf of Mexico)). Two other genes (*ERN2* and *CASP10*) also have functions relating to apoptosis, and they were also positively selected in the inshore *T. aduncus* (SABD) and *T. aduncus* (Sth Africa), respectively (Table S2.3). In addition, one gene (*BIRC6*), which is involved in apoptotic processes, was under positive selection within the offshore *T. t. truncatus* (Brazil) (Table S2.3). Genes with functions relating to the immune system and disease pathways were also common within inshore lineages, with eight genes (*CASP10, DSP, MRC1, NLRC5, TNFRSF11A, ADAMTS16, HDAC4* and *THBS2*) positively selected within inshore lineages, and one (*RHBDF2*) positively selected in one of the offshore lineages (*T. truncatus* (Ch/Jp)) (Table S2.3). The gene *CPAMD8* was also positively selected within multiple inshore lineages (*T. aduncus* (SABD), *T. aduncus* (Sth Africa) and *T. truncatus* (Gulf of Mexico)), and this has functions relating to the development of the eye (Table 2.3). In addition, other sensory and visual perception genes were under selection within the inshore *T. aduncus* (Sth Korea) (*ESPNL*) and *T. aduncus* (Sth Africa) (*CALHM1*), as well as one offshore lineage, *T. truncatus* (Aus) (*RP1L1*) (Table S2.3). A further 18 genes with functions related to developmental biology, including the development of organs, muscles, and tissues, and the morphogenesis of organs were also found to be under selection in both inshore (14 genes across five lineages) and offshore (7 genes across three lineages) (Table S2.4). Skin development and wound healing were also common GO terms among multiple lineages, with six genes (*GJB3, EVPL, FAM65A, SMOC2, WNT4*) being under positive selection across four lineages (two inshore and two offshore) (Table S2.3). Across the offshore lineages, similarities in gene functions were rare, except for five genes (*SZT2, FAM65A, LAMB1, ITPKA* and *INPP5J*) that may be involved in metabolism and starvation (Table S2.3).

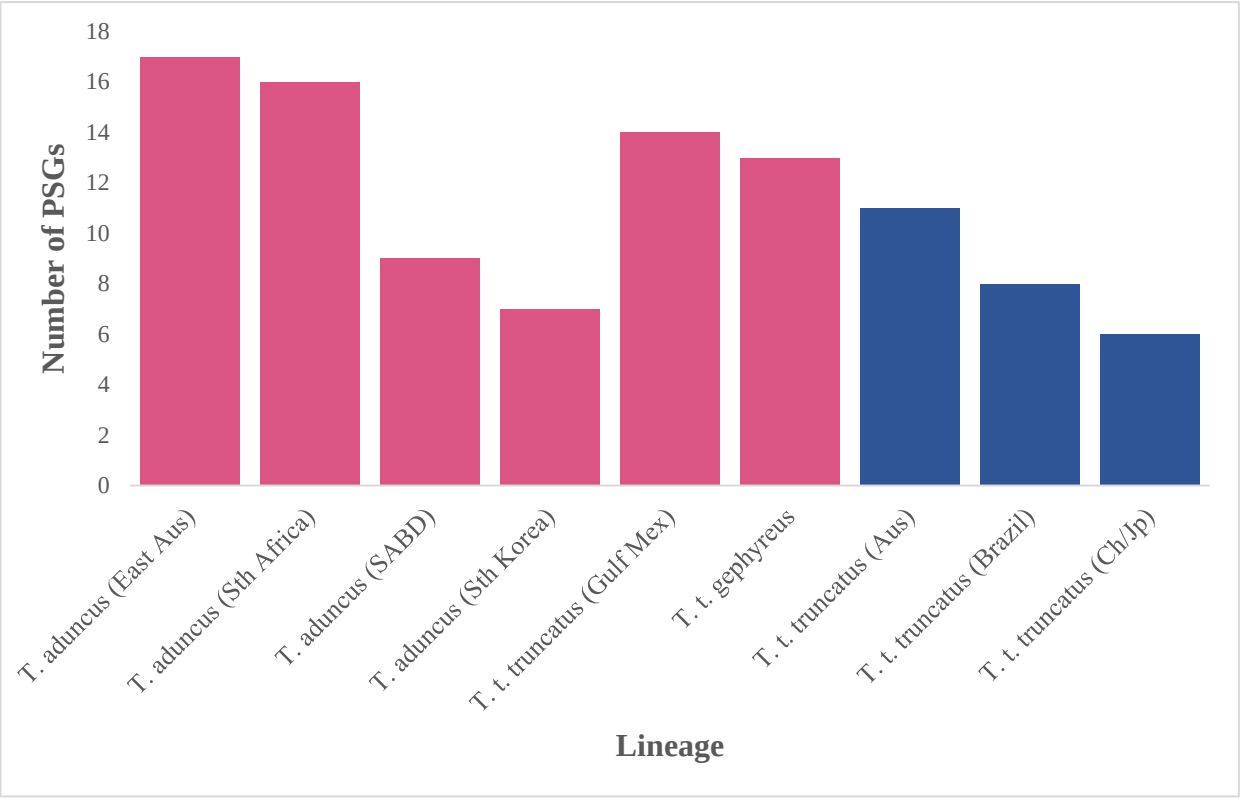

Figure 2.3: The number of positively selected genes (PSGs) in each bottlenose dolphin lineage from inshore (pink) and offshore (blue) environments.

Table 2.3: Positively selected genes (PSGs) in inshore (top) and offshore (bottom) bottlenose dolphin lineages

PSGs in inshore lineages			
Pathway	**Gene**	**Lineages**	**Biological GO terms**
Cell cycle, cellular responses to external stimuli, DNA repair, DNA replication, gene expression (Transcription), metabolism of proteins, reproduction	RPA1	*T. aduncus* (SABD, East Aus)	DNA damage, DNA recombination, DNA repair, DNA replication
Programmed cell death	DFFA	*T. t. gephyreus* *T. t. truncatus* (Gulf Mex) *T. aduncus* (East Aus, Sth Africa)	Apoptosis
Immune system	MRC1	*T. aduncus* (Sth Africa) *T. t. gephyreus*	Host-virus interaction
	TNFRSF11A	*T. aduncus* (SABD) *T. t. gephyreus*	Adaptive immune response
Metabolism of proteins	USP5	T. aduncus (SABD, East Aus)	Endocytosis, Ubl conjugation pathway
Transport of small molecules	SLC6A9	*T. aduncus* (Sth Africa) *T. t. gephyreus*	Amino-acid transport, Neurotransmitter transport, Symport, Transport
	SLCO4A1	*T. aduncus* (East Aus) *T. t. gephyreus*	Ion transport, Transport
Not found	CCDC138	*T. aduncus* (Sth Korea) *T. t. gephyreus*	Not found
	CPAMD8	*T. aduncus* (SABD, Sth Africa, Sth Korea)	eye development
	LOC103019283	*T. aduncus* (Sth Africa, Sth Korea)	Not found
	LOC103071834	*T. aduncus* (East Aus) *T. t. gephyreus*	Not found
	RECQL4	*T. aduncus* (SABD, Sth Africa)	DNA repair, DNA replication
	SFXN2	*T. aduncus* (East Aus, Sth Africa)	Amino-acid transport, Transport
PSGs in inshore and offshore lineages			
Pathway	**Gene**	**Lineages**	**Biological GO terms**
Extracellular matrix organisation	CAPN15	*T. aduncus* (East Aus, Sth Africa) *T. t. truncatus* (Aus)	Proteolysis
	PLOD3	*T. aduncus* (SABD) *T. t. gephyreus* *T. t. truncatus* (Aus)	Cellular response to hormone stimulus, collagen fibril organization, collagen metabolic process, endothelial cell morphogenesis, epidermis morphogenesis,
Not found	EGFLAM	*T. aduncus* (East Aus) *T. t. truncatus* (Ch/Jp, Brazil)	Animal organ morphogenesis
	CAND2	*T. aduncus* (SABD) *T. t. truncatus* (Brazil)	Transcription, Ubl conjugation pathway

2.6 Discussion

Reference genomes provide a wealth of biological information that can be used to clarify evolutionary history and facilitate conservation and management efforts. Here, a reference genome was assembled for the narrow endemic southern Australian bottlenose dolphin (SABD), providing an important resource to disentangle key evolutionary questions about this and related dolphin lineages. This assembly includes 23 chromosome-length scaffolds, with a low percentage of reads assembled onto short scaffolds. It also represents one of the highest N50 values for any currently available cetacean genome (Table S1.1). Gene content was similar to other cetacean genomes, with 93.6% of the complete single copy mammalian gene set identified in the SABD reference genome. This resource had a low level of fragmented and missing genes and a final annotated set of 15,953 orthologous genes.

In this study, the high-quality SABD reference genome was first used as a tool to investigate the phylogenomic relationships among bottlenose dolphin lineages from different environments across both Southern and Northern Hemispheres, and provide some support surrounding the proposed and/or accepted sub-species. As niche divergence and adaptation to different environments is thought to drive genomic differentiation, species and lineage-specific signatures of positive selection were also explored. Reconstruction of the phylogenomic tree revealed that *Tursiops* forms a monophyletic group, with strong divergence between two main clades, each represented by all *T. aduncus* or all *T. truncatus* lineages and ecotypes. Inferred topologies and levels of divergence within each clade supports a subspecies classification for *T. t. gephyreus* and *T. aduncus* (SABD). Functions of positively selected genes identified here were generally similar among species (*T. aduncus*, *T. truncatus* and *D. delphis*), with some of the same genes previously identified as under selection in other marine mammals or cetaceans (Sun et al. 2013, Foote et al. 2015, Chikina et al. 2016). These results provide support for convergent phenotypic evolution in marine mammals, particularly regarding their physiological adaptations. Likewise, similar gene functions were positively selected across the bottlenose dolphin lineages studied. Overlap of positively selected genes in the inshore lineages was far more frequent than overlap with, and between the offshore lineages. These results suggest that among the inshore lineages, the same genes, or genes with similar functions, may be evolving in parallel to similar selective pressures of the inshore environment and may be particularly important in the successful colonisation of these habitats.

2.6.1 Phylogenomic inferences

The taxonomy of Delphinidae and particularly bottlenose dolphins has been contentious, with over 20 different species previously proposed (Hershkovitz 1966). Confounding phylogenetic relationships within this genus are likely caused by rapid radiations, incomplete lineage sorting and hybridisation (Amaral et al. 2012, Moura et al. 2013, Gray et al. 2018). The phylogenetic tree presented here and

based on over 500 orthologous vertebrate genes provides support to earlier findings about evolutionary relationships within *Tursiops* based on nuclear data (Moura et al. 2020, Pratt 2020). Clear phylogenetic divergence between the accepted subspecies *T. t. gephyreus* and the offshore *T. t. truncatus* was evident, giving support to its current classification as a separate subspecies (Committee on Taxonomy of the Society for Marine Mammalogy 2020). SABD, which was previously proposed as a separate species, the Burrunan dolphin, *T. australis* (Charlton-Robb et al. 2011a), was more recently suggested to represent a subspecies of *T. aduncus* (Moura et al. 2020, Pratt 2020). In our study, the SABD lineage nested within *T. aduncus*, supporting the idea of the lineage not representing a separate species to other bottlenose dolphins, with further taxonomic work required to inform whether classification as a separate subspecies is warranted. While currently there is no set definition to classify a subspecies, a review on guidelines and standards for delimiting cetacean subspecies defines a subspecies as "*a population, or collection of populations, that appears to be a separately evolving lineage with discontinuities resulting from geography, ecological specialization, or other forces that restrict gene flow to the point that the population or collection of populations is diagnosably distinct*" (Taylor et al. 2007). In line with Moura et al. (2020), the *T. aduncus* lineages form reciprocally monophyletic groups, and therefore appear to be evolving separately. In addition, inshore bottlenose dolphin populations often show high site-fidelity with limited connectivity to adjacent populations. Therefore, other *T. aduncus* (e.g., Sth Africa and eastern Australia) and inshore *T. truncatus* lineages (e.g., Gulf of Mexico) require further studies to clarify whether they should be classified as different subspecies".

2.6.2. Positively selected genes in Delphininae species: Tursiops *and* Delphinus

Across the three Delphininae species examined (*T. aduncus*, *T. truncatus* and *D. delphis*), four genes were found to be under positive selection in more than one species (*LRPAP1, NDUFAF6, MYH7B* and *LOC103019283*). Of these four genes, *LRPAP1* and *NDUFAF6* may have a broad role in the metabolism pathway. *LRPAP1* is a molecular chaperone for LDL receptor proteins (Bu et al. 1995), that are important for cholesterol metabolism, familial hypercholesterolemia, and coronary heart disease. *LRPAP1* also interacts with the apolipoprotein gene *LRP1*, which was previously suggested as positively selected in the common bottlenose dolphin genome when compared to four terrestrial mammalian genomes (Sun et al. 2013). Given the role of these two genes in the metabolism of cholesterol and lipids, they may be particularly important in elevating lipid content and fat storage across dolphin lineages (Sun et al. 2013). Maintaining lipid content and fat is a crucial adaptation of marine mammals, as the thick layer of blubber reduces energy expenditure by adding buoyancy, and by acting as a thermal insulator (Hagen et al. 2000, Liwanag et al. 2012).

MYH7B was also under selection in two of the species (*T. aduncus* and *D. delphis*). This protein is a slow twitch myosin, is expressed in the heart and slow skeletal muscle of mammals, and has functions

relating to the formation of the cardiac muscle (Rossi et al. 2010). Positive selection within this gene has previously been detected within three marine mammal lineages (cetaceans, sirenians and pinnipeds) (Foote et al. 2015), and may be linked to cardiovascular regulation during diving (Foote et al. 2015). A gene from the same family, *MYH11*, which is expressed in smooth muscles, has also found to be under parallel selection in inshore bottlenose dolphins and thought to have important implications for heart functioning (Pratt 2020) . In this study, the *MYH7B* gene was under positive selection in the inshore (*T. aduncus*) and the nearshore *D. delphis*. However, given this exact gene has been found to be under selection in multiple marine mammal lineages, variation within this gene may be undergoing convergent evolution in marine mammals more broadly.

In addition to these four genes, genes with similar functions often showed signatures of positive selection among the three Delphininae species. For example, genes relating to the immune system, gene expression, signal transduction, metabolism, and developmental biology functions were often under selection in at least two of the species. Among mammals, enrichment of positively selected genes within immune system pathways suggests a strong interaction between pathogens and hosts (Shultz and Sackton 2019). In the marine environment, lower levels of diversity have been observed in immune genes of cetaceans compared to terrestrial mammals, and this is thought to be due to lower or less severe interactions with pathogens in the marine environment (Moreno-Santillan et al. 2016). Inactivation and loss of some genes involved in the immune system has also been observed in cetaceans, such as the presence of ORF disrupting mutations in interleukin-20 across nine cetacean species (Lopes-Marques et al. 2018), and *TRIM14* and *TREM1* in four species (Huelsmann et al. 2019). It is unclear whether this is due to the different pathogens found in the marine environment, or other adaptations of cetaceans that protect against pathogens, including skin immunity adaptations (Lopes-Marques et al. 2018, Huelsmann et al. 2019). In this study, however, the disease and immune system pathways had the greatest number of genes positively selected across the three species, with at least ten different disease and immune related genes found to be positively selected. As the climate continues to change and humans continue to disrupt marine ecosystems, cetaceans are being forced to live in highly stressful environments, and their ability to cope with pathogens may become vital to their survival. For example, in recent years the reporting of unusual mortality events across cetacean populations has raised questions regarding their health and the health of the oceans more broadly (Ross 2000, Wells et al. 2004, Gulland and Hall 2007). Of particular concern is the seeming rise in emerging infectious diseases, and the incidences of viral outbreaks across cetacean populations across the globe (Gulland and Hall 2007). Of the immune genes found to be under selection in dolphins of this study, three genes (*MRC1*, *SP110*, *EGFR*) have functions relating to host-virus interactions and may play a role in activating or inhibiting cellular responses to viruses (Table S2.3). The genes *NF1*, *EGFR*, *HDAC4*, *LAMA5* and *SOX13* also have functions relating to signal transduction and may be important for eliciting changes in cell state or activity in response to a stimulus (Table S2.3). Several signalling pathways are required to fight infections, including the

MAPK and Wnt signaling pathways (Partridge et al. 2010). Genes involved in the MAPK cascade have previously been suggested to be involved in immune responses to cetacean morbillivirus in bottlenose dolphins (Batley et al. 2019, Batley et al. 2021). While no MAPK genes were found here, *NF1* and *EGFR* are involved in the MAPK signaling cascade (Table S2.3), adding support that the MAPK cascade may play an important role in fighting infections in dolphins. Likewise, *SOX13* may be involved in regulating Wnt signaling pathways and the differentiation of T cells. Wnt proteins in the immune system are regulators of T cell development and activation (Staal et al. 2008), and therefore by regulating the Wnt signaling pathway, *SOX13* may be important in responding to infection and other stimuli by activating T cell proliferation in dolphins. Most studies investigating positive selection in marine mammal genomes have focused on key marine phenotypic adaptations (e.g., hearing and vision, hypoxia tolerance, buoyancy). However, our results suggest that selection in immune, disease and signal transduction pathways may also prove vital to their survival due to enhanced presence of anthropogenic stressors in the marine environment, particularly in coastal waters.

Between species, there was no obvious distinction between gene functions of PSGs identified. Briefly, a greater number of genes relating to DNA replication and the cell cycle pathway were positively selected in the *T. aduncus* genome, followed by *D. delphis*. Changes in gene expression of *T. truncatus* has indicated that cell cycle progression may be hindered by exposure to contaminants (Mollenhauer et al. 2009). In southern Australia, bottlenose dolphins have higher metal and toxin concentrations than *D. delphis*, and the inshore *T. aduncus* generally have higher concentrations than *T. truncatus* (Lavery et al. 2008, Gaylard 2017). However, the potential role of toxins and metals as a driving force of selection in cell cycle genes is understudied, and therefore the biological importance of cell cycle genes under selection in *T. aduncus* warrants further investigation.

These three species generally occupy distinct, but sometimes overlapping environmental niches, and are therefore likely subject to somewhat disparate selective pressures. Despite this, similar genes and gene functions showed signatures of positive selection across the three species. The minimal differences between species may be due to the small number of genes under positive selection within each species (*T. aduncus* = 11, *T. truncatus* = 12 and *D. delphis* = 23), which may reflect the species close phylogenetic relationships. Alternatively, these results may suggest that that similar gene functions and pathways may be hotspots of shared positive selection across delphinid species, or that differences in gene expression may be more important.

2.6.3. Positively selected genes within Tursiops

Inshore and offshore habitats generally differ in their environmental and ecological features creating contrasting selective pressures that can lead to local adaptation and reinforce genomic divergence. Despite this, similarities between gene functions positively selected across the nine bottlenose dolphin

lineages highlight the importance of developmental biology, skin development and wound healing genes for *Tursiops* in general. A total of 18 genes with functions relating to developmental biology were found to be under selection across the *Tursiops* lineages. The developmental genes identified here may be involved in a wide range of functions, including the ageing process, the development of organs, muscles, and tissues, and the skeletal and nervous systems. Specifically, five genes (*RECQL4*, *DOP1B*, *RAI1*, *FSTL4* and *RET*) had functions relating to multicellular organism development, and the progression of an individual from its early stages (e.g., zygote or young organism) to the later stages of life (e.g., adult) (Table S2.3). As such, they may have important roles in the ageing process of bottlenose dolphins. Dolphins are long-lived mammals and likely require some preventative mechanisms against age-related conditions, as has been observed in other long-lived marine animals (Keane et al. 2015, Marra et al. 2019, Tejada-Martinez et al. 2021). Genes involved in the longevity process have been found to be under selection across seven divergent cetacean species (Tejada-Martinez et al. 2021), and also in the longest-lived mammal, the bowhead whale, when compared to terrestrial mammals (Keane et al. 2015). However, there was no overlap in the genes related to the ageing process found in the two studies above and this study. Therefore, these ageing genes may be specific to the longevity of bottlenose dolphins. In addition to ageing genes, nervous system genes were also under selection across multiple bottlenose dolphin lineages. A great amount of variation is observed between the brain size of marine mammals, with toothed whales evolving larger brains than expected based on their body size (Marino et al. 2004). Subsequently, genes involved in neuronal development have been found to be under selection in cetaceans (McGowen et al. 2012, Chikina et al. 2016, Pratt 2020). In this study, five genes (*ZNF592*, *FGF13*, *HDAC4*, *SZT2* and *RET*), which are involved in neuronal development (Table S2.3), were positively selected in the studied lineages. Three similar genes (*ZNF597, ZNF345* and *FGF11*) have previously been identified as under positive selection in bottlenose dolphin genomes and linked to the neuronal development of cetaceans (McGowen et al. 2012, Chikina et al. 2016, Pratt 2020). It is difficult to link brain development genes with the phenotypic and behavioural traits of cetaceans, however, it is clear that genes involved in the development of the neurological system may be important in the evolution of brain size and complex social behaviours (McGowen et al. 2012). Other developmental genes not discussed here include those that may be important for the development and morphogenesis of muscles and organs, including the kidney and liver, which may be important for aquatic adaptation.

Adaptations for wound healing and skin development were also evident, with genes relating to these functions positively selected in both inshore and offshore bottlenose dolphin lineages. Wound healing is an essential process to the survival of mammals, being vital to their protection against chemicals and pathogens (Borena et al. 2015). Effective wound healing processes are particularly important in dolphins as they are exposed to a wide range of threats, including predation from larger predators, boat strikes and interactions with fisheries, as well as toxins and pathogens that can penetrate through

wounds (Van Bressem et al. 2009a, Davidson et al. 2012). In addition, bottlenose dolphins are known to frequently present tooth rake marks from interactions with conspecifics, particularly between adult males, likely as a consequence of competition over receptive females (Scott et al. 2005, Lee et al. 2019). High rates of cell proliferation in dolphins, therefore, enable them to heal from wounds quite rapidly (Noren and Mocklin 2012). In this study, at least six genes had functions relating to wound healing and skin development (*GJB3*, *EVPL*, *FAM65A*, *SMOC2*, *WNT4*), while four genes (*EGFR*, *WNT4*, *NF1* and *EVPL*) were also positively selected within *D. delphis* and *T. truncatus* at the species level, providing molecular evidence to support the idea of effective wound healing in dolphins.

Despite occupying quite different environmental niches, genes relating to developmental biology and wound healing were positively selected in both inshore and offshore lineages. These similarities suggest that these functions may have key evolutionary roles in bottlenose dolphins and that different genes with similar functions may be evolving in parallel.

2.6.4. Parallel evolution of genes in inshore bottlenose dolphin lineages

While inshore and offshore environments differ in their characteristics, inshore environments tend to generally share similar features. Similarities in structural complexity and resource availability, depth, sea surface temperatures, and freshwater inputs may create comparable selective pressures among inshore habitats. Across their range, inshore bottlenose dolphins show phenotypic traits adapted to this type of environment, including for feeding, diving behaviour and echolocation (Louis et al. 2020, Pratt 2020). The similar environmental features of inshore environments, and comparable phenotypic adaptations of inshore bottlenose dolphins suggest the potential role of parallel adaptive evolution in these inshore dolphins. Here, genes that were positively selected in multiple bottlenose dolphin lineages mostly involved those that inhabit inshore environments. For example, across all bottlenose dolphin lineages, 13 genes were positively selected within multiple inshore lineages, and in comparison, only four and no genes were positively selected in multiple (inshore and offshore) and offshore lineages only. These results support previous suggestions that similar selective pressures across the inshore habitats are resulting in the parallel evolution of bottlenose dolphins inhabiting these environments (Louis et al. 2020, Pratt 2020). The genes that were positively selected in multiple inshore lineages had functions relating to DNA damage, repair and apoptosis, the immune system, and eye development. DNA damage and inaccurate repair can lead to mutations and potentially disrupt genes (Chatterjee and Walker 2017). Therefore, accurate DNA repair is a vital protective process for inhibiting carcinogenesis (Chatterjee and Walker 2017). The genes *RECQL4* and *RPA1* had functions relating to DNA damage and repair (Table S2.3) and may be important in blocking cells from becoming cancerous in this long-lived species. Apoptosis or programmed cell death is a secondary response to DNA damage, and is used as a protective measure against damaged cells (Wang 2001). Apoptosis genes have been previously found to be under

selection in several cetaceans (Tejada-Martinez et al. 2021), and in another delphinid, the inshore Indo-Pacific humpback dolphin (*Sousa chinensis*) (Ming et al. 2019). In this study *DFFA* which has apoptotic functions was positively selected in all inshore lineages (excluding *T. aduncus* (Sth Korea)). As previously discussed, inshore lineages may be exposed to greater levels of toxin and heavy metal pollution, with inshore populations exhibiting very elevated levels of contaminants in some regions of the world (Lavery et al. 2008, Gaylard 2017). Toxins can distort DNA structure and potentially become cancerous, making DNA repair mechanisms particularly important. The finding of these genes being positively selected in inshore lineages may highlight the harshness of living in these inshore environments, resulting in the adaptation of protective measures against molecular damage and carcinogenesis. However, this finding is to the best of my knowledge, novel, and the biological importance of these genes in the inshore environment warrants further investigation.

The immune genes *MRC1* and *TNFRSF11A* were also positively selected in multiple inshore lineages. The environment and host-virus interactions are key drivers of the evolution of the immune system in dolphins (Fair et al. 2017), and may be the reason why some immune genes were positively selected in the inshore bottlenose dolphin genomes, but not in the offshores. Inshore environments are generally more stressful, particularly in areas of high human use, where dolphins are exposed to boat traffic, fishing, coastal development and pollution outlets (Stock et al. 2018). Therefore, prominent human-induced stressors that inshore lineage lineages are exposed to may be driving positive selection in these immune related genes. Finally, the gene *CPAMD8* was positively selected in three inshore lineages, and it has roles relating to eye development (Table 2.3). Likewise, over-enrichment of genes with functions relating to photoreceptor activity and eye development has been found in inshore Indo-Pacific bottlenose dolphins using reduced-genome sequencing techniques (Pratt 2020). This was the first evidence of differences in visual perception of inshore and offshore dolphins, and it was suggested to be influenced by differences in the turbidity and light levels between the two environments (Pratt 2020). Here, further support is provided for differences in the visual systems between inshore and offshore bottlenose dolphins.

2.7. Conclusion

This study makes available a new bottlenose dolphin reference genome, representing a potential subspecies of *T. aduncus*, the southern Australian bottlenose dolphin, which can be used to further study the evolution of bottlenose dolphins and to assist with the conservation management of this lineage. Whole genome sequences from other bottlenose dolphin lineages and a common dolphin clarified phylogenomic relationships within the genus *Tursiops*, providing further support for subspecies classification of *T. t. gephyreus* and possibly SABD. This study also represents the first to investigate

positively selected genes in bottlenose dolphins at both species and subspecies levels. Similarities in functions of the genes identified between species suggest that the immune system, signal transduction, metabolism, and developmental biology are likely hotspots of positive selection across delphinid species. In addition, developmental biology and wound healing genes positively selected across inshore and offshore bottlenose dolphins emphasise the importance of these functions within *Tursiops*. Finally, the discovery of the same genes under positive selection in multiple inshore lineages suggests comparable selective pressures across these environments, and potential parallel evolution in genes relating to DNA damage, repair and apoptosis, the immune system and eye development. This research broadly informs about the evolutionary mechanisms driving adaptation and genomic divergence in bottlenose dolphins and highlights key pathways and gene functions that may be important for species and lineage persistence.

Chapter 3: Niche divergence and parallel genome evolution in bottlenose dolphins

3.1 Contributions

Kimberley Batley – conception of study design, data analysis and interpretation, writing of manuscript.

Luciana Moller – primary supervisor – conception of study and guidance in design and interpretation, collection of samples from eastern and southern Australia, and drafting of manuscript.

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

Jonathan Sandoval-Castillo – guidance and assistance in bioinformatics and data analysis.

Christopher Brauer – guidance and assistance in data analysis and presentation.

Nikki Zanardo – collection of biopsy samples from live, free-ranging dolphins from South Australia.

Pedro Fruet – collection of samples from Brazil.

3.2 Abstract

The colonisation of newly available habitats can be an important driver of population divergence, which can also lead to reproductive isolation and speciation. In some cases, the colonisation of independent but closely related lineages in similar environments can result in the parallel evolution of traits that may pertain to niche specialisations. Bottlenose dolphins (genus *Tursiops*) have a widespread distribution, occupying both inshore and offshore environments across almost all ocean basins. Inshore and offshore populations show substantial patterns of morphological, ecological, and genetic differentiation thought to be a product of ecotypic divergence and niche specialisation. However, the mechanisms that have enabled the formation of ecotypes and the genomic consequences of niche divergence remain unclear. In this study, the concept of parallel evolution driven by niche divergence is explored by comparing the whole genomes of representative inshore and offshore bottlenose dolphin ecotypes from across the globe. A very strong relationship between genomic diversity, runs of homozygosity (ROH), and ecotype was observed, with the inshore ecotype less genetically diverse, and having a greater proportion of their genome comprised by ROH than the offshore ecotype. Parallel demographic histories within ecotypes were also observed, with the inshore ecotype generally showing a signal of bottlenecks during the Last Glacial Maximum, while populations of the offshore ecotype expanded during this period. A recent bottleneck detected for the inshore subspecies, the Lahille's bottlenose dolphin (*T. t. gephyreus*) from Brazil, may be the cause for the extremely low genomic diversity and many small ROH observed in this lineage. These results highlight the role of coastal niche divergence in the parallel evolution of a highly mobile and social marine mammal and provide support for natural selection facilitating local adaptation of closely related populations and species to similar environments.

3.3 Introduction

The colonisation of newly available or underutilised ecological niches is a key driving force in the divergence of populations, which can also lead to reproductive isolation and speciation (Schluter 2009). Selective pressures associated with ecological and environmental niche divergence have led to the diversification of several marine taxa, including penguins (Vianna et al. 2020), polar bears (Liu et al. 2014) and elasmobranchs (Sandoval-Castillo and Beheregaray 2020). However, understanding the role of natural selection as a driver of diversification and speciation remains a challenge due to the often unique environmental and evolutionary histories of populations and species (Elmer and Meyer 2011). In some cases, the colonisation of closely related lineages in comparable but physically isolated ecological niches can result in the parallel evolution of traits that may pertain to niche specialisations (Elmer and Meyer 2011). This recurrent or replicated adaptation of closely related lineages to similar environments is considered strong evidence of natural selection and provides insights into the genomic mechanisms underlying adaptation to ecological niches. For example, the study of parallel adaptation

of threespine sticklebacks to freshwater environments (Jones et al. 2012), rabbit populations to infection by the myxoma virus (Alves et al. 2019), and high-altitude species to the low availability of oxygen (Qiu et al. 2012) have provided an understanding of how selection has acted to produce similar phenotypes that relate to ecological niches.

Repeated colonisation and adaptation to similar environmental niches also appear to exist across some marine mammal populations. For instance, bottlenose dolphins show high levels of morphological, ecological and genetic differentiation across their vast geographical range, which is often suggested to result from niche divergence and ecotype formation (e.g. Moura et al. 2013, Gridley et al. 2018, Moura et al. 2020). Within the genus, only two separate species are currently accepted: the common bottlenose dolphin (*Tursiops truncatus*) and the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) (Committee on Taxonomy of the Society for Marine Mammalogy 2020). Although both species sometimes occupy overlapping habitats, they have contrasting evolutionary histories and face different selective pressures. The common bottlenose dolphin has a cosmopolitan distribution, occupying inshore, nearshore and offshore waters, and often exhibit hierarchical population structure, with high levels of divergence generally observed between inshore and offshore populations – or ecotypes (e.g. Hoelzel et al. 1998, Louis et al. 2014a, Fruet et al. 2017). These divergences are thought to be driven by the repeated colonisation of offshore *T. truncatus* into coastal habitats following sea level rise during interglacial periods (Natoli et al. 2004, Louis et al. 2014a). This is supported by higher levels of gene flow in offshore *Tursiops*, as well as morphological and ecological differences between the inshore and offshore ecotypes (Louis et al. 2014a, Louis et al. 2014b, Nykanen et al. 2019). In comparison, Indo-Pacific bottlenose dolphins are only found in inshore and nearshore areas, occupy coastal zones of temperate and tropical regions, and exhibit fine-scale population structure, small population sizes and high site fidelity, often to regions heavily utilised by humans (Möller et al. 2007, Charlton-Robb et al. 2014, Zanardo et al. 2016b, Pratt et al. 2018). Inshore bottlenose dolphins are repeatedly found to have lower genetic diversity than their offshore counterparts, likely due to a rapid decay in the gene pool following founding events by a small number of individuals into new coastal habitats (Hoelzel et al. 1998, Natoli et al. 2004, Lowther-Thieleking et al. 2015). The subsequent divergence is thought to be driven by adaptation to local prey resources and environmental conditions, which is then reinforced by natal site philopatry (Möller and Beheregaray 2004, Wiszniewski et al. 2009, Fruet et al. 2014).

Phenotypic and/or genomic divergence between bottlenose dolphin populations and ecotypes has led to the proposal of several subspecies in the Southern Hemisphere. In the Southwest Atlantic Ocean, observed morphological, ecological and genetic differentiation between inshore and offshore bottlenose dolphins has led to the general acceptance of the Lahille's bottlenose dolphin (*T. t. gephyreus*) as a true subspecies within the genus (Costa et al. 2016, Wickert et al. 2016, Fruet et al. 2017). This subspecies inhabits estuaries and coastal waters from southern Brazil to Argentina, with at least two distinct

populations (southern Brazil to Uruguay, and Bahia San Antonio, Argentina) (Fruet et al. 2014, Fruet et al. 2017). This inshore lineage currently has a small and declining population, and the lowest genetic diversity so far recorded for any bottlenose dolphin population (Fruet et al. 2014, Fruet et al. 2017). In Australia, inshore and offshore populations have been classified as separate species, with *T. aduncus* reflecting the inshore populations and *T. truncatus* representing the offshore populations (Möller and Beheregaray 2001). However, in coastal waters of southern Australia, a third putative species, the Burrunan dolphin (*Tursiops australis*) has been described by Charlton-Robb et al. (2011a), based on genetic evidence (Möller et al. 2008, Charlton-Robb et al. 2011a), but its morphological distinctiveness is apparently insufficient to support a separate species (Jedensjö et al. 2017, Jedensjö et al. 2020). Other recent genetic studies have suggested that this lineage is likely a subspecies of *T. aduncus* (Moura et al. 2020). Due to the current taxonomic disagreement, this lineage will be referred to here as the southern Australian bottlenose dolphin (SABD).

The rapid rate of anthropogenically induced climate change threatens marine biodiversity, with changes in ocean temperatures, sea level, sea ice coverage and salinity all predicted to impact cetaceans directly and indirectly (Learmonth et al. 2006, Hamilton et al. 2019, Albouy et al. 2020). Under these changing conditions stressors including extreme weather events, exposure to pathogens and contaminants, habitat modification and human interactions are all expected to intensify and lead to range shifts, and changes in abundance, population structure, disease susceptibility, reproductive success, and competition (Learmonth et al. 2006, Gulland and Hall 2007). Inshore bottlenose dolphin populations exemplify the biological features found in organisms at substantial risk of human impacts: low genetic diversity, small population sizes, low gene flow with neighbouring populations, and living in semi-enclosed environments with small home ranges and philopatric behaviour (Möller and Beheregaray 2004, Möller et al. 2007, Charlton-Robb et al. 2014, Fruet et al. 2014, Zanardo et al. 2016b, Pratt et al. 2018). In particular, *T. t. gephyreus* has been classified as vulnerable by the International Union for the Conservation of Nature (IUCN) due to their decreasing population trend, low genetic diversity and increasing levels of anthropogenic stressors including from fishing, pollution, boat traffic and habitat degradation (Secchi 2007, Vermeulen et al. 2019, Genoves et al. 2020). Within the geographical range of this lineage the total number of mature dolphins is estimated to be approximately 360 (Vermeulen et al. 2019), which is well below the "genetically safe" threshold of 5,000 mature individuals (Hoban et al. 2020a, Laikre et al. 2020). Yet, the causes of the small population size and low genetic diversity in *T. t. gephyreus* are unknown and warrants investigation into its evolutionary history. In addition, the species *T. aduncus* has been classified as Near Threatened by the IUCN due to human disturbances, development, and pollution (Braulik et al. 2019). In Australia, extreme weather events and pathogen exposure also threaten bottlenose dolphin populations. For example, a recent marine heatwave in Western Australia has led to the long-term decline in survival and reproductive rates of *T. aduncus* from the Shark Bay population (Wild et al. 2019), and the highly infectious cetacean morbillivirus has

recently been implicated in unusual mortality events of coastal *Tursiops* in western and southern Australia (Stephens et al. 2014, Kemper et al. 2016). Prior to 2009, the virus had not been implicated in any coastal *Tursiops* death in Australia, suggesting a potential changing environment and possibly new selective pressures faced by the inshore dolphin populations. By contrast, *T. truncatus* are currently IUCN-classified as Least Concern (Hammond et al. 2008, Wells et al. 2019) due to their larger population sizes, wider distribution and relatively high gene flow with neighbouring populations. Clarifying patterns of genetic diversity and demographic histories between inshore and offshore bottlenose dolphin populations will provide a better understanding of how these dolphins have recurrently evolved in response to divergent selective pressures, and how they may continue to adapt to continued environmental and climatic changes.

Contrasting demographic histories between inshore (*T. aduncus*) and offshore (*T. truncatus*) populations have been observed for the Northern Hemisphere (Yim et al. 2014, Vijay et al. 2018), however these studies only used a representative of one inshore population and did not investigate the parallel evolution of ecotypes. Louis et al. (2020) extended this by exploring the genomic basis underlying repeated divergence between inshore and offshore common bottlenose dolphins (*T. truncatus*) from two ocean basins in the Northern Hemisphere. In the Southern Hemisphere, little is known about the evolutionary history of bottlenose dolphins, and the mechanisms driving genetic diversity and adaptation. Pratt (2020) explored the genomic basis of ecotype formation in bottlenose dolphins using reduced representation genome sequencing, and implicated variation within genes associated with the cardiovascular, musculoskeletal and energy production systems in the evolution of inshore lineages. Here, these studies are expanded by exploring the niche divergence and potential parallel evolution of bottlenose dolphins using whole genomes of inshore and offshore lineages from across the globe. Specifically, 23 whole genomes from samples representing three inshore and three offshore bottlenose dolphin lineages from across the Southern Hemisphere were sequenced. These data were then combined with publicly available genomes from inshore and offshore bottlenose dolphin populations from the Northern Hemisphere (Yim et al. 2014, Foote et al. 2015, Vijay et al. 2018), an inshore *T. aduncus* genome from the same location as the proposed holotype lineage off South Africa (Moura et al. 2020), and with genomes from the nearshore, closely related common dolphin species (*Delphinus delphis*) for a comparative purpose. To the best of our knowledge, this study represents the first whole genome study of all currently recognised bottlenose dolphin lineages in the Southern Hemisphere and includes the first whole genome sequences for *T. t. gephyreus* and *D. delphis*. With this dataset, the concept of parallel evolution driven by niche specialisation is broadly tested and investigated. It is hypothesised that independent events of niche divergence in similar environments by the same ecotype (inshore *vs* offshore) will be reflected in similar genomic signatures of diversity and ROH and on concordant demographic histories.

3.4 Methods

3.4.1 Sampling design and genome sequencing

Biopsy samples from free-ranging bottlenose dolphins (Indo-Pacific bottlenose dolphins, *T. aduncus*, and common bottlenose dolphins, *T. truncatus*) from the Southern Hemisphere (Australia and Brazil) were collected between 1999 and 2015 using either a remote biopsy gun (Krützen et al. 2002) or a hand-held biopsy pole (Bilgmann et al. 2007a). At least five individuals from each bottlenose dolphin lineage were selected. This dataset was supplemented with six biopsy samples from the closely related species, *D. delphis*, from Australia. The latter species is generally highly mobile and abundant, travel in large schools, and inhabit coastal and open oceans with high productivity (Perrin 2009, Zanardo et al. 2016a). However, in Australia, these animals can exhibit site fidelity in shallow, urbanised areas, such as Gulf St Vincent in South Australia (Kemper et al. 2008) and therefore represent a 'nearshore ecotype' for comparative purposes. Genomic DNA was extracted from skin tissue following the salting out method as described in chapter 2 (Sunnucks and Hales 1996). Samples that passed the quantity and quality standards (≥ 20 ng/µl, A260/280 ratio of 1.8-2.0) were sent to the Australian Genome Research Facility, AGRF, and Novogene for library preparation and whole genome resequencing. Libraries were prepared with the NEBNext Ultra II DNA library prep kit for Illumina, and either sequenced on two lanes of the Illumina NovaSeq 6000 S2 platform (150 bp PE) at AGRF, or on a single lane of the same platform at Novogene. All samples (n = 88) were sequenced at between 5 and 11X coverage, however only 29 of these samples with the highest coverage, which spanned all available lineages were used (Chapter 2, Figure 2.1 and Table 2.1).

Genomes of bottlenose dolphins that are publicly available on the Sequence Read Archive (SRA) were downloaded to complement the data generated, as per Chapter 2 (Chapter 2, Figure 2.1). Specifically, three genomes of inshore *T. aduncus* (South Korea) and four offshore *T. truncatus* from the Northwest Pacific (Ch/JP), as well as an inshore *T. truncatus* from the Gulf of Mexico were downloaded from the SRA (Yim et al. 2014, Foote et al. 2015, Vijay et al. 2018). A single *T. aduncus* genome from the South African lineage that is thought to represent the same lineage as the holotype (Perrin et al. 2007) was also provided by Moura et al. (2020). The final dataset of 38 genomes were grouped into inshore and offshore ecotypes based on prior knowledge of the populations and lineages sampled. The Gulf of Mexico *T. truncatus* lacked metadata but was believed to be an inshore animal (Foote, pers. comm.), while *D. delphis* was grouped into a nearshore ecotype for comparative purposes. Details of samples included in this study and their respective ecotypes can be found in Table 2.1 (Chapter 2).

3.4.2 Bioinformatics and SNP calling

Individual raw resequencing reads were pre-processed following the pipeline adapted from GATK best practices, with modifications as described in (Batley et al. 2021). Briefly, individual sequencing reads

with low quality were trimmed and adapters removed using Trimmomatic (Bolger et al. 2014). The remaining reads were mapped to the *T. aduncus* (SABD) chromosome-length reference genome [generated and detailed in chapter 2] using Bowtie2 v2.2.7 (Langmead and Salzberg 2012). From the resulting mapped files, duplicates were marked and sorted, before locally realigning indels. Finally, replicate reads from different libraries were merged using SAMTOOLS (Li et al. 2009). As a female dolphin was used to form the reference genome, the Y chromosome was not sequenced for this individual. The X scaffold, however, was identified [chapter 2] and removed using SAMTOOLS, for all downstream analysis.

Single Nucleotide Polymorphisms (SNPs) were called from the SABD reference genome using BCFTOOLS (Li et al. 2009) and filtered to only include high-quality SNPs with VCFTOOLS (Danecek et al. 2011) following methods described in Batley et al. (2021). Since genome sequences became available at different times, SNPs had to be called on two separate occasions. All analysis involving the Northern Hemisphere genomes were completed using a filtered dataset of 19,408,848 SNPs generated using all 38 individuals, while analysis of the other samples was completed using 18,705,648 SNPs generated using the 30 Southern Hemisphere genomes (details under Results). The SNPs from the eight Northern Hemisphere samples were called with the same 30 Southern Hemisphere samples. The difference between the datasets may relate to regions that are not variable in the Southern Hemisphere samples, and therefore it is expected that the different datasets did not substantially influence the results. For SNPs retained at each filtering step see Table S3.1.

The 19,408,848 SNP dataset was then thinned to 77,813 SNPs using VCFTOOLS. To assess genomic structure within and between lineages, a principal component analysis (PCA) was run for this dataset (consisting of 38 individuals from ten lineages, and 32 individuals from nine lineages – excludes *D. delphis*) in RStudio v3.5.3 and with the package FACTOMINER (Lê et al. 2008).

3.4.3 Analysis of genomic diversity

Whole genome and autosomal heterozygosity were calculated using sample allele frequency likelihoods in ANGSD v0.931. This software was selected as it accounts for biases that may arise during genotype calling of low and medium coverage genomes by considering genotype likelihoods and statistical uncertainties in the analysis (Korneliussen et al. 2014). First, the site allele frequency likelihoods were generated following ANGSD's suggested filters, and those described in Westbury et al. (2019) to include only high-quality reads (-minqmap 25, -uniqueOnly 1, -dosaf 1, -fold 1 -minq 20). Using this output, the heterozygosity was calculated as the proportion of heterozygous genotypes across the autosomes. Levels of heterozygosity were compared between lineages, and to previously reported values for other mammalian species (Robinson et al. 2016, Morin et al. 2020b). The mean heterozygosity within and between lineages was tested for significant differences using two-sample t-

tests and one-way ANOVAs. Finally, to explore the distribution of heterozygosity across the genome, considering the filtered SNP datasets (19,408,848 and 18,705,648) and only the autosomes, heterozygosity was calculated in 100 kb non-overlapping windows using a customised script from Robinson et al. (2019). This was calculated by dividing the number of heterozygotes by the total number of called genotypes per window (Robinson et al. 2019).

3.4.4 Runs of Homozygosity

Autosomal ROH were identified for each individual using the window-based approach implemented in Plink v1.9 (Purcell et al. 2007). This is the most commonly used ROH detection method as it outperforms other approaches in detecting autozygosity (i.e. homozygous by descent) (Howrigan et al. 2011). To minimise the effects of linkage disequilibrium (LD) and to ensure the detection of autozygous ROHs, the SNP dataset was lightly pruned for LD prior to ROH detection using Plink. The SNPs with $r^2 > 0.9$ were assumed to be linked and removed from the dataset. To further account for homozygosity originating from LD, a minimum ROH length of 300 kb was selected, as empirical studies on humans have estimated that ROH with tract length up to 100 kb can originate from LD (International HapMap et al. 2007, Slatkin 2008, Ceballos et al. 2019).

A ROH was detected following criteria applied to killer whales, *Orcinus orca* (Hooper et al. 2020). In short, the sliding window was set to 300 kb, requiring a minimum of 50 SNPs, and density of 1 SNP every 50 kb. Given the high SNP density within the genomes (on average 1 SNP every ~ 228 bp), up to 5 heterozygous SNPs, and one missing SNP per window were allowed to account for genotyping errors. Finally, a length of 1,000 kb was required between two SNPs to be called a separate ROH.

The number and length of ROH were compared between the different lineages as these metrics are known to inform on demographic histories. Generally, longer ROH originate from recent inbreeding, whereas shorter ROH reflect distant ancestors. Specifically, based on constant mammalian recombination rates, ROH less than 1 Mb in length are thought to correspond to ancestral inbreeding 2-12 kya, while ROH longer than 2 Mb may relate to inbreeding events within the past ~2,000 years (see Hooper et al. (2020) for details). In addition, individual genomic inbreeding coefficients (FROH) were estimated, where the total number of ROH greater than 1 Mb was divided by the total length of autosomes (Hooper et al. 2020).

3.4.5 Demographic history and niche divergence

The Sequential Markovian coalescent + Plenty of Unlabeled Samples (SMC++) method (Terhorst et al. 2017) was employed to infer the demographic histories of *Tursiops* lineages from Australia, South Africa, SWAO, and the Northern Hemisphere, along with *D. delphis* from Australia. The SMC++ method was chosen as it can integrate information from the site frequency spectrum of multiple

unphased genome sequences simultaneously, improving the robustness of population size estimates, particularly for those with recent histories (Terhorst et al. 2017). Input data files were generated for each lineage independently using the filtered VCF file and the vcf2smc function in SMC++. A mask file for each chromosome was used to define large uncalled regions, so that these were distinguished from long ROH. When generating the input files for each lineage, the distinguished individual (one with highest genome coverage from each population within a lineage) was varied twice. This was done to incorporate additional information from multiple distinguished individuals from different populations to generate composite likelihoods, potentially improving power in the analysis (e.g. Louis et al. 2020). As population structure can bias the analysis (e.g. Sellinger et al. 2020), demographic histories between populations of the same lineage were also assessed following the same method but using a single distinguished individual due to the smaller sample size for population comparisons (Figure S3.1). Bootstrapping was performed by breaking the genome into twenty 5 Mb chunks per chromosome, and 22 chromosomes per bootstrap (100 bootstraps). Population size history was assessed using the 'estimate' function, a mutation rate of 1.5e-8 per site per generation (Moura et al. 2014a, Moura et al. 2020), and a generation time of 21.5 years for *Tursiops* and 12.8 for *D. delphis* (Taylor et al. 2007). Times of divergence between the *Tursiops* subspecies were then estimated to better inform on potential drivers of divergence, and whether divergence led to low diversity as a result of putative founder events. This involved comparisons among *T. t. gephyreus* and *T. t. truncatus*, and *T. aduncus* from the southern (SABD) and eastern coasts of Australia. Divergence was estimated by generating datasets containing the joint frequency spectrum for the two lineages tested and running the 'split' function using default parameters.

3.5 Results

New whole genomes for 23 inshore and offshore bottlenose dolphins and six *D. delphis* were sequenced at an average depth of 9x (Chapter 2, Table 2.1). The geographic distribution of these samples broadly covers all recognised species and subspecies of bottlenose dolphins from across the Southern Hemisphere (this study and Moura et al. (2020)), and are complemented with whole genomes of bottlenose dolphins from the Northern Hemisphere (Yim et al. 2014, Foote et al. 2015, Vijay et al. 2018). All sequenced genomes mapped to at least 96% of the SABD reference genome, with 20,207,341 filtered, high-quality SNPs anchored onto 23 chromosome-length scaffolds, and 6,736 small scaffolds that make up less than 0.4% of the genome. All downstream analyses were restricted to the 21 large autosomal chromosomes and either 18,705,648, or 19,408,848 SNPs, with an average missing data of less than 0.006% (SD ± 0.004).

3.5.1 Genomic structure and diversity

The PCA confirmed the clear genomic differentiation between the accepted bottlenose dolphin species (*T. truncatus* and *T. aduncus*) and *D. delphis* (Figure 3.1). PC 1 explained 27.19% of variance including splitting *T. truncatus* from *T. aduncus*, while PC 2 explained 22.28% of variance including splitting between *Tursiops* and *D. delphis*. Genomic separation was observed between the *T. aduncus* lineages, with a clear division between *T. aduncus* from eastern Australia and the putative subspecies (SABD) from southern Australia. *T. t. truncatus* generally clustered together, with no distinct separation between lineages, besides separation of *T. t. truncatus* (Gulf Mex) from the other *T. t. truncatus* lineages, and a strong division between the accepted inshore subspecies *T. t. gephyreus* and the offshore *T. t. truncatus* from Brazil (Figure 3.1).

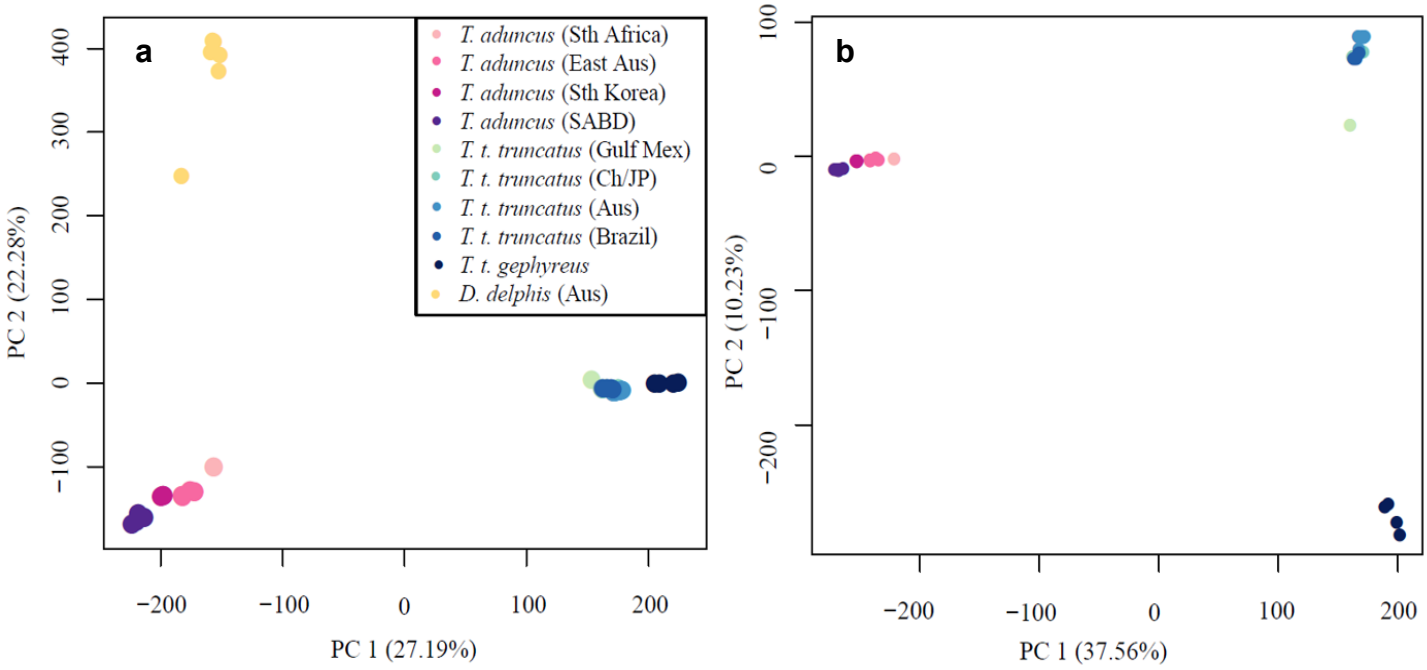

Figure 3.1: Patterns of genomic differentiation between a) species (*T. aduncus*, *T. truncatus* and *D. delphis*) and b) bottlenose dolphin lineages from across the globe inferred via Principal Components Analysis.

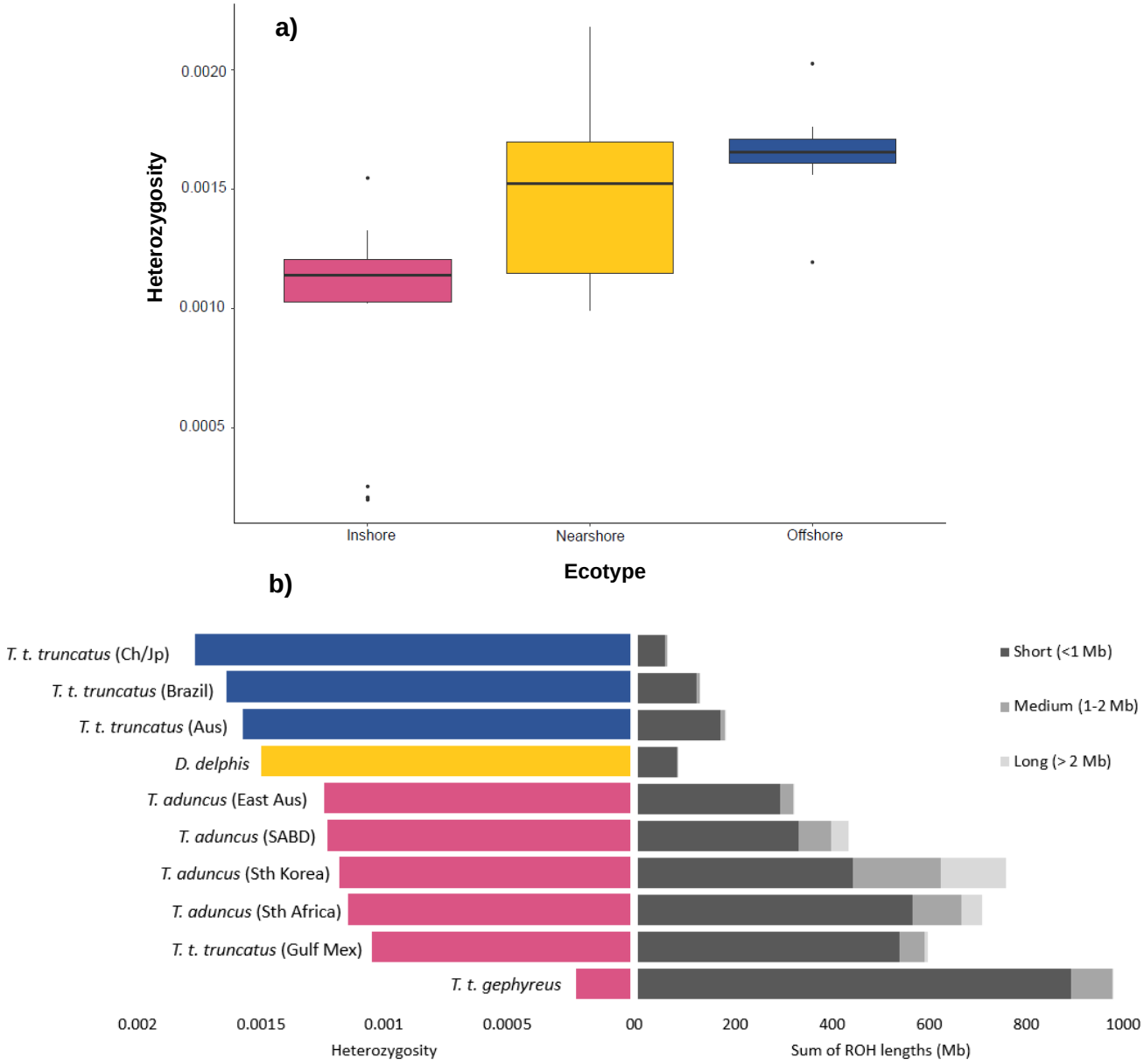

Figure 3.2: Average genome-wide heterozygosity per ecotype (a) and lineage (b) and the sum of ROH lengths in inshore (pink), nearshore (gold), and offshore (dark blue) dolphin lineages. Black horizontal bars represent the median (inshore 0.001141, nearshore 0.0015237, offshore 0.001657), black boxes encompass the interquartile range (inshore 0.000177, nearshore 0.0005541, offshore 0.000103), with lower whiskers representing Q_1-1.5 * IQR and the upper illustrating Q_3+1.5 * IQR.

Mean autosomal heterozygosity ranged between 0.00022 and 0.0017 (Figure 3.2), with a significant association observed between ecotype and genomic diversity (Figure 3.2, Table S3.2). Lineages of the inshore ecotype always exhibited significantly lower genomic diversities than offshore ecotypes and the nearshore *D. delphis* (Figure 3.2, Table S3.3). Among lineages of the inshore ecotype, the common bottlenose dolphin lineages recorded lower genomic diversity than *T. aduncus* lineages, with the inshore *T. t. gephyreus* recording extremely low genomic diversity (0.00022 ± SD 2.02E-05) that was

significantly lower than all other lineages (Table S3.3). In comparison, the adjacently distributed offshore lineage (*T. t. truncatus*) is 7.5-fold more diverse (0.0016 ± SD 6.94E-05) than *T. t. gephyreus*. A significant difference in the mean heterozygosity was observed between all inshore lineages and the *T. t. truncatus* from the Northwest Pacific (Ch/Jp), which exhibited the highest level of diversity of all dolphin lineages (Table S3.3).

Patterns of heterozygosity across the genome highlight distinct differences between ecotypes. In general, the genomes of inshore lineages displayed moderate levels of heterozygosity interspersed with regions of low heterozygosity. In comparison, offshore lineages and the nearshore *D. delphis* showed a more even distribution of higher heterozygosity, with negligible regions of low heterozygosity – particularly in common dolphins (Figure 3.3). Patterns of heterozygosity were similar within ecotypes, albeit the inshore *T. t. gephyreus* that displayed an even distribution of very low heterozygosity across the genome.

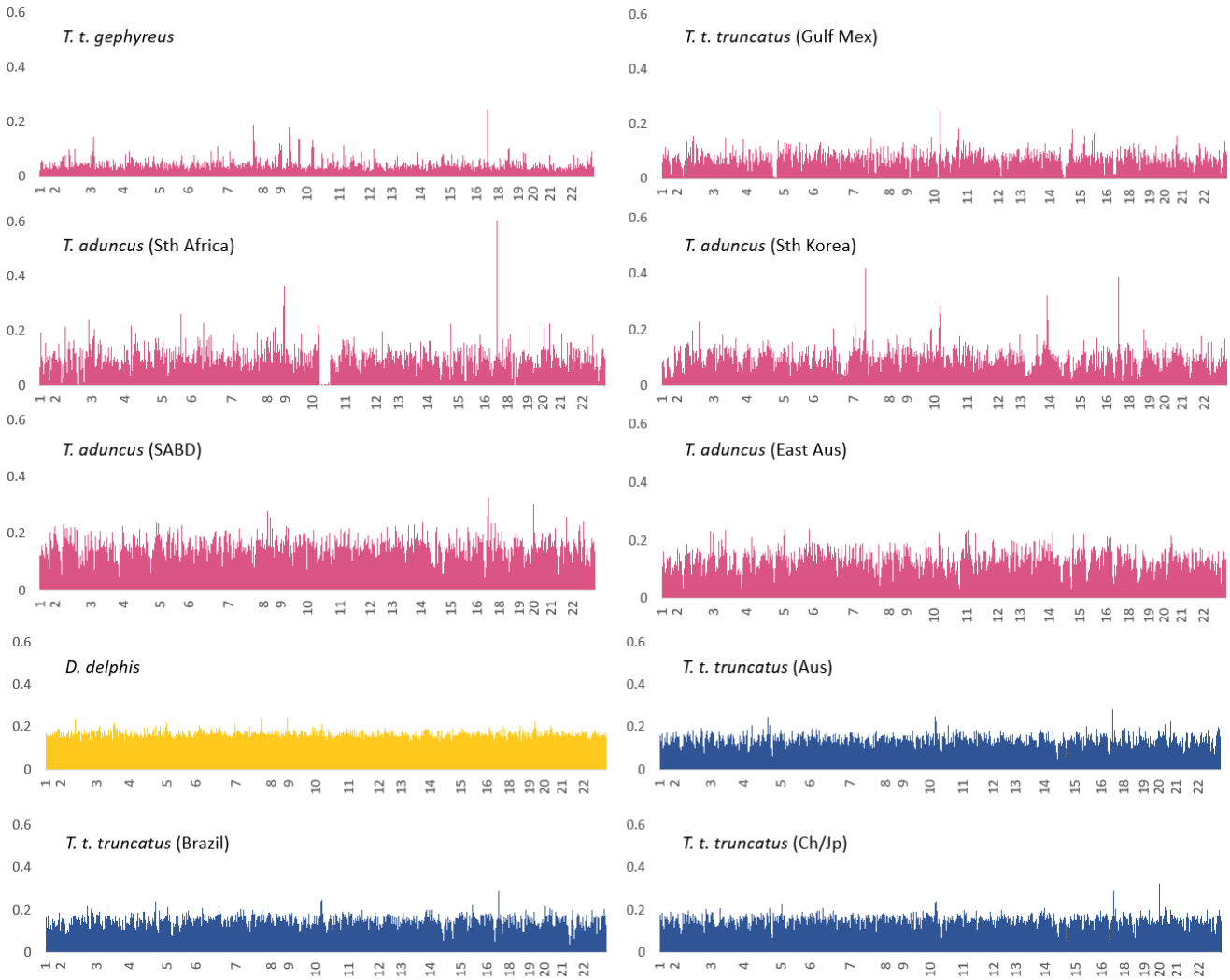

Figure 3.3: Average distribution of heterozygosity across the genomes of inshore (pink), nearshore (gold), and offshore (blue) dolphin lineages. Bar plots represent per-site heterozygosity in non-overlapping 100kb windows across the 22 autosomal chromosome-length scaffolds.

3.5.2 Runs of Homozygosity

Runs of homozygosity were identified in all individuals, except for one nearshore *D. delphis* from Gulf St. Vincent. Specifically, for the inshore ecotype the ROH covered a greater proportion of the genomes, with many short (<1 Mb) and some longer ROH (>2 Mb). Fewer short and no longer runs were observed for the offshore ecotype (excluding one *T. truncatus* from Aus, which showed higher levels of inbreeding) (Figure 3.4). The nearshore *D. delphis* had very few and very short ROH. Across all lineages, the genomes of *T. t. gephyreus* were covered by the greatest proportion of ROH, but the length of ROH were generally short, with few ROHs being larger than 2 Mb. In comparison, the *T. aduncus* (Sth Korea) genomes were covered by less ROH, but the frequency of long ROH (>2 Mb) was far greater than observed for all other lineages (Figure 3.4).

The inbreeding coefficient (FROH) was higher in all inshore lineages compared to the offshore lineages (Figure 3.5), however, large standard deviations observed suggest that, at an individual level, some exhibit higher levels of inbreeding (Figure 3.6, Figure S3.2 for individual comparisons). Within the inshore ecotype, *T. t. gephyreus* have the lowest heterozygosity and the greatest proportion of their genome covered by ROH, but the values of FROH were lower than in the other inshore lineages (*T. aduncus* from South Africa, South Korea and the SABD), suggesting that recent inbreeding may not be the cause for the low levels of diversity observed in that subspecies. The high FROH and long ROH observed in *T. aduncus* from South Africa, South Korea and the SABD suggest very recent inbreeding events. In comparison, the negligible number of long ROH observed for *T. aduncus* (East Aus), inshore *T. t. truncatus* (Gulf Mex) and all offshore and nearshore lineages, suggest a general lack of recent inbreeding events in these lineages.

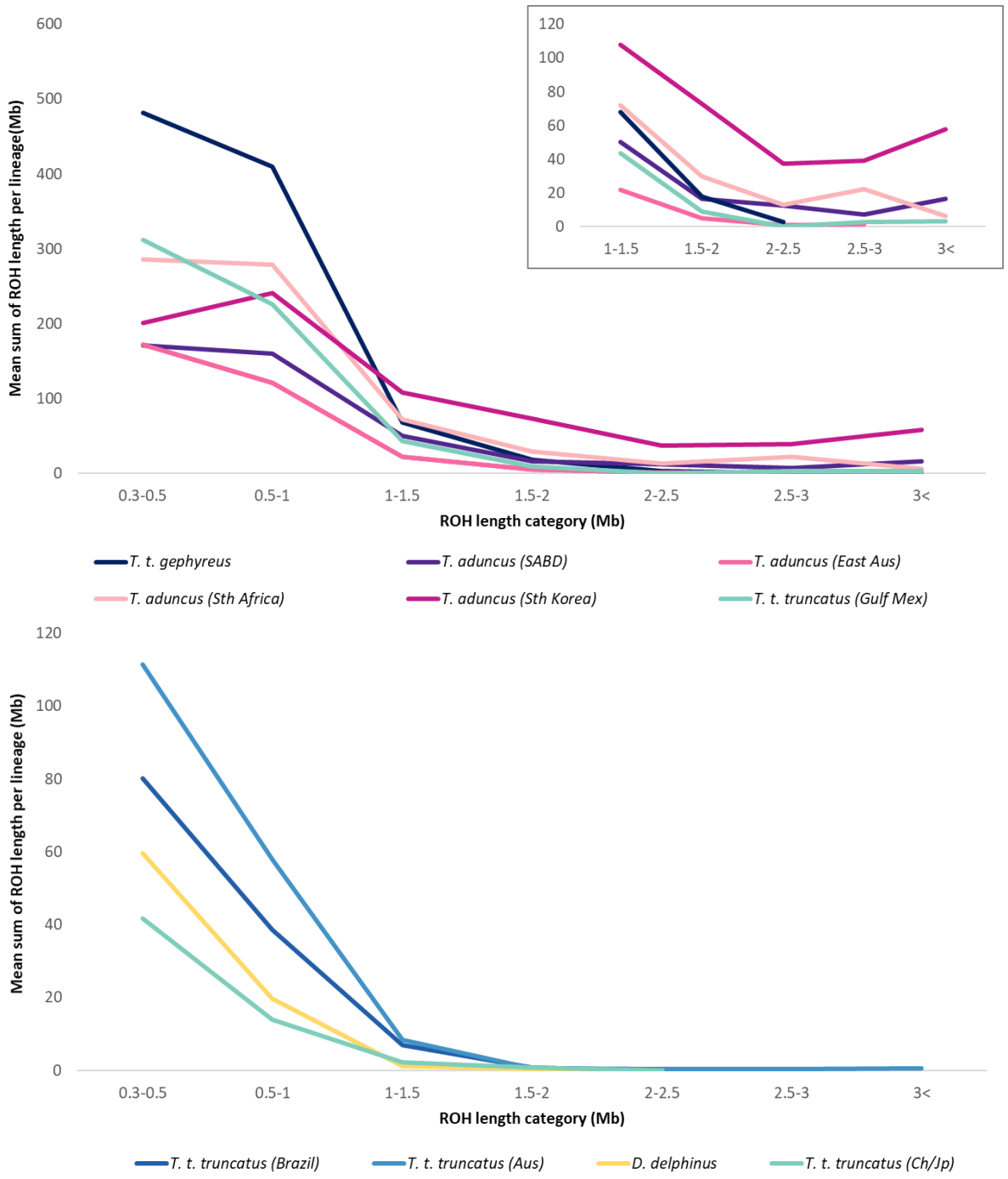

Figure 3.4: The sum of ROH per length category of inshore (top), nearshore and offshore (bottom) dolphin lineages.

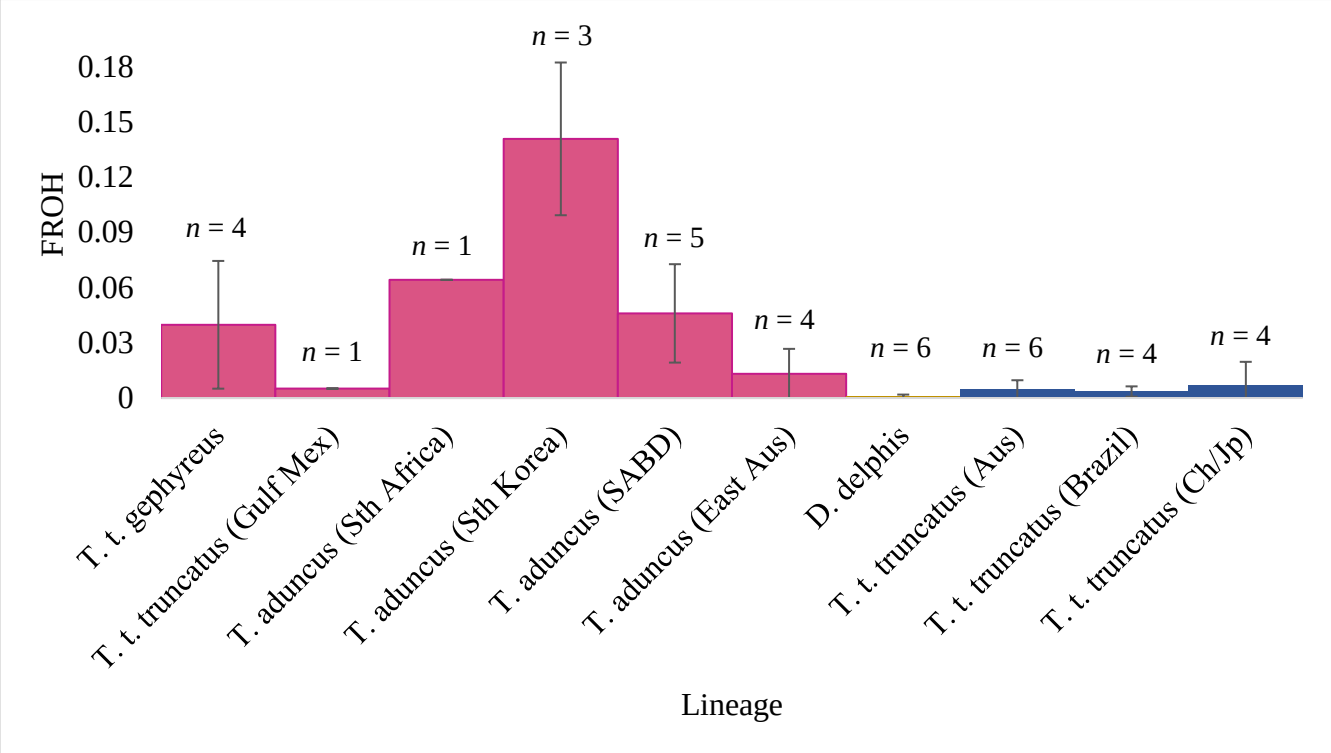

Figure 3.5: The average inbreeding coefficient (FROH) for inshore, nearshore, and offshore dolphin lineages examined. Pink bars represent lineages of the inshore ecotype, and dark blue bars represent lineages of the offshore ecotype. *D. delphis* genomes exhibit negligible inbreeding (FROH 0.0007 ± 0.0018).

3.5.3 Demographic history and niche divergence

The SMC++ analysis revealed consistent demographic histories within the offshore ecotype, irrespective of the lineage or ocean basin (Figure 3.6). The inshore ecotype showed more diverse patterns, but these were generally different to the patterns observed for the offshore ecotype including in both the deep (e.g., the LGM) and recent past (Figure 3.6). The offshore and nearshore ecotype experienced population expansions towards the start of the last glacial period, and more rapid expansions towards the end of the LGM. In comparison, *T. aduncus* lineages of the inshore ecotype generally experienced population declines during the LGM followed by stable, small population sizes. By contrast, the inshore *T. truncatus* lineages followed similar patterns to the offshore ecotype, with population expansions towards the end of the LGM, while *T. aduncus* from eastern Australia also displayed this pattern. All inshore, nearshore, and offshore ecotypes maintained stable populations throughout the last ~1,500 years, until the very recent past when changes in population sizes are observed for the inshore ecotypes. While lineages of the offshore and nearshore ecotype maintained stable population sizes, the N_e of all inshore lineages began to expand, while *T. t. gephyreus* appears to have experienced a population bottleneck. The SMC++ split analysis estimated that *T. t. gephyreus* diverged from their offshore counterpart towards the end of the penultimate glacial period (~151 kya, 95% CI 62,798-77,987) (Figure S3.3), while the putative subspecies of *T. aduncus* from Australia's

southern coast (SABD) appears to have maintained a small population size since diverging from the Australian east coast's *T. aduncus* ~51 kya (95% CI 45,145-51,622) (Figure 3.6).

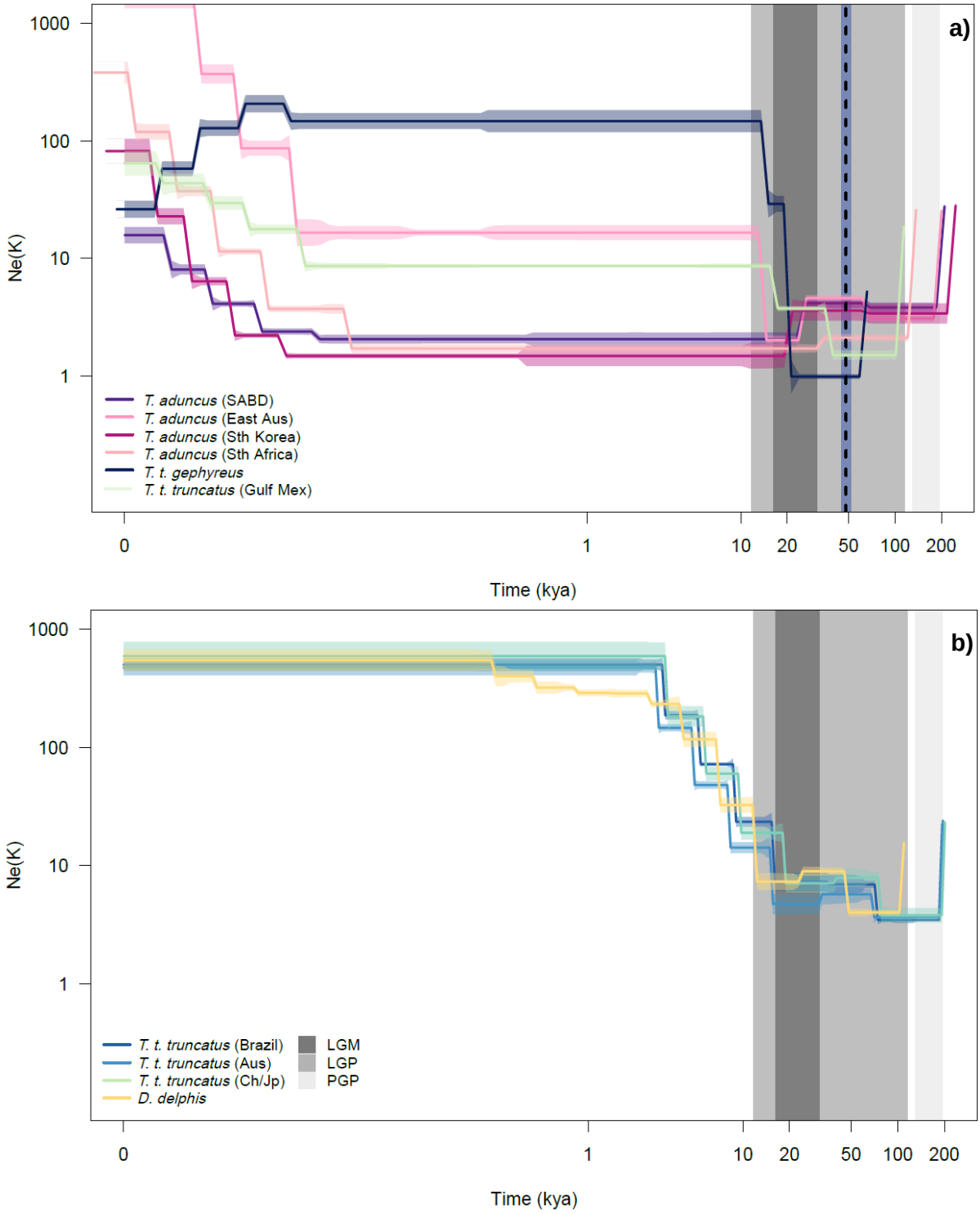

Figure 3.6: Demographic history of inshore, nearshore and offshore dolphin lineages from three ocean basins; **a)** inshore ecotypes; **b)** nearshore and offshore ecotypes. The dashed line reflects the estimated time of divergence between *T. aduncus* (SABD and East Aus). Light grey area: Penultimate Glacial Period (130-194 kya), mid-grey area: Last Glacial Period (11.7-115 kya) and dark grey: Last Glacial Maximum (16.3-31 kya). Glacial periods follow de Jong et al. (2020).

3.6 Discussion

Repeated colonisation of independent but closely related lineages to similar environmental niches provides a great opportunity to understand the role of niche divergence in the parallel evolution of genomes. For bottlenose dolphins, divergence between inshore and offshore ecotypes offers insights into the genomic basis of ecotype formation. Here, data from 38 whole genomes and over 18 million SNPs are used to explore the genomic consequences of repeated niche divergence between closely related dolphin lineages. To the best of our knowledge, this represents the largest whole genome study of dolphins at a global scale. A very strong association between genomic diversity, inbreeding and ecotype was detected. Lineages of inshore ecotypes were consistently less genetically diverse than lineages of offshore ecotypes. Demographic reconstructions highlighted similar histories of niche divergence in lineages of the same ecotype, but different histories between ecotypes. Results also indicated that a recent bottleneck may be the cause of exceptionally low diversity observed in an inshore ecotype with a narrow range (*T. t. gephyreus*), shedding light on the role that historical factors play in shaping genome-wide diversity. This study provides evidence for parallel genome evolution due to niche divergence in a highly mobile marine mammal and points to the role of natural selection in shaping adaptive potential of bottlenose dolphins.

3.6.1 Genome-wide diversity and inbreeding

Patterns of genome-wide diversity and ROH differed between ecotypes (inshore and offshore) and the nearshore *D. delphis*. Overall, inshore dolphins appear to be most vulnerable to environmental changes, with genome-wide levels of heterozygosity considerably lower than offshore lineages. Genetic studies have repeatedly observed lower genetic diversity in inshore populations compared to their offshore counterparts (Hoelzel et al. 1998, Natoli et al. 2004, Lowther-Thieleking et al. 2015, Fruet et al. 2017), and this is further supported in this study based on whole genomes. This global pattern is thought to represent founding events by a small number of individuals from offshore populations, followed by negligible gene flow with their offshore counterparts and enhanced drift (Hoelzel et al. 1998, Natoli et al. 2004, Möller et al. 2007, Louis et al. 2014a, Lowther-Thieleking et al. 2015, Ellegren and Galtier 2016, Pratt 2020). Of greatest concern is the inshore subspecies *T. t. gephyreus* found here to be at least five times less diverse than all other inshore lineages. This lineage exhibits the lowest diversity for any *Tursiops* population studied to date (Fruet et al. 2014, Fruet et al. 2017, Pratt 2020). In comparison to other mammals, this lineage has similar levels of heterozygosity to some of the most vulnerable and endangered species worldwide, including the cheetah (*Acinonyx jubatus*) (Dobrynin et al. 2015), snow leopard (*Panthera uncia*) and white African lion (*Panthera leo*) (Cho et al. 2013), and the Tasmanian devil (*Sarcophilus harrisii*) (Miller et al. 2011, Hendricks et al. 2017) (Figure S3.4). Although populations with low genetic diversity can persist, they need the capacity to adapt to stressful conditions, particularly given the increasing pressures from climate change. For example, the low diversity

observed in the cheetah and the Tasmanian devil have been associated with increased disease susceptibility (Dobrynin et al. 2015, Morris et al. 2015). The extremely low diversity of *T. t. gephyreus*, coupled with their susceptibility to anthropogenic impacts (e.g., fisheries by-catch) and reported declining population trend raises serious concern over the population's capacity to persist (Fruet et al. 2014, Vermeulen et al. 2019).

Despite the low diversity of *T. t. gephyreus*, heterozygosity was evenly distributed across its genome and the length of ROH were generally small. This is particularly the case in comparison to the other inshore lineages that displayed higher levels of heterozygosity, interspersed with regions of low heterozygosity, and a greater number of long ROH. This pattern of heterozygosity and ROH observed for all other inshore lineages are consistent with populations that have experienced recent inbreeding events, or low levels of gene flow following a bottleneck or founder event (Brüniche-Olsen et al. 2018). For *T. t. gephyreus*, the colonisation of inshore habitats by a small number of individuals after the LGM was suggested as a possible reason for their low diversity (Fruet et al. 2017, Pratt 2020). The evenly distributed diversity across the genome of *T. t. gephyreus* suggests a small and isolated population, as proposed for Tibetan and Ethiopian wolves (Robinson et al. 2019), while a high number of small ROH may perhaps represent a recent bottleneck (Ceballos et al. 2018). In addition, the finding of small ROH, very few long ROH and low inbreeding (FROH) in comparison to other inshore dolphin lineages is consistent with reports of negligible levels of inbreeding in *T. t. gephyreus* based on a small representation of the genome (Fruet et al. 2014, Pratt 2020). The difference in heterozygosity and ROH patterns observed between *T. t. gephyreus* and other inshore lineages imply that additional demographic factors may be involved.

In contrast, lineages of the offshore ecotype generally exhibited an even distribution of heterozygosity, with a very small number of regions of lower heterozygosity and very few or nil long ROH. This pattern may be indicative of large populations with high gene flow, and potentially some ancestral inbreeding. Offshore lineages throughout the Southern Hemisphere have been found to be more genetically similar to each other than to adjacent inshore populations (Pratt 2020). Connectivity between offshore lineages may extend to the Northern hemisphere, with shared mtDNA haplotypes reported for offshore dolphins from Brazil and the northeastern Atlantic Ocean (Quérouil et al. 2007, Oliveira et al. 2019). These studies support the demographic patterns inferred from genome-wide heterozygosity and ROH of large populations, with evidence of long-range gene flow. The lack of ROH and even distribution of high heterozygosity across the nearshore *D. delphis* genome is also consistent with large, long-term populations with minimal signs of inbreeding as found in the Xinjiang and Minnesota wolves (Robinson et al. 2019). High diversity and lack of inbreeding is generally characteristic of *D. delphis* populations worldwide and support the idea that the high diversity observed in this species is driven by large, long-term populations with high gene flow (Amaral et al. 2009).

3.6.2 Parallel demographic histories of bottlenose dolphin ecotypes

To clarify the parallel evolution of closely related lineages, and the history of bottlenose dolphin ecotypes, demographic histories were reconstructed using SMC++. Concordant with heterozygosity and ROH results, the offshore ecotype exhibited consistent demographic histories, and patterns that generally contrast to the inshore *T. aduncus* lineages. As the availability of coastal habitats were disrupted during the last glacial period, it is expected that many populations inhabiting those environments would have experienced bottlenecks, as generally observed for the inshore ecotype and other cetacean species (e.g. gray whales (*Eschrichtius robustus*) (Bruniche-Olsen et al. 2018), sperm whales (*Physeter macrocephalus*) (Warren et al. 2017) and killer whales (*Orcinus orca*) (Moura et al. 2014a)). In contrast, population expansions of offshore bottlenose dolphins during the last glacial period were demonstrated for lineages of the Northern Hemisphere based on whole genomes (Yim et al. 2014, Vijay et al. 2018, Louis et al. 2020), and likewise found for all offshore lineages and the nearshore *D. delphis* examined here. This pattern may relate to either stable or improved conditions of offshore environments during the last glacial period, the original larger population sizes of offshore ecotypes, and their higher gene flow over greater spatial scales. During the last glacial period, bottlenecks in populations of large predatory species, such as killer whales and large sharks (O'Brien et al. 2012, Moura et al. 2014a), may have led to less prey competition and predation upon *T. truncatus* (Vijay et al. 2018). Alternatively, the population expansions may reflect increased connectivity of offshore animals during this time, rather than true expansions in N_e (Nykanen et al. 2019, Louis et al. 2020). All offshore lineages and the nearshore *D. delphis* also showed continual expansions after the LGM, which may reflect the maintenance of higher gene flow due to greater spatial scale movements and home ranges, more fluid social structures, and larger population sizes (Möller 2012, Moura et al. 2013, Gaspari et al. 2015).

In comparison, inshore lineages were shown to have experienced bottlenecks during the LGM. This, however, excludes the two inshore *T. truncatus* (*T. t. gephyreus* and Gulf of Mexico), which followed similar patterns to the offshore lineages during the LGM only, and may reflect the increased connectivity during a period where available inshore habitats were scarce. Previous studies on inshore dolphins from the North Atlantic Ocean proposed that bottlenecks followed by population expansions after the LGM likely reflected founding events of coastal areas, with habitat preference and environmental conditions then driving genetic divergence between the two ecotypes (Hoelzel et al. 1998, Natoli et al. 2004, Louis et al. 2014b, Louis et al. 2020). Due to the low diversity consistently observed within inshore lineages, this has been previously hypothesised for other areas of the world where inshore and offshore bottlenose dolphin ecotypes exist (Fruet et al. 2017, Pratt 2020). However, the bottlenecks observed for all inshore (*T. aduncus*) lineages seem to have occurred prior to the peak of the LGM, during a period of increasing ice volume and sea level, and thus reduced habitat availability

(Ludt et al. 2015). Species with restricted ranges may have been particularly vulnerable to these changes. Despite a general lack of physical barriers to the dispersal of dolphins in ocean environments, small home ranges and natal philopatry are typical of inshore *Tursiops* (Möller 2012). Inshore and offshore bottlenose dolphin environments generally differ in water depth, currents, salinity, and temperature, as well as resource availability and productivity (Möller 2012). Therefore, as inshore lineages were possibly forced to retreat into deeper, cooler, and less productive waters during the LGM, they may have found it difficult to adapt to the different ecological and environmental conditions of the offshore environment.

It is noteworthy that the bottleneck observed in the South African lineage is far less pronounced than in *T. aduncus* from other ocean basins. Interestingly, during the LGM, a decline of killer whales was inferred for most populations across their global range, but with the exclusion of southern Africa (Moura et al. 2014a). The productivity of waters off southern Africa were relatively stable during periods of environmental change in the Pleistocene and may have been an important factor influencing demographic stability in populations of killer whales, and potentially of *T. aduncus*, off South Africa (Moura et al. 2014a). This highlights the importance of ocean productivity in the persistence of marine populations during periods of climatic change. Similarly, environmental changes during the last glacial period were more amenable on the east coast of Australia compared to the southern coast, with sea temperatures and currents differing only slightly between glacial and interglacial stages (Lawrence and Herbert 2005, Hostetler et al. 2006). Although a bottleneck during the LGM was also observed for the eastern Australian *T. aduncus*, it was the only *T. aduncus* lineage to exhibit a population expansion after it. After the LGM, the glacial coastline on Australia's east coast retracted, and the Eastern Australian Current re-strengthened and warmed (e.g. Lawrence and Herbert 2005, Hostetler et al. 2006), which may have led to greater connectivity within this dolphin lineage. These unique oceanographic conditions of Australia's east coast during the last glacial period and the potential enhanced connectivity may have had an overall impact on levels of heterozygosity of this lineage, including the shorter ROH lengths, lower FROH and higher diversity in comparison to the other *T. aduncus* lineages. By contrast, the lack of recovery of the other *T. aduncus* lineages could reflect greater re-arrangement of coastal habitats after the LGM in the respective ocean regions, such as Australia's southern coast, which favoured restricted distributions, strong genetic differentiation, and small population sizes, most typical of the inshore lineages (Moura et al. 2020).

3.6.3 Niche divergence of bottlenose dolphin ecotypes

Niche divergence and subsequent local adaptation is suggested to be an important driver of genetic differentiation between bottlenose dolphin populations (Hoelzel et al. 1998, Natoli et al. 2004, Louis et al. 2014b, Louis et al. 2020). In the Southern Hemisphere, strong evidence for genetic differentiation between *T. aduncus* from southern and eastern Australia has led to the proposed subspecies, the SABD

(Moura et al. 2020, Pratt 2020). Here, clear separation was observed between the two lineages and this distinction is hypothesised to be driven by the colonisation of newly released habitats. To test this hypothesis, divergence between the two lineages was estimated. Divergence was predicted to have occurred at approximately 51 kya (95% CI 45,145-51,622), during the last glacial period, but not particularly in the LGM. This period was characterised by fluctuating atmospheric temperatures, resulting in many glacial and interglacial periods, and the associated emergence of land bridges (Lambeck and Chappell 2001). The Bassian land-bridge connected Tasmania and mainland Australia from ~43 to 14 kya, but several short periods (~76 kya and 68 to 62 kya) of the eastern land connection may also have existed (Lambeck and Chappell 2001), and could have potentially led to initial allopatric differentiation. This land barrier has been proposed to be a driving force in the population divergence, and in some cases speciation of coastal marine species (e.g. jelly fish (Dawson 2005); Nerita (Waters et al. 2005); sea-stars (O'Loughlin et al. 2003, Waters et al. 2004); seadragons (Wilson et al. 2016)) and potentially other delphinids (*D. delphis* (Bilgmann et al. 2008)). Contemporary oceanographic features likely maintained the disjunction between the east and west biota in southern Australia (Waters 2008), while natal philopatry and adaptation to their local environmental and ecological niches may have reinforced divergence between the dolphins.

In the Southwest Atlantic Ocean (i.e., Brazil), divergence between the inshore and offshore *Tursiops* ecotypes has been hypothesised to have occurred after the LGM, as a small number of individuals moved into the coastal habitats (Fruet et al. 2017, Pratt 2020). However, in this study it was estimated that the two ecotypes diverged approximately 151 kya. Given the incongruence in the estimated time of divergence and the confidence intervals, further effort needs to be placed on accurately determining the time of divergence between these two lineages. Nevertheless, it is apparent that the inshore lineage diverged from the offshore *T. truncatus* during the last glacial period. This suggests that a post-LGM induced founder event is not the most likely reason for the extremely low diversity observed for this subspecies, but rather a more recent bottleneck. While the resolution of analyses of very recent demographic changes is limited, the inferences of a recent bottleneck are supported by the pattern of many small ROH, which can be indicative of a bottleneck. Previous studies have also recognised that this subspecies is typically represented by very small populations, and there is a general declining trend for the subspecies across its range (Fruet et al. 2014, Vermeulen et al. 2019). Along Brazil's southernmost coast, there are also high levels of mortality of dolphins as bycatch in fisheries, particularly young dolphins before recruitment into the adult population (Fruet et al. 2012, Fruet et al. 2014, Venuto et al. 2020). In addition, substantial coastal development, prey depletion, pollution, bioaccumulation of PCBs, and increasing observations of skin lesions and diseases are known threats to individuals of these populations (Tagliani et al. 2007, Van Bressem et al. 2007, Van Bressem et al. 2015, Secchi et al. 2016, Vermeulen et al. 2019). The low genetic diversity potentially caused by a recent bottleneck to this subspecies, their small population sizes, the narrow range of the lineage, and

the multiple increasing threats raise serious concerns for the conservation of this coastal bottlenose dolphin subspecies.

3.6.4 Conservation management

Genetic diversity is essential for maintaining global biodiversity and evolutionary processes, especially under increased threats of climate change (Laikre 2010). It provides populations with the potential to adapt to changes and stressors, reduces the harmful effects of inbreeding, and enhances disease tolerance and resistance (Lotze et al. 2011, Wernberg et al. 2018, Hoban et al. 2020b). Yet over the past century, the genetic diversity in wild populations has declined, mostly in response to climate change, habitat loss, emerging infectious diseases, and subsequent small population sizes (Laikre 2010, Leigh et al. 2019, Hoban et al. 2020b). It is therefore particularly important to identify vulnerable species and populations to ensure effective management and conservation measures are implemented, such as those related to maintaining or rescuing genetic diversity and stabilising or increasing population sizes. Inshore dolphins are particularly vulnerable to climatic and anthropogenic changes given their small ranges, high site fidelity in coastal and often urbanised regions, and because they generally live in small and isolated populations with low levels of genetic diversity (Möller et al. 2007, Charlton-Robb et al. 2014, Fruet et al. 2014, Zanardo et al. 2016b, Pratt et al. 2018). This was exemplified by our finding of low genomic diversity, small population sizes and population bottlenecks without recovery (particularly in *T. t. gephyreus*), that may have directly or indirectly been caused by climatic events, habitat loss and/or anthropogenic stressors. Given coastal dolphins often live in close proximity to areas with high human use, they are highly vulnerable to the bioaccumulation of heavy metals and toxins, habitat loss, boat strikes, noise pollution, coastal development, fisheries by-catch, disease outbreaks, climate change and extreme weather events (Fruet et al. 2012, Bilgmann et al. 2019, Vermeulen et al. 2019, Bonneville et al. 2021). Anthropogenically accelerated climate change threatens to enhance these stressors, and the lack of population size recovery found in inshore populations highlight the need to apply management measures to reduce human-induced impacts and maintain genetic diversity and population sizes. In the case of *T. t. gephyreus*, findings from this study suggest a recent population bottleneck as a potential main cause for their extremely low genomic diversity. This is in agreement with the International Union for the Conservation of Nature (IUCN) current classification of this subspecies as Vulnerable, citing a declining population trend, small population size and increased susceptibility to anthropogenic stressors as the main threats to the persistence of populations (Vermeulen et al. 2019). The reduction of anthropogenic stressors on this subspecies should therefore be a major management priority. Where conservation efforts have focused on minimising human exploitation and destruction, some patterns of recovery in depleted marine species have been observed, particularly in coastal and estuarine populations (Lotze et al. 2006, Lotze et al. 2011). It is recommended that managers should design conservation strategies that aim to reduce anthropogenic stressors and maintain connectivity between

the five management units identified for this subspecies in the SWAO and monitor changes in their genetic diversity and population sizes over time. Indeed, population viability analysis suggested that the implementation of a dolphin protection area, in a section of major by-catch risk for dolphins belonging to three of the management units, would likely lead to an increase in population size of about 20% over 60 years (Fruet et al. 2021).

While nearshore and offshore dolphins exhibit large population sizes with high gene flow and genetic diversity, and may have a considerable capacity for adapting to increased selective pressures, it is still important that knowledge about population structure and status be available in the event of unforeseen impacts, such as large-scale oil spills and emerging infectious diseases (e.g. Batley et al. 2019, Batley et al. 2021).

3.6.5 Limitations and future directions

Here, we used SMC++ to infer the demographic histories of bottlenose and common dolphin lineages. This program is particularly useful in that it integrates information from multiple unphased genomes to improve the robustness of estimates. In this study, between 4 and 6 genomes of low to moderate coverage (between 5 and 28x) were available for most lineages, and only one genome for the South African *T. aduncus* and inshore Gulf of Mexico *T. t. truncatus* (20x and 34x, respectively). We therefore compared patterns ascertained from our study with previously inferred demographic histories that used some of the same genomes and multiple methods. Vijay et al. (2018) inferred demographic histories of *Tursiops* from the Northern Hemisphere using both PSMC and SMC++, and our results are consistent with their findings of contrasting demographic histories between *T. aduncus* and *T. t. truncatus* (Vijay et al. 2018). This is particularly important for the inshore *T. t. truncatus* (GM), where only one genome was available for the SMC++ analysis. The authors used PSMC to infer the demographic history of this genome, which only integrates information from one individual and therefore requires a high coverage genome. This method has improved accuracy for older time periods but given the low-medium coverage of our data, SMC++ was the most appropriate method. This was also the case for the South African *T. aduncus*, with our results resembling those inferred in Moura et al. (2020). While it is undesirable to use one genome for the SMC++ analysis, these comparisons suggest that SMC++ may still be useful for detecting demographic histories based on single genomes. However, while patterns are similar, the N_e estimates were generally very large with SMC++. This could be due to combining individuals from more than one population into a single lineage for the demographic history analysis. The model assumes that all individuals are from a single panmictic population, which was not the case here, particularly for the inshore lineages. However, the N_e estimates when comparing multiple individuals from a single population were just as large. To test whether sample size influenced the large N_e estimates, we ran the estimate function for 30 individuals from a single *T. aduncus* population (Gulf St. Vincent, South Australia [genomes from Batley et al. (2021)]). Interestingly, the population size patterns were similar

between the comparisons, but the N_e estimates when using 30 individuals were slightly larger, suggesting a more pronounced expansion (Figure S3.5). Recent abundance estimates for this population based on line-transects are between 657 and 2,201 individuals (Bilgmann et al. 2019), indicating that the N_e estimates are likely overstated here. Furthermore, this algorithm produced estimates up until the present day for all inshore lineages, but not for the nearshore or offshore lineages. Although SMC++ can infer more robust histories for more recent time scales, it is still limiting in the very recent past, and the timing of patterns may be somewhat inaccurate. The recent coalescent estimates for inshore dolphins may be a result of the small population sizes and low genetic diversity. It is therefore important to take caution in interpreting these recent estimates, particularly in relation to the expansions of the inshore lineages. The population decline in *T. t. gephyreus* may be an artefact of the extremely low genetic diversity or may be a true representation of the recent population trend. Given this uncertainty, and the multiple limitations involved in estimating demographic histories, it is important that future work aims to elucidate these patterns using other algorithms and higher coverage genomes of individuals across the subspecies range. For example, PSMC using a single high coverage genome will add confidence to older estimates, while other algorithms, such as IBDNe may prove more accurate for estimating population sizes from around four to 200 generations, even when populations are small (Browning and Browning 2015).

3.7 Conclusion

This whole genome study clarified the genomic consequence of repeated niche divergence between closely related dolphin lineages. A strong association between genomic diversity, ROH and ecotype was detected, with lineages of the inshore ecotype being significantly less genetically diverse than those of the offshore counterpart and the nearshore *D. delphis*. Of great concern is the remarkably low genomic diversity for the inshore lineage *T. t. gephyreus*, which was 7.5-fold lower than its offshore counterpart, and as low as some of the most vulnerable and endangered mammal species worldwide. Patterns of historical demography highlighted parallel evolutionary histories within ecotypes, with the inshore *T. aduncus* lineages generally experiencing population bottlenecks during the LGM, while the inshore *T. truncatus* lineages and the offshore ecotype experienced population expansions during the same period. Recent estimates of population size in all inshore lineages highlighted that a bottleneck may be the cause of the extremely low diversity observed in *T. t. gephyreus*. These estimates may be an artefact of small sample sizes and it is therefore advised that very recent estimates be taken with caution. Overall, small population sizes, low genetic diversity and high site fidelity of inshore dolphins render them particularly vulnerable to environmental change and anthropogenic stressors. Ongoing population size and genetic monitoring will aid the efforts to conserve the more vulnerable inshore populations, particularly during a time of environmental change where species responses are likely to

vary. This study also marks the first to incorporate genomes from both inshore and offshore bottlenose dolphins from the Southern and Northern Hemispheres. Importantly, it uncovered evidence of parallel evolution across the genomes of bottlenose dolphins and emphasizes niche divergence and natural selection as drivers of local adaptation in bottlenose dolphins around the world.

Chapter 4: Whole genomes reveal multiple candidate genes and pathways involved in the immune response of dolphins to a highly infectious virus

This chapter is published in the special issue on the "Use of Whole Genome Sequences in Molecular Ecology" in *Molecular Ecology* (**Batley, K.C.,** Sandoval-Castillo, J., Kemper, C.M., Zanardo, N., Tomo, I., Beheregaray, L.B., Möller, L.M. (2021). "Whole genomes reveal multiple candidate genes and pathways involved in the immune response of dolphins to a highly infectious virus." *Mol Ecol*. doi: 10.1111/mec.15873). This chapter has been reproduced with permission.

4.1 Contributions

Kimberley Batley – conception of study design, data analysis and interpretation, writing of manuscript.

Luciana Moller – primary supervisor – conception of study and guidance in design and interpretation, collection of samples southern Australia, and drafting of manuscript.

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

Jonathan Sandoval-Castillo – guidance and assistance in bioinformatics and data analysis.

Catherine Kemper – Collection of stranded dolphins, and conduction of post-mortem examinations.

Ikuko Tomo – Collection of stranded dolphins, and conduction of post-mortem examinations.

Nikki Zanardo – Collection of biopsy samples from live, free-ranging dolphins.

4.2 Abstract

Wildlife species are challenged by various infectious diseases that act as important demographic drivers of populations and have become a great conservation concern particularly under growing environmental changes. The new era of whole genome sequencing provides new opportunities and avenues to explore the role of genetic variants in the plasticity of immune responses, particularly in non- model systems. Cetacean morbillivirus (CeMV) has emerged as a major viral threat to cetacean populations worldwide, contributing to the death of thousands of individuals of multiple dolphin and whale species. To understand the genomic basis of immune responses to CeMV, we generated and analysed whole genomes of 53 Indo- Pacific bottlenose dolphins (*Tursiops aduncus*) exposed to Australia's largest known CeMV- related mortality event that killed at least 50 dolphins from three different species. The genomic data set consisted of 10,168,981 SNPs anchored onto 23 chromosome- length scaffolds and 77 short scaffolds. Whole genome analysis indicated that levels of inbreeding in the dolphin population did not influence the outcome of an individual. Allele frequency estimates between survivors and nonsurvivors of the outbreak revealed 15,769 candidate SNPs, of which 689 were annotated to 295 protein coding genes. These included 50 genes with functions related to innate and adaptive immune responses, and cytokine signalling pathways and genes thought to be involved in immune responses to other morbilliviruses. Our study characterised genomic regions and pathways that may contribute to CeMV immune responses in dolphins. This represents a stride towards clarifying the complex interactions of the cetacean immune system and emphasises the value of whole genome data sets in understanding genetic elements that are essential for species conservation, including disease susceptibility and adaptation.

4.3 Introduction

Climatic variations, natural and anthropogenic alterations to ecosystems, changes in host behaviour, and the movement of pathogens and vectors have all contributed to the emergence of infectious diseases (EIDs) in wildlife populations (Williams et al. 2002, Morens et al. 2004, Cunningham et al. 2017, Titcomb et al. 2019). Infectious diseases have become a major conservation concern due to pathogens' abilities to rapidly evolve, their short generation times and often complex transmission dynamics, as well as being able to cause swift and widespread mortality, diminish genetic diversity and contribute to population declines and extinctions (Altizer et al. 2003, Blanchong et al. 2016, Stejskalova et al. 2017). Disease outbreaks are beginning to become a cause for concern in cetacean populations worldwide, especially for species that exhibit high social connectivity and gregarious behaviour, and for populations that are immunologically naïve, small, threatened, or immune-supressed (Gulland and Hall 2007, Van Bressem et al. 2009a, Weiss et al. 2020). In recent years, the reporting of EIDs and strandings in cetaceans has increased, with one highly contagious and virulent pathogen emerging as a major threat to their populations; cetacean morbillivirus (CeMV) (Sacristán et al. 2015). CeMV belongs to the genus *Morbillivirus,* which affects both terrestrial mammals [humans (measles virus), canines (canine distemper virus), cattle (rinderpest virus), goats and sheep (peste des petits ruminants virus), and two novel morbilliviruses in cats and bats] and marine mammals [true seals (phocine distemper virus) and cetaceans] (Alfonso et al. 2016, Ohishi et al. 2019). These viral species are distinct, but share a common phylogenetic origin, similar genome structure, symptoms of infection and pathomorphology (da Fontoura Budaszewski and von Messling 2016, Diaz-Delgado et al. 2019). This, along with observed cross-species transmissions (Stejskalova et al. 2017, Jo et al. 2018, Padalino et al. 2019), suggests that knowledge gained on immune responses for one viral species may be applicable more generally to morbilliviruses.

Since its discovery in the late 1980's, CeMV has become of great conservation concern given the increased reporting of unusual mortality events (i.e. unexpected and significant die-offs of a marine mammal population that warrants a rapid response by managers (MMPA 1972, Kemper et al. 2016)) in a larger number of known host species and populations (Di Guardo et al. 2005, Van Bressem et al. 2009a).The virus has been implicated in the death of tens of thousands of cetaceans worldwide (Van Bressem et al. 2014), but until recently had only been recognised as a contributing factor in the death of a small number of bottlenose dolphins (*Tursiops* spp.) across Australia (Stone et al. 2011, Stone et al. 2012, Stephens et al. 2014). However, in 2013 CeMV was identified in dolphins that died during an unusual mortality event involving at least 50 individuals of three species (Indo-Pacific bottlenose dolphin, *Tursiops aduncus*; common bottlenose dolphin, *T. truncatus;* and common dolphin, *Delphinus delphis*) in South Australia, becoming the largest confirmed CeMV outbreak in Australia and the first recorded deaths from CeMV in this state (Kemper et al. 2016). This unusual mortality event lasted

approximately seven months (March to September), and the initial months of the outbreak coincided with climatic anomalies that resulted in abnormally high sea surface temperatures (Kemper et al. 2016). Indo-Pacific bottlenose dolphins were the most affected, with 31 testing genetically positive for the virus, and majority of these were neonates, calves, and juveniles. Twenty-nine were from Gulf St Vincent (GSV), a population that is relatively small (700-1,200 dolphins, Bilgmann et al. 2019), exhibits high social connectivity (Zanardo et al. 2016b), shows relatively low genetic diversity (Pratt et al. 2018), and is considerably vulnerable to epizootic events (Reed et al. 2020). These characteristics, coupled with the CeMV-related mortality event, provides a unique opportunity to understand the importance of host genetic factors affecting disease susceptibility in cetacean species.

Host genetic factors are known to be key drivers in the plasticity of immune responses in natural populations, being major determinants of an individual's susceptibility ("the state of being very likely to be influenced, harmed or affected by something" (Susceptibility 2021)) and resistance ("the ability not to be affected by something, especially adversely" (Resistance 2021)) to infection (Karlsson et al. 2014, Stejskalova et al. 2017). For example, inbreeding can reduce fitness through homozygosity in deleterious recessive alleles, lack of genetic diversity can reduce adaptive potential, and homozygosity in immune-related genes may hinder pathogen recognition (Smith et al. 2009, Blanchong et al. 2016). Yet studies investigating the role of host genetic factors in disease susceptibility and resistance in wildlife populations are relatively limited. Association-based studies provide a favourable framework for identifying associations between genomic locations, regions or genes, and complex traits in natural populations. Studies addressing the role of host genetics in combating infection have generally targeted a small number of genomic regions of known functional importance, and genes with strong effect. For example, the Major Histocompatibility Complex (MHC) are among some of the most targeted and well-studied immune associated genes in model and non-model species, including cetaceans (Martin and Carrington 2005, Acevedo-Whitehouse and Cunningham 2006, Cammen et al. 2015b, Elbers et al. 2018, Pagan et al. 2018, Manlik et al. 2019). For example, by comparing two populations of Indo-Pacific bottlenose dolphins with varying levels of reproductive output and population viability, Manlik et al. (2019) found that the population with low reproductive output had lower levels of MHC diversity and therefore was possibly at greater risk of succumbing to human induced pressures. In the case of immune responses to the measles virus, specific alleles within the human leukocyte antigen (HLA) genes class I and II (*B; DQA, DQB, DRB*) have been associated with varying antibody titers following vaccination against the virus (Haralambieva et al. 2015). However, many other non-MHC genes have been proposed to be involved in host immune responses to morbilliviruses. For example, the signalling lymphocyte activation molecule (SLAM) has been identified as an immune cell receptor for measles, canine distemper, rinderpest and peste des petitis ruminants viruses, and is suggested to be a universal receptor for entry and propagation of morbillivirus in all mammals, including cetaceans (Sato et al. 2012, Shimizu et al. 2013, Melia et al. 2014, Ohishi et al. 2019). Other genes, including viral binding

genes, cytokine receptor genes, pathogen-associated sensing genes and antiviral genes were also suggested to be involved in immune responses to morbilliviruses (Hashiguchi et al. 2011, McCarthy et al. 2011, Haralambieva et al. 2015, Stejskalova et al. 2017).

Advancements in next generation sequencing, computational power and improved availability of genomic data has enabled the move from a targeted to a non-targeted approach of association-based studies. This approach enables the search for multiple genetic variants across the genome under selection and associated with a trait, without the need of prior knowledge. This framework is frequently utilised in humans, model organisms and agricultural systems (Elbers et al. 2018), and while still limited, advancing technologies have now enabled association studies in wildlife populations. In particular, whole genome data has been utilised to investigate immune responses of endangered and vulnerable Australian marsupials (Tasmanian devil, *Sarcophilus harrisii;* and the koala, *Phascolarctos cinereus)* to two highly damaging diseases that continue to threaten populations across their distribution (Wright et al. 2017, Johnson et al. 2018). The move to non-targeted approaches and large genomic datasets improves our ability to address the genetic basis of adaptation in wildlife populations and allows us to understand the role that genetic variants play in the plasticity of immune responses, and in the susceptibility of individuals and populations to infectious diseases.

In this study, we expand substantially on previous work based on reduced representation sequencing (RRS) (Batley et al. 2019) to investigate the genomic basis of resistance and susceptibility of Indo-Pacific bottlenose dolphins to CeMV using whole genomes. Using a much larger dataset, we searched for regions of the genome under selection between *case* (non-survivors) and *control* (survivors) from the viral outbreak to identify genetic variants, genes and pathways associated with resistance and susceptibility to CeMV. Our study provides the first whole genome-based information to enable the screening of other cetaceans for potential genetic risk factors, ultimately enabling the identification of populations and species particularly vulnerable to large-scale CeMV outbreaks.

4.4 Materials and methods

4.4.1 Study Species, Sites and Sample Collection

Indo-Pacific bottlenose dolphins that died and stranded during an unusual mortality event throughout South Australia between March and September 2013, were collected by the South Australian Museum for post-mortem examinations. Histopathological examinations, Reverse-Transcription Polymerase Chain Reaction (RT-PCR) and/or immunohistochemical assays confirmed that CeMV infection and related pathologies were the main contributing factor in the dolphin deaths (Table S4.1 and see Kemper

et al. 2016). Muscle tissue from 29 Indo-Pacific bottlenose dolphins from GSV and adjacent waters, and one from Spencer Gulf that died during the unusual mortality event, tested positive for CeMV and generally exhibited CeMV related pathologies (e.g., pneumonia with syncytial cells, lymphoid depletion with systemic secondary infection by bacteria, fungi, or parasites) were provided by the South Australian Museum and formed the *case* group. *Case* samples were classified into age classes, with the majority of the strandings being young dolphins (neonates, calves, and juveniles: < 1.6 m in length) (young n = 28, adults n = 2). These samples were frozen at -80˚C and kept at the South Australian Museum before being transferred to 90% ethanol and to the Molecular Ecology Laboratory at Flinders University (MELFU).

Samples were complemented with biopsy samples from free-ranging Indo-Pacific bottlenose dolphins from GSV and adjacent waters collected between 2014 and 2015 (Bilgmann et al. 2007a, Zanardo et al. 2016b, Pratt et al. 2018), which putatively survived the outbreak (i.e., *control* samples). These skin and blubber samples were collected using either the PAXARMS biopsy system (Krützen et al. 2002) or a hand-held biopsy pole (Bilgmann et al. 2007a). Age classes (calves, juveniles, and adults) of sampled individuals were estimated *in situ* based on body size and association with an adult dolphin (see Zanardo et al. 2016b for details). This resulted in a total of 34 *control* samples, with samples from young (n = 11), complemented with random adult samples from GSV and close adjacency (n = 23). These *control* samples are considered putative survivors since they belong to the same genetic and socially cohesive population (Zanardo et al. 2016b, Pratt et al. 2018) as the one most impacted during the unusual mortality event and were collected within 18 months of the outbreak. Biopsy samples were preserved in a salt-saturated solution of 20% dimethyl sulphoxide (DMSO) and stored at -80˚C at the MELFU. Dolphins were genetically sexed using a polymerase chain reaction (PCR) (Banks et al. 1995). The phenotypic data for samples that passed DNA quality controls and were subsequently selected for whole genome sequencing is available in Table S4.1.

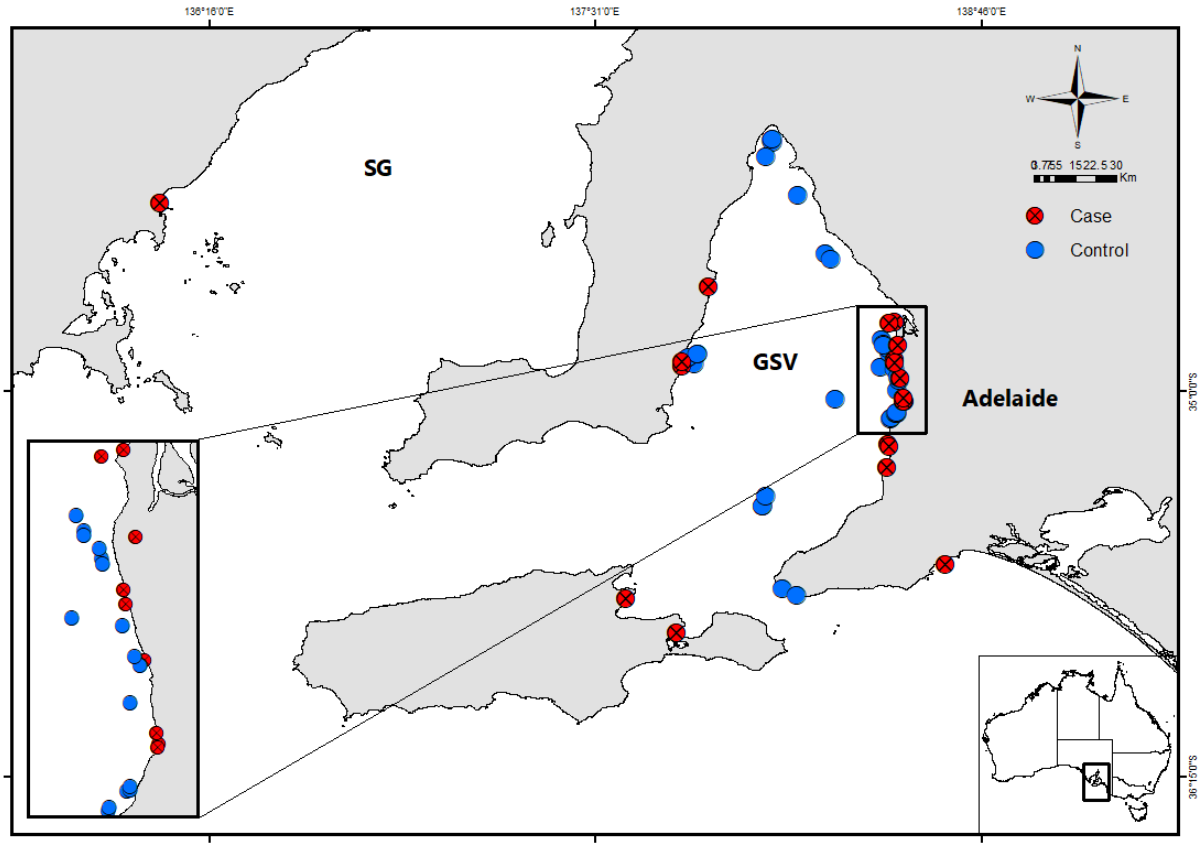

Figure 4.1: Sampling locations of Indo-Pacific bottlenose dolphins, *Tursiops aduncus*. *Case* (n= 19) are non-survivors and *control* (n= 34) are survivors from the 2013, Gulf St. Vincent outbreak used in the whole-genome association study of resistance and susceptibility to CeMV.

4.4.2 DNA Extractions and whole genome sequencing

Genomic DNA was isolated from *control* samples following the salting out method (Sunnucks and Hales 1996), while genomic DNA was extracted from *case* samples using the Qiagen DNeasy blood and tissue kit following the manufacturer's protocol. The purity of extractions was verified using a ND-1000 spectrophotometer (Nanodrop, Thermo Scientific), and quantity assessed using a fluorometer (Qubit, Life Technologies). The DNA integrity was further assessed by gel electrophoresis (2% agarose gels, produced in-house). All extractions were expected to pass quality controls based on standards set by the Australian Genome Research Facility (AGRF), where libraries were prepared and sequenced. Specifically, samples were required to have a quantity ≥ 20 ng/µl, a high molecular weight (≥ 20 kb), free of RNA (assessed on agarose gels) and an A260/280 (protein contamination) ratio between 1.8-2.0. Extractions that did not pass quality controls were re-extracted a maximum of three times, using the same method but with altering amounts of tissue to potentially increase the concentration and improve the quality of DNA. As expected, extractions that failed the quality controls were typically of *case* samples, since these were obtained from carcasses rather than free-ranging dolphins. *Case* samples

with a low concentration for all extractions were then combined, and concentrated using a centrifuge vacuum concentrator (Hetovac, Heto Lab). Extractions from 53 samples that passed all quality controls (*case*, n = 19 and *control*, n = 34) were subsequently selected for library preparation and whole genome sequencing at AGRF (Table S4.1). Libraries were prepared using the NEBNext Ultra II DNA library prep kit and sequenced on two lanes of the Illumina NovaSeq 6000 S2 platform (150 bp PE). Samples were sequenced at ~7x coverage, excluding one sample from GSV that was sequenced at a higher depth of coverage (~28x) to form a reference genome (Batley et al. unpublished data). While throughout this paper we refer to the species as the Indo-Pacific bottlenose dolphin (*T. aduncus*), it has been previously suggested to represent a separate species, endemic to southern Australian waters, *T. australis* (Charlton-Robb et al. 2011b). However, recent studies suggest this is more likely to be a subspecies of *T. aduncus* (Moura et al. 2020), and therefore we refer to the reference genome here as the southern Australian bottlenose dolphin (SABD). Details regarding the construction, quality and statistics of this reference genome will be available in Batley et al. (in prep).

4.4.3 Read processing, SNP calling and filtering

Raw sequencing data was pre-processed following the pipeline adapted from GATK best practices (Van der Auwera et al. 2013), with modifications. Firstly, reads were trimmed if read quality was below 23 in a sliding window of five nucleotides, while adapters were removed using Trimmomatic v0.38 (Bolger et al. 2014). The remaining reads were mapped to the chromosome-length scaffolded *S*ABD reference genome using Bowtie2 v2.2.7 (Langmead and Salzberg 2012). The resulting SAM files were then converted to BAM files, duplicate marked and sorted using Picard (Picard Toolkit 2019). Indels were then locally realigned to correct mapping errors using GATK before merging the replicate reads from different libraries with SAMTOOLS (Li et al. 2009).

SNPs were called from the mapped reads of all individuals using the SABD reference genome in a two-part process using BCFTOOLS (Li 2011). This involved generating genotype probabilities at each genomic position before calling the SNPs. SNPs were then filtered with VCFTOOLS (Danecek et al. 2011) and using parameters described in Brauer et al. (2016). In short, reads with a minor allele frequency <3% and genotyped in <80% of the samples were excluded. Indels were removed and only SNPs with a quality and depth ratio of 2%, mapping quality >30 and mean depth <12 were retained. Finally, Hardy-Weinberg equilibrium was calculated within the two groups (*cases* and *controls*), and SNPs that were out of HWE in each group were excluded. SNPs were called altogether, including those available from whole genomes of common dolphins and common bottlenose dolphins (data not presented here), but as this study focused on Indo-Pacific bottlenose dolphins from southern Australia, only SNPs that are unique to this lineage were retained (see Table S4.2 for SNPs retained at each step).

4.4.4 Whole genome association study

4.4.4a Potential effects of inbreeding, relatedness, sex, and age-classes.

As inbreeding can reduce disease resistance due to the loss of genetic diversity (Acevedo-Whitehouse et al. 2003), levels of inbreeding were calculated to test for potential effects of inbreeding. The inbreeding coefficient, F, was calculated within and between *cases* and *controls* using the het command in Plink v1.9, based on an unlinked SNP dataset (189,178 SNPs). The mean F of each group was compared using an independent samples t-test.

Levels of relatedness within and between *cases* and *controls* as well as differences in the representation of sexes and age classes between groups were also calculated to assess the potential influence of these factors on the outcome of an individual. Pairwise relatedness between individuals based on the unadjusted Ajk statistic method of Yang et al. (2010) was estimated using VCFTOOLS. Pairwise relatedness within and between groups, as well as the mean number of individuals of each sex and age class between groups were then compared using an independent sample t-test.

4.4.4b Identifying SNPs under selection

Allele frequency differences between the two groups were calculated to identify SNPs potentially involved in resistance or susceptibility. This analysis used 7,720,686 SNPs and was based on two association tests implemented in Plink v1.9; the chi-square test and Fisher's exact test. SNPs with a highly significant *P*-value ($p \leq 0.001$) were selected as outlier SNPs, as per Batley et al. (2019). These two tests were complemented by the Weir and Cockerham's F_{ST} (Weir and Cockerham 1984), which estimates differentiation between groups based on allele frequency shifts. F_{ST} was calculated between *cases* and *controls* using the --weir-fst-pop command in VCFTOOLS. SNPs with an F_{ST} value greater than five standard deviations from the mean (0.0024 ± 5SD) were selected as outlier SNPs (Axelsson et al. 2013, Kardos et al. 2015). Outlier SNPs from each of the three tests were compared, and those identified as outliers in at least two tests were selected as candidate SNPs to reduce false positives. The two tests implemented in Plink also output Odds Ratios (OR), which were used to test the odds of the minor allele being in association with an outcome (i.e. non-survival).

4.4.4c Annotation of candidate SNPs

To annotate and explore the function of candidate SNPs, 600 bp flanking regions of the candidate SNPs were aligned to *T. truncatus* proteins (GCF_001922835.1) using blastx v2.2.28. This used an alignment length of above 30 amino acids, similarity above 50%, and an e-value threshold of 8e-07. For all alignments to the proteins, the genomic region of the SNP (intronic or exonic) and their predicted

functional effect (missense vs synonymous changes) were investigated using SnpEff (Cingolani et al. 2012). Specifically, a VCF file of all candidate SNPs with flanking regions that aligned to the protein database was generated and the SNPs were annotated against the SABD reference genome. The SABD reference annotation was used here as SNPs were initially mapped to the SABD reference genome and SnpEff required knowledge on SNP location; however, the annotation was not available for the initial protein annotation. Functions of the putative candidate genes were explored using Gene Ontology (GO) terms provided by UniProtKB (UniProt 2019), and their involvement in immune pathways and gene interactions were explored with human Ensembl identifiers and Reactome (Fabregat et al. 2018).

4.4.4d Candidate immune gene approach

Several genes potentially involved in immune responses to morbilliviruses have been proposed (Hashiguchi et al. 2011, McCarthy et al. 2011, Haralambieva et al. 2015, Stejskalova et al. 2017), but some of these genes were not identified as candidate genes in our dataset. To investigate whether SNPs within these genes are in fact neutral between *cases* and *controls*, or alternatively, under selection but did not align to the protein coding regions, the allele frequency and genotype counts for each SNP within each gene were compared between *cases* and *controls*. To achieve this, each gene location was extracted from the SABD reference genome and SNPs within the specified regions were extracted using VCFTOOLS. As the SABD annotation is in a draft format, genes that were not found in the SABD annotation were downloaded from NCBI (*T. truncatus*; GCF_001922835.1) and mapped to the SABD reference genome using blast v2.2.28. Allele frequency differences were calculated using a chi-square test, and genotypes of the top performing SNP within each gene (i.e. SNP with the greatest differentiation between *cases* and *controls*) were counted using Plink.

4.5 Results

Whole genome sequencing produced a total of 4,274,472,237 reads for 53 Indo-Pacific bottlenose dolphins from South Australia (Figure 4.1). After quality filtering, 3,310,493,013 reads (mean = 31,231,066 ± 13,586,174) remained, of which an average of 96.81% of reads mapped to the SABD reference genome. Calling SNPs from the genome resulted in a total of 33,386,256 SNPs, of which 17,226,558 remained after quality filtering (Table S4.2). Of these SNPs, 10,168,981 (on 23 chromosome-length scaffolds and 77 smaller scaffolds, Figure S4.1) were unique to *T. aduncus.* The final dataset available for analysis therefore, consisted of 10,168,981 SNPs for 53 individuals with an average of 1.02% missing data (SD ± 1.32%). Missing data did not differ significantly between *cases* and *controls* (*cases* = 0.95% ± 0.61%; controls = 1.06% ± 1.6%, $P = 0.389$).

4.5.1 Potential effects of inbreeding, relatedness, sex, and age-classes

There was no significant difference in the mean inbreeding coefficient between the two groups (*cases* = 0.0574 ± 0.049; *controls* = 0.0289 ± 0.061, P = 0.087), suggesting that genome-wide levels of inbreeding did not influence susceptibility of *case* dolphins to CeMV during the outbreak.

The mean relatedness of pairs of individuals within and between groups was not significantly different (*cases* = -0.0185 ± 0.056; *controls* = -0.0184 ± 0.051; *case-control* = -0.0226 0.036; all $P > 0.05$). Likewise, there was no significant difference between the sex composition between groups (*cases*: M = 10, F = 9; controls: M = 21, F = 9; P = 0.527). There was, however, a significant difference between the representation of different age classes in the two groups, but due to the limited number of adult *case* samples (n = 2), the influence of age could not be accounted for in the analysis.

4.5.2 Identifying SNPs under selection and annotation of candidate SNPs

Methods to detect SNPs under selection between *case* and *control* individuals identified outlier SNPs in all three tests, with 13,000 outlier SNPs detected using the Fisher's exact test, 17,398 SNPs for the chi-square test and 36,726 SNPs for the F_{ST} test of differentiation. Of these outliers, 5,105 SNPs were present in two tests, and a further 10,664 SNPs were present in all three tests. A total of 15,769 SNPs (< 0.16% of all SNPs) found on 22 chromosome-length scaffolds showed putative signatures of selection between *case* and *control* individuals and were considered candidate SNPs.

Of the 15,769 candidate SNPs and associated flanking regions, 689 aligned and annotated to the common bottlenose dolphin (*T. truncatus*) protein dataset and/or the SABD annotation. These SNPs annotated to 295 protein coding genes and six uncharacterised proteins (Table S4.3). Investigation of all candidate genes and their involvement in different pathways found that 131 candidate genes were related to 856 different biological sub-pathways that can be grouped into 25 pathways (Table S4.4). The key pathway of interest is the immune system (37 genes) (Figure 4.2, Table S4.4), however other pathways of interest include disease (26 genes), signal transduction (32 genes), and cell-cell communication (4 genes) (Table S4.4). The remaining 164 genes either did not have Ensembl identifiers or could not be characterised into pathways. However, inspection of GO terms suggests that a further 13 genes could be involved in immune system pathways (Figure 4.2, Table S4.5).

Within the immune system pathways, genes were characterised into the innate, adaptive and cytokine signalling pathways. Nine genes (*PDIA3, FBXW10, FBXL7, UBA5, SEC31A, AREL1, LMO7, IKBKB* and *ASB11*) grouped into the MHC class I pathway of the adaptive immune system, which is important for recognising and fighting intracellular pathogens. Fc receptor proteins (FcRs) were also well characterised with ten genes (*DOCK1, MHY9, ACTR3, GRB2, NFATC2, CARD11, RASGRP2, IKBKB,*

MAPK8 and *CALM1*), while several F-box proteins were also identified (*LMO7*, *FBXL7*, *FBX10* and *FBXW11*). The *FBXW11* was identified through the candidate gene approach, as it had been previously proposed to be involved in CeMV susceptibility and resistance (Batley et al. 2019). Likewise, *MAPK8* was also identified as a candidate gene in this study (as well as in Batley et al. 2019), while the MAPK cascade was well characterised with four candidate genes (*FGF2*, *GRB2*, *BTC* and *CALM1*) and a further 9 genes with GO terms relating to the MAPK cascades (*PDE6H*, *NTRK2*, *KIDINS220*, *INHBA*, *NPSH1*, *PLCE1*, *HRH4*, *TGFB3*, *IKBKB*). Finally, pathways and GO terms highlighted the importance of the regulation and expression of interleukins and T cells (see Table S4.4 for all pathways and sub-pathways).

Further inspection of the annotated SNPs, and the gene regions they fall within, revealed that majority of the SNPs fell within introns (n = 485). In total, 59 SNPs were found in exonic regions, in which 29 caused a missense mutation and 30 SNPs resulted in synonymous substitutions (Table S4.6). Of the SNPs that annotated to immune genes, twelve SNPs were found within exons, however just six of these within three genes (*CD300LF*, *NFATC2* and *NFKBIZ*) caused a missense change, while six SNPs within four genes resulted in no amino acid change (*DOCK1*, *FBXW10*, *MASP1*, *MHY9*, *HRH4*, *KCTD5*) (Figure 4.2). The odds-ratio (OR) suggest that for the SNPs that caused a missense change, the minor allele increased the odds of succumbing to CeMV (Figure 4.2). Other genes of interest that were annotated include *IL4α*, which had an OR of 16.25 (Figure 4.2) and *PATJ*, both of which have previously been suggested to potentially be involved in immune responses to morbilliviruses (McCarthy et al. 2011, Haralambieva et al. 2015, Batley et al. 2019).

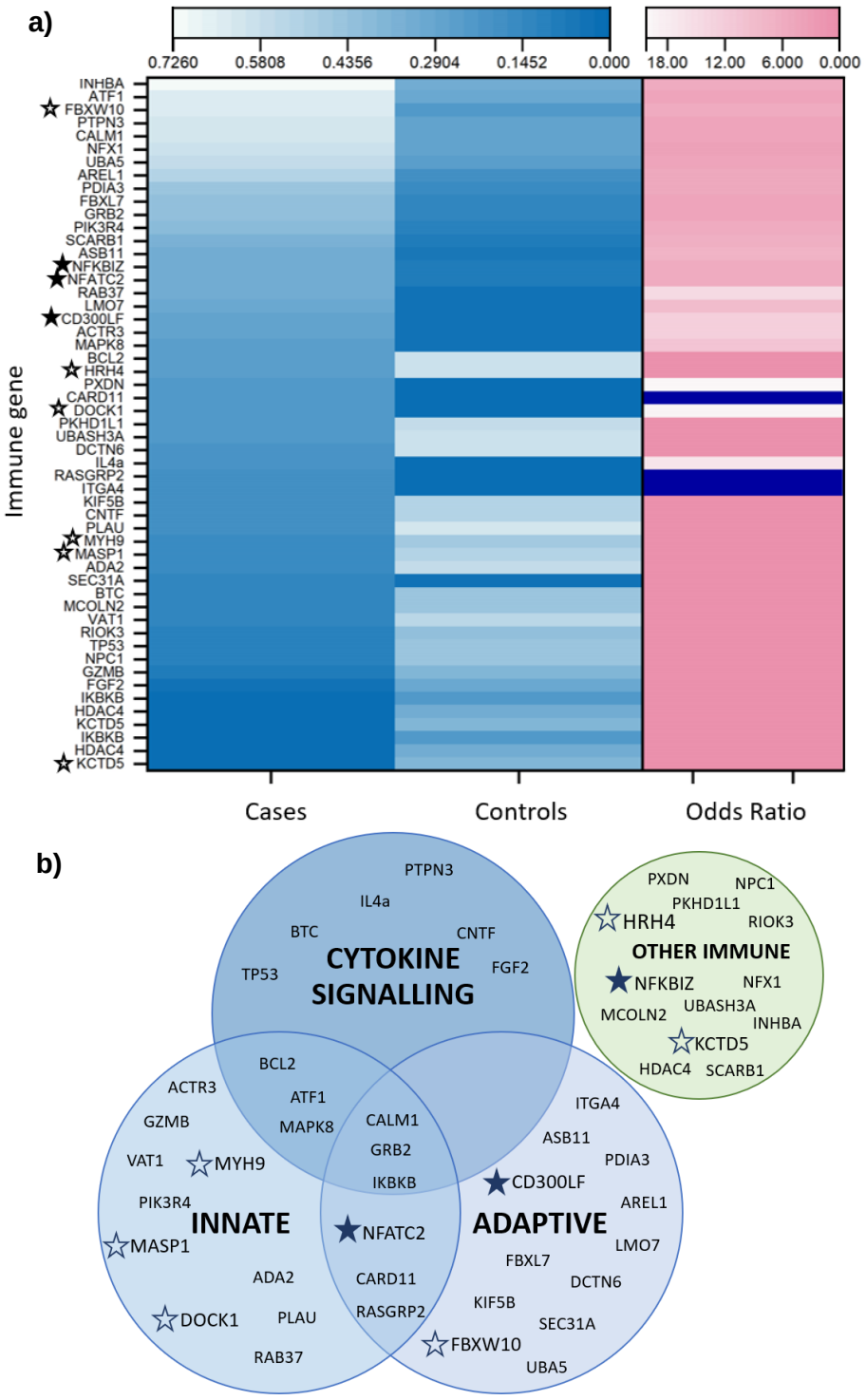

Figure 4.2: 36 immune-related genes putatively associated with cetacean morbillivirus resistance and susceptibility; **a)** allele frequency differences between *case* and *control* individuals and their corresponding odds ratio. Blue odds ratios represent non-applicable odds ratios (as allele frequency in *controls* = 0); **b)** Immune sub-pathways of the candidate immune-related genes. Other immune refer to the genes that had GO terms relating to immune functions. Stars represent the seven genes that include exonic SNPs (filled = missense change; non-filled = synonymous change).

4.5.3. Candidate immune genes

At least 29 genes have been suggested to potentially play a role in immune responses to morbilliviruses in general (Hashiguchi et al. 2011, McCarthy et al. 2011, Haralambieva et al. 2015, Stejskalova et al. 2017), but only three of these (*PATJ*, *MAPK8* and *IL4α*) were found to be under putative selection between *cases* and *controls* in this study. For the remaining 26 genes, 22 aligned to the SABD reference genome. Within the aligned genes, 7,041 SNPs were extracted, of which 26 SNPs on four genes (*RARb*, *FBXW11*, *ANK3* and *ACOX3*) showed significant allele frequency differences ($P < 0.001$) between *cases* and *controls*. These SNPs were identified as outliers in the tests for selection but did not align to the common bottlenose dolphin proteins, and are therefore considered to be located within intronic, promoter or enhancer regions of the genes. The majority of the 22 genes were highly polymorphic (Table S4.7), however inspection of genotype counts for the top performing SNP within each gene (i.e. SNP with greatest allele frequency differences between *cases* and *controls*) highlighted a lack of heterozygosity within 11 of the immune genes across all samples (Figure 4.3, Table S4.7). For these 11 SNPs, at least 84% of all samples were homozygotes. For *TLR8* and *TLR3*, all *case* samples were homozygotes, while only four control samples were heterozygotes. The genes *DQα*, *BSG* and *SLC11A1* also showed low levels of variation, with only four samples being heterozygotes.

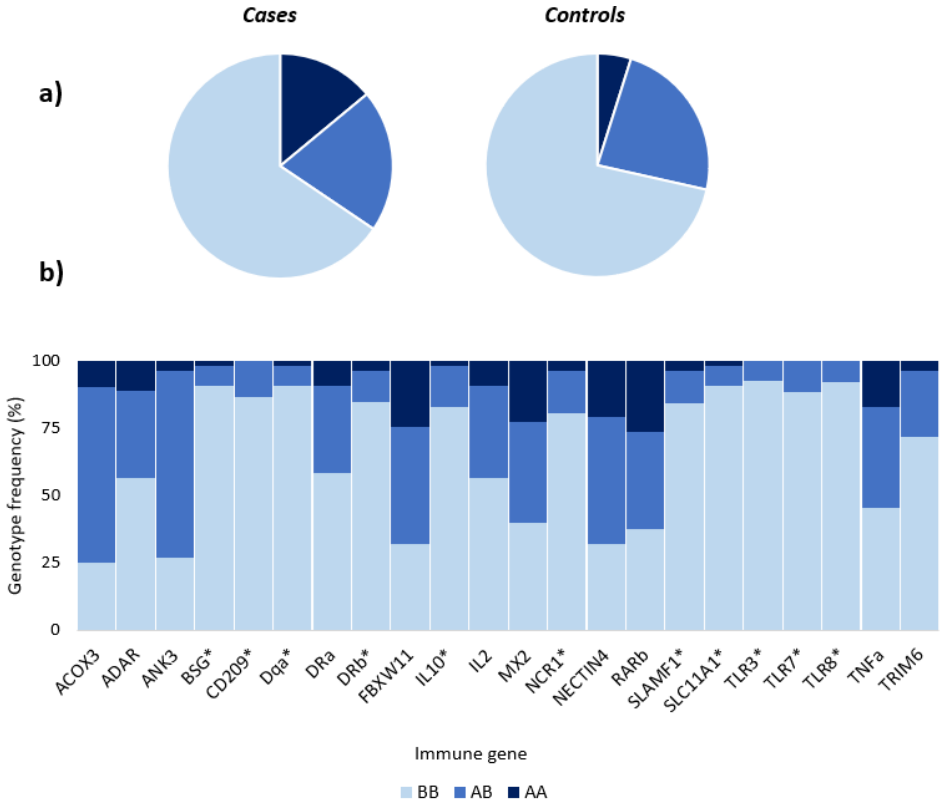

Figure 4.3: Genotype distribution for the most differentiated SNP (x^2) between *case* and *control* bottlenose dolphins for 23 genes previously suggested to be involved in morbillivirus immune responses; a) average genotype frequency for all 23 immune genes in *cases* and *controls*; b) genotype frequencies for each immune gene across all samples (*cases* and *controls*). * denotes the genes with exceptionally high homozygosity (> 84% of individuals).

4.6. Discussion

Host genetic factors play an important role in mobilising immune responses to invading pathogens, and may influence the outcome of an individual; yet relatively few studies have assessed the importance of these factors and immunogenetic diversity in wildlife disease risk (Smith et al. 2009, Bossart et al. 2019). Here, we used whole genome datasets to characterise genomic regions underlying resistance and susceptibility of dolphins to a highly contagious and fatal virus, CeMV. First, we provide additional support for previously proposed genes suggested to be associated with morbilliviruses and immune responses in general. This includes genes that were found to be under selection between *case* and *control* individuals from the same population using a reduced-representation genome sequencing approach (Batley et al. 2019). We further expand on this, by uncovering host genetic variants across the entire dolphin genome, and in genes and pathways associated with immune functioning, including in MHC class I pathways involved in recognising pathogens. We also identified a lack of immunogenetic diversity in the studied dolphin population within immune-related genes previously recognised as generally important in the fight against pathogens. CeMV is of growing concern given ongoing climate change threatening to lead to more stressful environments for populations and species, potentially leading to immune suppression, and altering host and virus distributions (Burge et al. 2014). Since its discovery, CeMV has been reported to be the causative agent of several unusual mortality events across multiple cetacean species and populations (Van Bressem et al. 1999, Di Guardo et al. 2005, Van Bressem et al. 2014). Through the identification of genes potentially involved in CeMV immune responses, our work clarifies how host genetic factors drive CeMV outcomes and provides knowledge about the diversity of immune responses, their interactions, and pathways in dolphins. More broadly, this work provides an example of how advancing technologies can enable greater insights into the role of host genetic factors in the variation of a trait across the entire genome, while also providing support for RRS approaches in conservation genomics.

4.6.1 Comparison between RRS and WGS for identifying genes associated with disease resistance

As genomic technologies and capabilities continue to advance, conservation genomic techniques including reduced-representation and whole genome sequencing to assess variation of a trait will become more popular for wildlife populations. While the focus of this study is to understand host genetics variants in CeMV resistance and susceptibility, where possible it is important to compare and validate the potential use and constraints of RRS and whole genome sequencing approaches for addressing important conservation questions (Wright et al. 2020).

In this study, we increased the number of loci from 35,493 SNPs (Batley et al. 2019) to 7,720,686 SNPs across the genome to investigate associations between CeMV resistance and susceptibility and host genetic factors. In concordance with the RRS dataset, estimates of inbreeding were not elevated in *case*

samples compared to *controls* at the whole genome level, supporting previous suggestions that the outcome of an individual during this mortality event was not influenced by genome-wide inbreeding. Levels of inbreeding within wildlife populations have been associated with disease emergence, immunocompetence and increased disease susceptibility and severity (Valsecchi et al. 2004, Smith et al. 2009). While the GSV bottlenose dolphin population exhibits relatively low genetic diversity (Pratt et al. 2018), inbreeding estimates from both RRS and WGS did not suggest significant levels of inbreeding. This provides support for a lower density of SNPs (RRS) being sufficient for estimating inbreeding in this population.

In the case of the Tasmanian devil (*Sarcophilus harrisii*), which exhibits remarkably low genetic diversity, SNP density was too low in a RRS data set (> 9,000 SNPs) to conduct a robust association study to identify genes associated with breeding success (Wright et al. 2020). While the SNP density was far greater for the bottlenose dolphin RRS dataset of Batley et al. (2019), it was limited in its coverage across the genome, covering roughly 1% of its genome. This means that in addition to genes potentially not being represented in the RRS dataset, gene regions may have been missed (e.g. exons). Despite this limitation, variation within introns of five candidate genes (*PATJ* [*INADL*], *MAPK8*, *FBXW11*, *ANK3* and *ACOX3*) between *cases* and *controls* were identified as potentially important genes for CeMV susceptibility. While these results are informative and important for understanding disease susceptibility in this population, complex traits are often determined by many genes with small effect (Santure and Garant 2018), and the RRS approach to investigate a genotype-phenotype relationship may restrict the potential to identify variation in a large number of genes. The whole genome dataset included a much larger SNP dataset, and analysis identified significant differentiation between *cases* and *controls* in the same five genes as Batley et al. (2019), and in an additional 294 protein coding genes and uncharacterised proteins, with variation in at least 50 immune-related genes observed. Unlike the RRS approach, in this study variation was observed within exon regions of the genome and SNPs within three immune genes (*CD300LF*, *NFATC2* and *NFKBIZ*) caused missense mutations. RRS datasets remain extremely important in conservation genomics, particularly when it is not feasible to sequence whole genomes, or when reference genomes are not available. For example, RRS was informative in elucidating the negligible influence of inbreeding on CeMV susceptibility and provided a stride towards understanding the role of host genetic factors in CeMV immune responses. However, our comparison highlights the more comprehensive functional knowledge gained through a whole genome analysis.

4.6.2. Gene functions and immune system pathways

The immune system plays a key part in the outcome of an individual and we therefore focus on pathways and genes associated to immune functions, however, other pathways and gene functions were also

disclosed (see Table S3 and S4 for details). A wide range of well characterised immune related pathways were found to be putatively under selection, including a similar number of genes from both the innate and adaptive immune systems, as well as cytokine signalling pathways. These pathways are distinct, but interconnected (Gelain and Bonsembiante 2019), reflecting the highly complex interactions and networks of the mammalian immune system.

4.6.2a Innate immune system

The innate immune system is the first line of host defence and is rapid and non-specific in its response to pathogens, involving the interplay of the complement system, pattern recognition receptors (PRRs), cytokines and a diverse range of immune cells that detect and remove pathogens (Ohishi et al. 2011, Gui et al. 2013). A comparison between healthy and CeMV seropositive common bottlenose dolphins from estuaries in Florida and South Carolina revealed an upregulation of the innate immune system in seropositive dolphins, and in particular in lysozyme activity and monocytic phagocytosis (Bossart et al. 2019). In this study, the innate immune system was well characterised, with 19 genes found to be under selection between *cases* and *controls*. In particular, ten genes were grouped into Fc receptor proteins (FcRs) that have important functions in the activation and down-regulation of immune responses through their ability to bind to antibodies and stimulate cellular and humoral immune responses (Takai 2002, 2005). Genes within this pathway (*DOCK1*, *MHY9*, *ACTR3*, *GRB2*) may be important for recognising foreign pathogens and stimulating phagocytosis to engulf and eliminate infectious agents (Acevedo-Whitehouse and Cunningham 2006), while an additional three genes (*NFATC2*, *CARD11*, *CALM1*) may be important for the release of inflammatory mediators (Turner and Kinet 1999). In humans, measles virus proteins have been reported to interact with FcRs to generate immunosuppression through impairment of the cells' function, decreased production of interleukins, and the loss of antigen specific T cell proliferation (Marie et al. 2001). Of particular interest, the gene *NFATC2* is part of a family that appear to be key mediators of immune responses, specifically by regulating the transcription of cytokine genes (*TNF-a* and *IL-13*) (Turner et al. 1998, Klein et al. 2006, Fric et al. 2014). These cytokine genes have been associated with defence against the measles virus (Haralambieva et al. 2015), and also may have been important in fighting infection in this population. While these genes (*TNF-a* and interleukins) are known to be important for morbilliviruses' host defence, this finding highlights the need to look beyond cytokine receptors, at activators and initiators of these proteins, as they may play a role in an individuals' response and outcome.

Neutrophils are among some of the most common white blood cells that circulate in the human body, participating in the inflammatory responses by releasing cytotoxic proteins during degranulation (Lacy 2006, Naegelen et al. 2015). Neutrophils participate in inflammatory responses by releasing cytotoxic proteins during degranulation (Lacy 2006, Naegelen et al. 2015). The morbillivirus infected dolphins

in this study had a high prevalence (e.g. 18 out of 24 non-survivors examined) of lymphoid depletion in the spleen and lymph nodes (Kemper et al. 2016). This develops immunodeficiency resulting in secondary infections including from bacteria, protozoa, parasites and fungi (Di Guard and Mazzariol, 2016). The presence of neutrophils in morbillivirus infected dolphin is a sign of an acute inflammatory response against those pathogens (Duignan et al. 1992, Diaz-Delgado et al. 2017, Diaz-Delgado et al. 2019). Here, we found evidence of selection within five genes (*GZMB, PLAU, ADA2, RAB37* and *VAT1*) that are involved in neutrophil degranulation. These results suggest that variation within these genes may play an important role in the release of cytotoxic proteins during neutrophil degranulation and may be key contributors to the coordination of an inflammatory response against the secondary infectious pathogens that follow CeMV.

4.6.2b Adaptive immune system

The adaptive immune system, also known as the specific and non-rapid system, is mediated by B and T lymphocytes, and recognises pathogens by high affinity receptors (Werling and Jungi 2003). In regards to CeMV infection in cetaceans, Bossart et al. (2019) observed a reduced adaptive immune response in CeMV seropositive dolphins, with a reduction in T cell lymphocyte proliferation and in T helper cells. Here, we found significant differentiation between *cases* and *controls* in 15 genes that were characterised into the adaptive immune system. Of particular interest is the MHC class I pathway, which generally is involved in the fight against viruses. In this study nine candidate genes were characterised into this pathway (*PDIA3, FBXW10, FBXL7, UBA5, SEC31A, AREL1, LMO7, IKBKB* and *ASB11*). The MHC complex is of known immune importance, being involved in resistance and susceptibility to disease through antigen processing and presentation (Acevedo-Whitehouse and Cunningham 2006, de Sa et al. 2019). Genes here were characterised into antigen processing of the MHC class I pathway (*LMO7, AREL1, FBXL7, FBXL10, UBA5, IKBKB* and *ASB11*) whereby foreign proteins are degraded into short peptides for presentation to the MHC class I system (Strehl et al. 2005). The genes *PDIA3* and *SEC31A* are involved in antigen presentation, folding, and loading of MHC class I receptors that coordinates the movement of high-affinity peptides to MHC class I molecules (Santos et al. 2007, Scholz and Tampe 2009). In addition, three genes (*DCTN6*, *KIF5B* and *SEC31A*) were characterised into the antigen presentation of the MHC Class II pathway and may be important for presenting antigens to T-lymphocytes that initiate an immune response (Moreno-Santillan et al. 2016). Across vertebrates, the MHC complex is one of the most well studied immune-related regions. It has been implicated in responses to measles vaccination (Haralambieva et al. 2015), and suggested to be functionally important in CeMV infection (Stejskalova et al. 2017). Although we found no evidence of selection within key MHC genes (e.g. *DQA, DQB*), variation in several downstream genes suggests that the MHC Class I and II pathways may be involved in a dolphin's ability to fight CeMV infection.

Some cell receptors may also play a role in modifying the response of immune cells. We found significant variation between *cases* and *controls* within four genes (*ITGA4*, *HRH4*, *IKBKB* and *CD300LF*) that may be involved in the regulation of immune functions. The gene *CD300LF*, found to contain four SNPs that cause a missense mutation may positively regulate the IL4-mediated signalling pathway by acting as a coreceptor for IL-4 (Moshkovits et al. 2015); a cytokine signalling gene that has been previously suggested to be important in morbillivirus immune responses, and found to be putatively under selection in this study. *ITGA4* may also promote viral resistance by permitting T-lymphocytes to migrate to sites of inflammation.

A major immune response of humans to measles is controlled by T-lymphocytes that recognise measles antigens (Haralambieva et al. 2015). These T-lymphocytes also play a key role in immune responses of dolphins to CeMV, with seropositive dolphins showing a reduction in T cell proliferation in comparison to healthy dolphins (Bossart et al. 2011, Bossart et al. 2019, Diaz-Delgado et al. 2019). Throughout the 2016 outbreak in GSV, all stranded dolphins showed clinical signs of lymphoid depletion (Kemper et al. 2016), suggesting that T cell proliferation may have been reduced, hampering the ability of an individual to fight the infection. Seven genes (*CARD11*, *NFATC2*, *GRB2*, *NFKBIZ*, *HDAC4*, *UBASH3A* and *IKBKB*) were found to be putatively under selection and may be involved in other adaptive pathways that relate to the signalling and differentiation of T and B cells. The candidate gene *NFKBIZ*, which contained a SNP that caused a missense mutation, may be of functional importance in the T cell receptor signalling pathway and in the regulation of inflammatory responses.

4.6.2c Cytokine signalling in the immune system

Cytokines and their receptors are very important in the modulation of immune responses and are key components of host defence. Given their role in combating pathogens, cytokines have been the focus of several vaccination efforts against measles (Haralambieva et al. 2015), and were proposed as candidate genes for morbillivirus resistance and susceptibility (McCarthy et al. 2011). One of these cytokine signalling receptor genes, *IL4a*, was found to be under selection in our study, and selection in a further eleven genes were also characterised into this system (*CNTF*, *BCL2*, *CALM1*, *GRB2*, *ATF1*, *BTC*, *PTPN3*, *IKBKB*, *TP53*, *MAPK8* and *FGF2*). These findings provide further support that variation within cytokine signalling genes such as interleukins, and particularly *IL4a*, play an important role in host immune responses to morbilliviruses in general.

4.6.3 Other pathways

Other candidate genes found under putative selection in dolphins are involved in multiple pathways that may indirectly be linked to immune responses. For example, signal transduction is an important process where extracellular signals, such as hormones or growth factors change the cell state or activity (Nair

et al. 2019). Here, three candidate genes (*FGF2*, *GRB2*, *BTC* and *CALM1*) are involved in the MAPK family signalling pathway, and another nine (*PDE6H, NTRK2, KIDINS220, INHBA, NPSH1, PLCE1, HRH4, TGFB3, IKBKB*) were associated to the MAPK cascades. These are involved in the initiation of the innate immune system, activation of the adaptive immune system, and cell death after infection (Dong et al. 2002). *MAPK8* was previously suggested to be involved in dolphin susceptibility and resistance to CeMV, and was related to a response to heat stress (Batley et al. 2019). Likewise, it was found to be under selection in this whole genome study. Two other genes (*INHBA* and *BMPR1B*) are involved in signalling by the transforming growth factor family members that have important functions in the regulation of inflammatory responses and in T cell regulation and differentiation (Li et al. 2006). Transforming growth factors have been associated with immune responses to a range of diseases (Akdis et al. 2016), but to the best of our knowledge, they have not been implicated in immune responses to morbilliviruses.

4.6.4 Candidate immune genes

Numerous genes have been proposed to be involved in immune responses to morbilliviruses, including binding genes, pathogen-associated molecular pattern sensing genes, cytokine-cytokine receptor genes, antiviral genes, and vitamin A and D receptor genes (McCarthy et al. 2011, Haralambieva et al. 2015, Stejskalova et al. 2017). Due to the high number of gene annotations, we mainly focused on SNPs that annotated to protein coding regions and therefore may have missed variation between *cases* and *controls* in intronic regions of important immune genes. We therefore assessed genetic variation and investigated putative signatures of selection in 22 genes previously proposed to be important in resistance and susceptibility to morbilliviruses. We found significant differentiation in intronic regions in four of such candidate genes (*RARb, FBXW11, ANK3* and *ACOX3)*. While the identified SNPs are within introns, the potential role of these genes in fighting CeMV should not be discarded. A large proportion of the mammalian genome is made up of introns (Chorev et al. 2017), and in the SABD reference genome less than 0.6% of SNPs are within exons (Batley et al. unpublished data). While many introns act in a neutral manner with apparently no function, intronic SNPs might indirectly influence gene function and immune response genes through the alteration of splicing (Dhiman et al. 2008, Seoighe and Korir 2011, Singh et al. 2018, Guigó and Ullrich 2020).

Genetic diversity is essential for natural populations to adapt to rapid and ongoing changes to their environment (Manlik et al. 2019). Maintaining genetic diversity is particularly important for populations to recognise and fight infectious diseases (Hendricks et al. 2017). Immune genes are considered amongst some of the most polymorphic genes in wildlife populations (Morris et al. 2015, Ruan et al. 2016, Dooley et al. 2018), with diversity suggested to be maintained through pathogen-host balancing selection (Morris et al. 2015), and an excess of homozygous alleles likely impairing an

individual's ability to successfully fight pathogens (Smith et al. 2009, Shafer et al. 2012, Blanchong et al. 2016). The dolphin population studied here has relatively low levels of standing genetic variation compared to neighbouring populations (Pratt et al. 2018, Pratt 2020), and this may have negatively influenced their susceptibility to CeMV. While we observed a high level of polymorphism in many immune genes, we found a lack of heterozygosity in some that are thought to be functionally important. This lack of diversity was observed across *case* and *control* samples, and therefore may not have led to *case* dolphins being more likely to succumb to CeMV, but the population being more susceptible. Within the GSV, *T. aduncus* and *D. delphis* are considered resident species (Kemper et al. 2008). *D. delphis* are very gregarious and form a larger population (Zanardo et al. 2016a, Parra et al. in review) and although a small number of *D. delphis cases* were recorded during the outbreak, the virus did not seem to have a similar impact in this population (Kemper et al. 2016). *D. delphis* from GSV are more genetically diverse than *T. aduncus* from the same bioregion (Bilgmann et al. 2014, Pratt et al. 2018), and this difference in diversity may have influenced their ability to fight and survive CeMV infection.

4.7 Conclusions

This whole-genome association study disclosed the importance of key immune response genes and pathways in susceptibility and resistance of dolphins to the highly infectious and fatal CeMV. While the RRS study provided an important first step in uncovering genes potentially involved in CeMV immune responses, by expanding to a whole genome level, we have uncovered novel genes and pathways that have not previously been the target of morbillivirus immune response studies. In particular, the genes *CD300LF*, *NFATC2* and *NFKBIZ* may be involved in the regulation and expression of interleukins and T cells, while the gene pathways FcRs and MAPK cascade may be important for recognising pathogens and activating immune responses, and the initiation and activation of the immune system, respectively. In addition, we found evidence for putative selection in genes previously suggested to be potentially involved in responses to morbilliviruses, adding evidence that knowledge gained on immune responses by one species can be more broadly applied to other morbilliviruses. The results highlighted the importance of cytokines, T cells (particularly Th2) and *IL4*, in fighting infection by these viruses. Overall, our work highlights the complex interactions between the innate, adaptive, and signalling processes of the mammalian immune system in fighting infection by viruses and adds to our understanding of major marine mammal immune responses. The unravelled interactions of the immune systems emphasise the significance of whole genome studies to characterise the interplay of immune responses and genes involved in combating infections. Additional whole genome studies of larger CeMV outbreaks should clarify the role of these genes and pathways across virus strains, and cetacean populations and species.

Chapter 5: General Discussion

5.1 Introduction

Globally, we are losing biodiversity at a rapid rate and are on our way to entering the sixth mass extinction event (Barnosky et al. 2011, Ceballos et al. 2015, Ceballos et al. 2017). Biodiversity loss, including global and local extinctions of species and populations, and even changes in abundance has severe and often permanent ramifications for ecosystem structure and function, and has become one of the most serious environmental problems (Ceballos et al. 2020). The growing human population is often linked to this crisis, with humans placing great stress on the natural environment through overexploitation, habitat modification or destruction, the introduction and spreading of invasive species, pathogens and parasites, pollution, and climate change (Pimm et al. 2014, Ceballos et al. 2017, Mazor et al. 2018, Ceballos et al. 2020). Some species, including bottlenose dolphins may be particularly vulnerable to anthropogenic pressures due to their population biology and life history traits of slow reproductive rates and delayed age at maturity (Fair and Becker 2000, Silber et al. 2017), as well some tendency to reside in coastal regions, and exhibit small population sizes with low levels of genetic diversity and reduced connectivity (Möller et al. 2007, Charlton-Robb et al. 2014, Fruet et al. 2014, Zanardo et al. 2016b, Pratt et al. 2018).

Under growing anthropogenic pressure and changing selective pressures, species and population persistence will depend on their capacity to adapt to new and unfavourable conditions or track their favoured ecological niche. It is therefore important that drivers of evolutionary divergence, genomic diversity, and adaptation are evaluated to better understand the potential of biodiversity to persist through ongoing habitat and climatic changes. This study used a comparative genomics framework to better understand the evolutionary history and adaptive potential of inshore and offshore bottlenose dolphins. First, a reference genome for the putative subspecies, the southern Australian bottlenose dolphin (SABD) was generated. With this tool, the phylogenomic relationships of bottlenose dolphins, and protein-coding genes evolving under positive selection between species and lineages were identified. Parallel evolution driven by niche specialisation was then investigated by exploring the relationship between genomic diversity and runs of homozygosity (ROH), and by comparing the demographic histories of inshore and offshore bottlenose dolphins. In addition, lineages with reduced genomic diversity that may be more vulnerable to anthropogenic impacts and climate change were identified. Finally, in one population of SABD impacted by a cetacean morbillivirus (CeMV) related unusual mortality event, the role of host genetic factors in disease resistance and susceptibility was investigated. The comparative genomics approach employed throughout this study enabled great insight into the evolution and adaptation of bottlenose dolphins, and findings from this work can be integrated into management action plans to promote the effective conservation of vulnerable dolphin lineages.

5.2 The SABD and the value of its reference genome

Within the past decade, considerable efforts have been made to generate reference genomes for all vertebrate species, including cetaceans, and to improve the continuity of those already available (e.g. the Cetacean Genomes Project and DNAZoo). These collaborative efforts, in addition to independent researchers, have generated at least 77 cetacean genomes for 36 different species since 2012 (Table S1.1). These genomes provide the necessary resources to address many evolutionary and biological questions that are essential for species conservation, and are a much valuable tool for mapping resequencing data and calling SNPs (Morin et al. 2020a). The SABD was first described as a separate species (*Tursiops australis*) in 2011 (Charlton-Robb et al. 2011a) based on genetic data (Möller et al. 2008, Charlton-Robb et al. 2011a), but a combination of greater genomic resolution and restricted morphological evidence suggests this is likely a subspecies of the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) (Jedensjö et al. 2020, Moura et al. 2020, Pratt 2020). To further investigate the evolution of this narrow endemic, putative subspecies, a high-quality reference genome was assembled into 23 chromosome-length scaffolds, with an N50 of 121 Mb, and containing 93% of genes from the mammalian orthologous database (mammalia_odb9) (Chapter 2). This genome constitutes one of the first chromosome-length scaffolded assemblies for a cetacean species and is to the best of my knowledge, among the highest quality cetacean genomes currently available. At the time of starting this project, there was no available Indo-Pacific bottlenose dolphin reference genome, and the common bottlenose dolphin (*Tursiops truncatus*) assembly was fragmented into thousands of scaffolds (Table S1.1). Therefore, the SABD assembly became the primary resource for the comparative genomics approach in this study by first becoming a tool to map the resequencing data of a further 88 genomes from all currently known bottlenose dolphin species and subspecies from the Southern Hemisphere, and a further three lineages from the Northern Hemisphere (Chapters 2 and 3). This much-needed tool provided great insight into niche divergence and parallel evolution, adaptation, and disease susceptibility of bottlenose dolphins, and will continue to contribute to our knowledge on the evolution and conservation of this putative subspecies (summarised in Table 5.1).

Table 5.1: Summary of the contributions to science that have been facilitated through the assembly of the SABD reference genome

Genomic analysis	Conservation questions	Conservation implications	Expected outcomes
Phylogenomics	Does phylogenomic position support the proposed species classification of *T. australis*?	Genomic position within *T. aduncus* clade did not support species classification; genomic differentiation may warrant subspecies classification for SABD	SABD to be managed as a separate entity from *T. aduncus*
Positive selection	Do signatures of positive selection relate to the adaptation of species to different selective pressures?	Genes with similar functions between species suggests hotspots of shared positive selection among delphinids	Evidence of natural selection facilitating parallel adaptation of lineages to similar inshore environments
	Do selective pressures associated with ecotypes create signatures of positive selection?	Same genes positively selected in multiple inshore lineages; driven by similar selective pressures associated with inshore habitats (parallel evolution)	
Genomic diversity and demographic history	Does genomic diversity vary between species and ecotypes?	General relationship between ecotype, genomic diversity and ROH; inshore dolphins more vulnerable to change	Evidence of natural selection facilitating adaptation to ecotype; identification of vulnerable populations; improved knowledge to aid effective conservation measures (mitigate human disturbance, monitor genetic diversity and population size)
	Do independent lineages share similar histories of niche divergence?	Parallel demographic histories within ecotypes generally observed; environmentally or human induced bottlenecks rather than founder effects in inshore populations	
Whole genome association study	Do host genetic variants make some dolphins more susceptible to CeMV?	Identification of novel immune genes potentially involved in CeMV immune responses; no observed variation in other immune genes; support for knowledge gained on immune responses to one morbillivirus species being applied to others	Improved understanding of host genetic factors in CeMV susceptibility; provided candidate genes to screen other cetacean populations and species affected by CeMV; better understanding of immune gene diversity and cetacean immune response genes
	Is RRS data sufficient in identifying host genetic factors associated with a trait?	Sufficient in estimating inbreeding and genomic diversity; provides initial insight into variation across the genome; may lack resolution to investigate functional changes	

5.3 Phylogenomic relationships of bottlenose dolphins

Incomplete lineage sorting resulting from recent and rapid radiations, as well as hybridisation among Delphininae species had led to a long history of confounding phylogenomic relationships (Amaral et al. 2012, Moura et al. 2013, Gray et al. 2018). For bottlenose dolphins, at least 20 species have been proposed (Hershkovitz 1966), but currently only two species are recognised (*T. truncatus* and *T. aduncus*) (Committee on Taxonomy of the Society for Marine Mammalogy 2020). Ecotypic divergence and niche specialisations of bottlenose dolphins have further complicated efforts to resolve phylogenomic relationships, with multiple species and subspecies proposed across the Southern and Northern Hemispheres (Charlton-Robb et al. 2011a, Costa et al. 2019). The phylogenomic relationships of bottlenose dolphin lineages across ocean basins from both Hemispheres were investigated based on 500 vertebrate orthologous genes. The bottlenose dolphin genus formed a monophyletic group, with the two accepted species forming distinct and well supported clades. The proposed species, the Burrunan dolphin (*T. australis*) was nested within *T. aduncus*, but genomic divergence from other *T. aduncus* lineages (Figure 2.2, Figure 3.1), support suggestions that SABD could be named as a subspecies (Moura et al. 2020, Pratt 2020). Estimates of divergence times between the SABD and eastern Australian *T. aduncus* (~51 kya, 95% CI 45,145-51,622) suggests an initial event of allopatric differentiation potentially driven by the Bassian land-bridge between mainland Australia and Tasmania (Lambeck and Chappell 2001), with contemporary oceanographic features, natal philopatry and local adaptation potentially reinforcing divergence (Chapter 3).Appropriate classification is vital to ensuring targeted conservation and management action plans are in place and are tailored to the lineages ecological and environmental conditions. This is particularly important for inshore lineages with relatively narrow ranges (such as SABD) that are vulnerable to anthropogenic pressures (e.g. toxin and heavy metal accumulation (Lavery et al. 2008), tourism interactions (Peters et al. 2012) and viral outbreaks (Kemper et al. 2016)). It is therefore recommended that conservation strategies for the divergent SABD lineage and their classification (taxonomy and IUCN Red List) be reassessed to ensure the persistence of this metapopulation (Pratt et al. 2018) into the future.

Support was provided for the subspecies classification of *T. t. gephyreus*, with the lineage nested within *T. truncatus* but showing substantial genomic divergence from other lineages (Figure 2.2, Figure 3.1). This is in line with morphological (Costa et al. 2016) and genetic evidence (Costa et al. 2019, Oliveira et al. 2019) that recommended a subspecies classification rather than the proposed species status (e.g. Wickert et al. 2016, Hohl et al. 2020). Until this study, little was known about the evolutionary history of this subspecies, but it has been hypothesised that it diverged after the LGM when individuals colonised the newly available inshore habitats (Fruet et al. 2017). This study found no signal of a post-glacial founder event for *T. t. gephyreus* and estimated an older time of divergence. Further analysis

with alternative methods may clarify whether the older, or an earlier, time of divergence is more likely for the separation of this subspecies.

5.4 Bottlenose dolphin evolution and adaptation

Divergent selection and adaptive divergence associated with environmental heterogeneity is a known driver of population divergence and eventual speciation (Schluter 2009). This is particularly the case for bottlenose dolphins, where species, sub-species and population-level genetic differentiation is suggested to be caused by adaptation to local environments (Natoli et al. 2005, Bilgmann et al. 2007b, Möller et al. 2007, Wiszniewski et al. 2009, Pratt 2020). To explore the role of divergent selective pressures associated with occupying different habitats, lineage-specific signatures of positive selection were explored (Chapter 2). A dataset comprising of 9,464 single copy and complete cetacean orthologous genes for nine bottlenose dolphin lineages, and for a single lineage of *D. delphis* and one of *O. orca* revealed that four genes were positively selected in more than one species (excluding the *O. orca*), while different genes with similar functions were also positively selected between species. This was further highlighted by evidence of positive selection in *MYH7B* in the *D. delphis* and *T. aduncus* genomes, which has previously been found to be under selection in other marine mammals (Foote et al. 2015). This gene has functions relating to the development of the cardiac muscle and may be important in heart development of marine mammal species. Different genes involved in the immune system, signal transduction, metabolism and developmental biology were also positively selected in multiple species and may therefore be hotspots of shared positive selection across delphinid species.

At the lineage-level, different genes with similar functions were positively selected across bottlenose dolphin lineages. These include genes involved in developmental biology and wound healing. Wound healing genes were also positively selected within *D. delphis* and *T. truncatus* at the species level and may therefore be important in the evolution of delphinids. Wound healing is an important biological process that protects the individual from foreign substances and infectious agents. Some dolphin populations are exposed to a wide range of threats that may act as strong selective pressures, including predation, boat strikes and incidental fishing (Fair and Becker 2000, Donaldson et al. 2010), and therefore, selection in these genes may support the effective wound healing that is often observed in dolphins (Noren and Mocklin 2012). A further 18 genes were positively selected across the bottlenose dolphin lineages, which had functions relating to developmental biology, including the development of multicellular organisms (e.g. ageing), neuronal development, and the development of different muscles and organs. Genes involved in developmental biology may be linked to a wide range of traits, including the evolution of brain size and complex behaviours (McGowen et al. 2012) and in response to many aquatic pressures (e.g. shallow and deep diving, osmotic regulation, sensory perception) (Nery et al. 2013, McGowen et al. 2014, Foote et al. 2015). While the phenotypic traits linked to the 18

developmental biology genes under selection requires further investigation, it is evident that genes with these functions are important in the evolution of dolphins, potentially to the aquatic environment and their evolved complex behaviours.

5.5. Parallel evolution in the genomes of inshore lineages

While inshore and offshore environments differ in their environmental features, inshore environments generally share similar features that create comparable selective pressures among inshore habitats. This was evident when comparing genes under selection between inshore and offshore bottlenose dolphin lineages, with 13 genes positively selected among multiple inshore lineages, while no genes were positively selected in more than one of the offshore lineages (Chapter 2). This finding supports the idea that similar selective pressures associated with the inshore environment are resulting in the parallel evolution and adaptation of inshore bottlenose dolphins (Pratt 2020). The genes likely to be under parallel evolution in the inshore lineages relate to DNA damage, repair and apoptosis, and the immune system and eye development. These genes may be evolving in parallel in response to the greater stress associated with living near areas of high human use. For example, toxin and heavy metal pollution can distort DNA structure, and potentially lead to cancer (Chatterjee and Walker 2017). Bioaccumulation of toxins and heavy metals have been recorded to be higher in inshore populations in southern Australia (Lavery et al. 2008, Gaylard 2017), and genes involved in DNA repair and apoptosis could therefore be important in reducing the risk of mutagenesis in the inshore lineages. There is no study that suggests inshore populations are exposed to a greater diversity or abundance of pathogens, however, disturbance of inshore environments have been reported to influence marine mammal mortality events and the spread of disease (Fury and Reif 2012, Meager and Limpus 2014). For example, poxvirus-like lesions in inshore dolphin populations in Australia have been linked to flooding events (Fury and Reif 2012), while the CeMV outbreak in South Australia was thought to have been influenced by stress induced by abnormally high sea surface temperatures within the gulf environments (Kemper et al. 2016, Batley et al. 2019). Therefore, the added human-induced stress associated with the inshore environment may be driving selection in immune functioning genes. Selection within immune and DNA damage, repair and apoptosis genes in marine mammals have been previously reported (Ming et al. 2019, Tejada-Martinez et al. 2021). However, no evidence of these gene functions being positively selected in offshore lineages may suggest that selective pressures associated with the stressful conditions of the inshore habitat are driving this parallel evolution in the inshore lineages only.

The genomic consequence of repeated niche divergence was further highlighted by comparing patterns of genomic diversity, ROH and demographic histories between inshore and offshore lineages (Chapter 3). A very strong relationship between genomic diversity, ROH and ecotype was observed, with inshore lineages exhibiting significantly lower genomic diversity than the offshore lineage. Likewise, a greater

proportion of their genomes were covered by ROH, reflecting the recent inbreeding events or reduced levels of connectivity following a bottleneck (Brüniche-Olsen et al. 2018). Parallel demographic histories were observed within ecotypes, with the inshore lineages generally experiencing population bottlenecks during the LGM, while offshore lineages expanded during the same period. The two inshore *T. truncatus* (*T. t. gephyreus* and Gulf of Mexico) followed similar patterns to the offshore lineages during the LGM only, potentially reflecting the increased connectivity during a period where available inshore habitats were scarce. The comparable environmental features of the inshore habitat may be creating similar selective pressures, resulting in the consistent finding of parallel evolution in the genomes of inshore lineages. These results highlight the importance of niche divergence in the evolution and adaptation of bottlenose dolphins and provide strong evidence of natural selection facilitating local adaptation in inshore populations.

5.6. Host genetic factors and disease susceptibility

As the climate continues to change and habitats are altered, modifications to disease dynamics will likely challenge the persistence of populations and species (Smith et al. 2009). Changes to pathogens and host ranges, and increased host susceptibility induced by anthropogenic and environmental stress are likely to lead to shifts in the prevalence, severity, and transmission of disease in wildlife populations (Drew Harvell et al. 2002, Williams et al. 2002, Hoegh-Guldberg and Bruno 2010). This has become evident in recent times, with the emergence of SARS-CoV-2, with many variants of concern rapidly emerging and spreading across the globe. Regarding wildlife populations, an apparent increase in the diversity and incidences of disease outbreaks has been observed, with the emergence of morbillivirus in many marine mammal populations around the world among some of the most pressing examples (Smith et al. 2009).

Despite host genetic factors, including levels of inbreeding and diversity, and variations within genes being important drivers of an individual's susceptibility and resistance to infection (Karlsson et al. 2014, Stejskalova et al. 2017), few studies have addressed the role of host genetics and disease susceptibility in wildlife populations. Therefore, whole genomes from surviving and non-surviving Indo-Pacific bottlenose dolphins (SABD) from one genetic population that was affected by a cetacean morbillivirus (CeMV) related unusual mortality event were sequenced. These were included in an association-based study to understand the role of host genetic factors in CeMV susceptibility. Estimates of inbreeding confirmed previous findings from reduced representation data that inbreeding was not a major driver of CeMV susceptibility, with inbreeding being similar within and between survivors and non-survivors (Batley et al. 2019). The population, however, has low genomic diversity and a high proportion of their genome covered by ROH compared to other dolphin species from the same region (*D. delphis* and *T. t. truncatus*), which apparently had only a few animals succumbing to the disease during the outbreak

(chapter 3). In particular, *D. delphis* are thought to be a resident species in Gulf St Vincent, are highly gregarious and form a larger population (Kemper et al. 2008, Zanardo et al. 2016a, Parra et al. in review). They have greater genetic diversity, and minimal ROH, and its greater diversity may have enhanced their ability to fight and survive CeMV infection.

Significant genetic differentiation between the two groups (survivors vs non-survivors) was observed for thousands of candidate SNPs and assumed to be potentially involved in CeMV susceptibility or resistance in this population. Gene annotations and their pathways revealed that genes involved in the immune system were particularly important. Building on work by Batley et al. (2019), the whole genome approach improved resolution to detect variation within genes across the genome, but also to investigate the gene region of candidate SNPs (e.g. exons or introns) and their impact (e.g. synonymous or non-synonymous substitutions). Given their importance in fighting infection, variation within immune genes and pathways were of the most interest, with twelve of the SNPs within immune pathways found within exon regions. Half of these SNPs caused a missense change, while the remaining half resulted in synonymous substitutions. Most of the candidate SNPs fell within intron regions (or potentially promotor/enhancer regions), however the importance of SNPs within introns in CeMV susceptibility should not be disregarded. A large proportion of the mammalian genome is covered by introns (Chorev et al. 2017). For example, 25% of the human genome is made up of introns (Jo and Choi 2015), and only 0.6% of all SNPs within the SABD genome annotated to exons. The importance of intronic variations in species evolution and adaptation are only beginning to be realised, and therefore, candidate SNPs in this study that annotated to immune genes in both intron and exon regions of the SABD genome were assumed to be putatively involved in CeMV resistance and susceptibility.

Regarding the immune genes with significant differentiation between survivors and non-survivors, the three main immune pathways were well represented (i.e. innate, adaptive and cytokine signalling), highlighting the complex interplay of the immune system in combatting CeMV infection. Novel genes and pathways that have not been the target of previous immune response studies were differentiated between survivors and non-survivors, while support for genes previously suggested to be involved in fighting morbillivirus was also provided (e.g. *IL4a*, McCarthy et al. (2011); *PATJ*, *MAPK8*, *FBXW11*, *ANK3* and *ACOX3*, Batley et al. (2019)). Specific MHC class I genes were not significantly differentiated in coding regions of the genes, however at least nine genes were characterised into this pathway, and may be important in recognising foreign molecules and initiating an immune response. Genes involved in cytokine and T cell signalling were well represented and are assumed to be important mediators of immune responses to morbilliviruses. Viral species within the genus *Morbillivirus* share similar characteristics, and likewise it has been suggested that the signalling lymphocyte activation molecule (SLAM) is a universal receptor for entry and propagation of morbillivirus in all mammals, including cetaceans (Sato et al. 2012, Shimizu et al. 2013, Melia et al. 2014, Ohishi et al. 2019). While

variation was not observed within SLAM in this study, the finding of differentiation within *IL4a* and genes involved in cytokine and T cell signalling provide further support that knowledge gained on immune responses for one viral species may be applicable more broadly to morbilliviruses.

These findings support the use of reduced representation genome sequencing in the initial stages of identifying host genetic variants associated with a trait as a proof of concept, and in estimating levels of inbreeding and diversity. Whole genome sequences provide greater resolution to identify genes across the genome and the relationship between variation and amino acid changes, providing an opportunity to look at variation within genes previously hypothesised to be important for a particular trait.

5.7 Vulnerability of inshore lineages to a changing environment

Inshore lineages are particularly vulnerable to a changing climate and increased anthropogenic and selective pressures due to low genomic diversity and small population sizes (chapter 3), ecological factors including small home ranges, high site fidelity to urbanised regions, specialised diets and feeding strategies (Möller et al. 2007, Zanardo et al. 2016b, Pratt et al. 2018), and biological traits such as slow reproductive rates and delayed age at maturity (Fair and Becker 2000, Silber et al. 2017).

Throughout this thesis, molecular evidence highlighted the heightened vulnerability and reduced adaptive potential of inshore bottlenose dolphins. For instance, signatures of positive selection suggested that adaptations of inshore lineages may potentially be driven by greater anthropogenic stress associated with site fidelity to areas of high human use (chapter 2). The low genomic diversity and small populations sizes of inshore lineages may reduce their adaptive potential. This is highlighted by population bottlenecks experienced by the inshore lineages during the LGM (chapter 3). This finding across multiple inshore lineages suggests that as the ocean cooled and sea levels dropped, these lineages may have been unable to adapt to the deeper, cooler, and less productive waters that they were likely forced to retreat to. In particular, extremely low genomic diversity was recorded for the inshore *T. t. gephyreus*, being similar to other vulnerable species (e.g. the cheetah (*Acinonyx jubatus*), snow leopard (*Panthera uncia*) and Tasmanian devil (*Sarcophilus harrisii*)) (Miller et al. 2011, Cho et al. 2013, Dobrynin et al. 2015), and this may be linked to a recent bottleneck. Genomic diversity can enhance an individual's potential to adapt to changes, reduce the harmful effects of inbreeding, and influence disease tolerance and resistance (Lotze et al. 2011, Wernberg et al. 2018, Hoban et al. 2020b). In addition to the low genomic diversity observed for the inshore lineages, within one population of SABD, negligible diversity was found in immune genes that were previously thought to be important in fighting viral infections, and particularly CeMV (Hashiguchi et al. 2011, McCarthy et al. 2011, Haralambieva et al. 2015, Stejskalova et al. 2017) (Chapter 4). Negligible genomic diversity in immune

genes may hinder a population's ability to recognise and fight infectious diseases (Smith et al. 2009). Therefore, low genetic diversity coupled with negligible diversity in key immune genes and potential stress induced by abnormally high sea surface temperatures may have hindered the SABD population's ability to fight CeMV infection, leading to the larger mortality observed in this population.

The Lahille's bottlenose dolphin and Indo-Pacific bottlenose dolphin are currently classified as Vulnerable and Near Threatened, respectively, by the International Union for Conservation of Nature (IUCN), citing residential and commercial development, human intrusion and disturbance, and pollution as key threats (Braulik et al. 2019, Vermeulen et al. 2019). While the IUCN recognises the importance of genomic diversity in maintaining global biodiversity (Reed and Frankham 2003, Garner et al. 2020), molecular evidence of species vulnerability are rarely incorporated into species conservation strategies (Laikre 2010, Garner et al. 2020). In this thesis, evidence from three genomic subfields (i.e. evolutionary, population and functional genomics) highlight the vulnerability of inshore lineages to anthropogenic stressors, emphasising the importance of integrating genomic findings into policy and action plans that promote sound conservation strategies. It is suggested that where possible, conservation efforts be focussed towards minimising human impacts, promoting connectivity and population replenishment, and monitoring genetic diversity and population sizes over time, particularly in the vulnerable inshore populations.

5.8 Limitations and future directions

This work provides baseline knowledge on the evolution, demographic history, and adaptation of dolphins, and to the best of my knowledge, represents one of the first, and largest comparative genomic studies for cetacean species and subspecies. As the field of molecular ecology and conservation genomics continues to progress, our understanding of the power and constraints of whole genome data will grow. Many low coverage genomes were included in this study, and while this provides great resolution to understand genomic diversity, inbreeding and adaptation within a population, the use of multiple low coverage genomes to reliably detect patterns of demographic history is relatively unknown. While patterns were similar within species and to previous studies (Vijay et al. 2018, Moura et al. 2020), the analysis was restricted to within the past 200 kya and produced estimates up until present day (for inshore lineages only). These recent estimates have large ramifications for the management and conservation of these lineages, and it is therefore recommended that future work further assess the patterns observed, as well as estimated times of divergence. This can be achieved by expanding the number of individuals included, and at a higher coverage (at least 15x), using other algorithms that are better suited to estimating demographic histories for small populations, or by using a single representative genome per lineage (at least 20x) in a PSMC analysis.

To the best of my knowledge, this work also represents the first whole genome association study of disease susceptibility and resistance in a marine mammal species. Future work should aim to screen other marine mammal populations, particularly those that have been affected by Morbillivirus, to confirm the role of the identified candidate immune genes in the resistance and susceptibility of populations and different species to the virus. By doing so, markers can be developed to screen marine mammals, ultimately identifying those at risk of succumbing to one of the greatest infectious disease threats to these animals worldwide. This work also highlighted an apparent lack of genetic diversity in several immune genes in one population of SABDs, which may have large implications for the health of the population. It is first recommended that future studies expand on this knowledge gained by taking a targeted approach to investigate variability in a greater number of characterised immune genes, including MHC genes that are important for immune responses and reproductive success (Manlik et al. 2019). Secondly, it is recommended that variation in immune genes in this population is compared to other populations and species, to further understand the implications of this lack of diversity.

Concluding remarks

This thesis used 98 whole genomes and a comparative genomic approach to advance our knowledge on eco-evolutionary patterns and the adaptive potential of bottlenose dolphins. The resulting work informs about the role of niche divergence and natural selection in the differentiation and adaptation of bottlenose dolphins, but also addresses the vulnerability of lineages to past climatic events and current selective pressures. The thesis advances our knowledge on eco-evolutionary patterns and processes, as well as adaptive potential of bottlenose dolphins, which can be incorporated into policy and action plans to promote more sound conservation management strategies.

Reference List

Acevedo-Whitehouse, K., and A. A. Cunningham. 2006. Is MHC enough for understanding wildlife immunogenetics? Trends in Ecology & Evolution **21**:433-438.

Acevedo-Whitehouse, K., F. Gulland, D. Greig, and W. Amos. 2003. Disease susceptibility in California sea lions. Nature **422**:35.

Akdis, M., A. Aab, C. Altunbulakli, K. Azkur, R. A. Costa, R. Crameri, S. Duan, T. Eiwegger, A. Eljaszewicz, R. Ferstl, R. Frei, M. Garbani, A. Globinska, L. Hess, C. Huitema, T. Kubo, Z. Komlosi, P. Konieczna, N. Kovacs, U. C. Kucuksezer, N. Meyer, H. Morita, J. Olzhausen, L. O'Mahony, M. Pezer, M. Prati, A. Rebane, C. Rhyner, A. Rinaldi, M. Sokolowska, B. Stanic, K. Sugita, A. Treis, W. van de Veen, K. Wanke, M. Wawrzyniak, P. Wawrzyniak, O. F. Wirz, J. S. Zakzuk, and C. A. Akdis. 2016. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor beta, and TNF-alpha: Receptors, functions, and roles in diseases. Journal of Allergy and Clinical Immunology **138**:984-1010.

Albouy, C., V. Delattre, G. Donati, T. L. Frolicher, S. Albouy-Boyer, M. Rufino, L. Pellissier, D. Mouillot, and F. Leprieur. 2020. Global vulnerability of marine mammals to global warming. Sci Rep **10**:548.

Alfonso, C. L., G. K. Amarasinghe, K. Bányai, Y. Bào, C. F. Basler, S. Bavari, and A. Bukreyev. 2016. Taxonomy of the order Mononegavirales. Archives of Virology **161**:2351-2360.

Altizer, S., D. Harvell, and E. Friedle. 2003. Rapid evolutionary dynamics and disease threats to biodiversity. Trends in Ecology & Evolution **18**:589-596.

Alves, J. M., M. Carneiro, J. Y. Cheng, A. Lemos de Matos, M. M. Rahman, L. Loog, P. F. Campos, N. Wales, A. Eriksson, A. Manica, T. Strive, S. C. Graham, S. Afonso, D. J. Bell, L. Belmont, J. P. Day, S. J. Fuller, S. Marchandeau, W. J. Palmer, G. Queney, A. K. Surridge, F. G. Vieira, G. McFadden, R. Nielsen, M. T. P. Gilbert, P. J. Esteves, N. Ferrand, and F. M. Jiggins. 2019. Parallel adaptation of rabbit populations to myxoma virus. Science **363**:1319-1326.

Amaral, A. R., L. B. Beheregaray, M. M. Coelho, S. M., R. K.M., and L. M. Möller. 2009. Worldwide phylogeography of the genus Delphinus revisited. Report of the International Whaling Commission (SC/61/SM11).

Amaral, A. R., J. A. Jackson, L. M. Moller, L. B. Beheregaray, and M. Manuela Coelho. 2012. Species tree of a recent radiation: the subfamily Delphininae (Cetacea, Mammalia). Mol Phylogenet Evol **64**:243-253.

Andrews, K. R., J. M. Good, M. R. Miller, G. Luikart, and P. A. Hohenlohe. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. Nature Reviews Genetics **17**:81-92.

Árnason, U., F. Lammers, V. Kumar, M. A. Nilsson, and A. Janke. 2018. Whole-genome sequencing of the blue whale and other rorquals finds signatures for introgressive gene flow. Science Advances **4**:eaap9873.

Attard, C. R. M., L. B. Beheregaray, C. Jenner, P. Gill, M. Jenner, M. Morrice, J. Bannister, R. LeDuc, and L. Möller. 2010. Genetic diversity and structure of blue whales (*Balaenoptera musculus*) in Australian feeding aggregations. Conservation genetics **11**:2437-2441.

Attard, C. R. M., L. B. Beheregaray, and L. M. Moller. 2018a. Genotyping-by-sequencing for estimating relatedness in non-model organisms: Avoiding the trap of precise bias. Molecular Ecology Resources **18**:381-390.

Attard, C. R. M., L. B. Beheregaray, J. Sandoval-Castillo, K. C. S. Jenner, P. C. Gill, M. M. Jenner, M. G. Morrice, and L. M. Moller. 2018b. From conservation genetics to

conservation genomics: a genome-wide assessment of blue whales (*Balaenoptera musculus*) in Australian feeding aggregations. Royal Society Open Science **5**:170925.

Autenrieth, M., S. Hartmann, L. Lah, A. Roos, A. B. Dennis, and R. Tiedemann. 2018. High quality whole genome sequence of an abundant Holarctic odontocete, the harbour porpoise (*Phocoena phocoena*). Molecular Ecology Resources.

Axelsson, E., A. Ratnakumar, M. L. Arendt, K. Maqbool, M. T. Webster, M. Perloski, O. Liberg, J. M. Arnemo, A. Hedhammar, and K. Lindblad-Toh. 2013. The genomic signature of dog domestication reveals adaptation to a starch-rich diet. Nature **495**:360-364.

Baines, C., A. Lerebours, F. Thomas, J. Fort, R. Kreitsberg, S. Gentes, R. Meitern, L. Saks, B. Ujvari, M. Giraudeau, and T. Sepp. 2021. Linking pollution and cancer in aquatic environments: A review. Environ Int **149**:106391.

Banks, J., K. Levine, M. Syvanen, J. Theis, and A. Gilson. 1995. DNA tissue gender typing. Standard operating procedure of the Wildlife Forensic Laboratory of the California Department of Fish and Game. Sacramento, CA: California Department of Fish and Game.

Banuet-Martinez, M., W. Espinosa-de Aquino, F. R. Elorriaga-Verplancken, A. Flores-Moran, O. P. Garcia, M. Camacho, and K. Acevedo-Whitehouse. 2017. Climatic anomaly affects the immune competence of California sea lions. PLoS ONE **12**:e0179359.

Barbier, E. B. 2017. Marine ecosystem services. Current Biology **27**:R507-R510.

Barceló, A., J. Sandoval-Castillo, K. A. Stockin, K. Bilgmann, C. M. Attard, N. Zanardo, G. J. Parra, K. Hupman, I. M. Reeves, E. L. Betty, G. Tezanos-Pinto, L. B. Beheregaray, and L. M. Möller. 2021. A Matter of Scale: Population Genomic Structure and Connectivity of Fisheries At-Risk Common Dolphins (*Delphinus delphis*) From Australasia. Frontiers in Marine Science **8**.

Barnosky, A. D., N. Matzke, S. Tomiya, G. O. Wogan, B. Swartz, T. B. Quental, C. Marshall, J. L. McGuire, E. L. Lindsey, K. C. Maguire, B. Mersey, and E. A. Ferrer. 2011. Has the Earth's sixth mass extinction already arrived? Nature **471**:51-57.

Batley, K. C., J. Sandoval-Castillo, C. Kemper, N. Zanardo, I. Tomo, L. B. Beheregaray, and L. M. Moller. 2021. Whole genomes reveal multiple candidate genes and pathways involved in the immune response of dolphins to a highly infectious virus. Molecular Ecology.

Batley, K. C., J. Sandoval-Castillo, C. M. Kemper, C. R. M. Attard, N. Zanardo, I. Tomo, L. B. Beheregaray, and L. M. Möller. 2019. Genome-wide association study of an unusual dolphin mortality event reveals candidate genes for susceptibility and resistance to cetacean morbillivirus. Evolutionary Applications **12**:718-732.

Bearzi, M., C. A. Saylan, and A. Hwang. 2009. Ecology and comparison of coastal and offshore bottlenose dolphins (*Tursiops truncatus*) in California. Marine and Freshwater Research **60**:584–593.

Beichman, A. C., K. P. Koepfli, G. Li, W. Murphy, P. Dobrynin, S. Kilver, M. T. Tinker, M. J. Murray, J. Johnson, K. Lindblad-Toh, E. K. Karlsson, K. E. Lohmueller, and R. K. Wayne. 2019. Aquatic adaptation and depleted diversity: a deep dive into the genomes of the sea otter and giant otter. Molecular Biology and Evolution.

Beling, D. 2021. Cetacean Genomes Status. SWFSC. Downloaded on 05/01/2021.

Bernaldo de Quiros, Y., A. Fernandez, R. W. Baird, R. L. Brownell, Jr., N. Aguilar de Soto, D. Allen, M. Arbelo, M. Arregui, A. Costidis, A. Fahlman, A. Frantzis, F. M. D. Gulland, M. Iniguez, M. Johnson, A. Komnenou, H. Koopman, D. A. Pabst, W. D. Roe, E. Sierra, M. Tejedor, and G. Schorr. 2019. Advances in research on the impacts of anti-submarine sonar on beaked whales. Proceedings of the Royal Society B **286**:20182533.

Bilgmann, K., O. J. Griffiths, S. J. Allen, and L. M. Möller. 2007a. A Biopsy Pole System for Bow-Riding Dolphins: Sampling Success, Behavioral Responses, and Test for Sampling Bias. Marine Mammal Science **23**:218-225.

Bilgmann, K., L. M. Möller, R. G. Harcourt, R. Gales, and L. B. Beheregaray. 2008. Common dolphins subject to fisheries impacts in Southern Australia are genetically differentiated: Implications for conservation. Animal Conservation **11**:518-528.

Bilgmann, K., L. M. Möller, R. G. Harcourt, S. E. Gibbs, and L. B. Beheregaray. 2007b. Genetic differentiation in bottlenose dolphins from South Australia: Association with local oceanography and coastal geography. Marine Ecology Progress Series **341**:265-276.

Bilgmann, K., G. J. Parra, L. Holmes, K. J. Peters, I. D. Jonsen, and L. M. Moller. 2019. Abundance estimates and habitat preferences of bottlenose dolphins reveal the importance of two gulfs in South Australia. Scientific Reports **9**:8044.

Bilgmann, K., G. J. Parra, N. Zanardo, L. B. Beheregaray, and L. M. Möller. 2014. Multiple management units of short-beaked common dolphins subject to fisheries bycatch off southern and southeastern Australia. Marine Ecology Progress Series **500**:265-279.

Blanchong, J. A., S. J. Robinson, M. D. Samuel, and J. T. Foster. 2016. Application of genetics and genomics to wildlife epidemiology. The Journal of Wildlife Management **80**:593-608.

Blehert, D. S., A. C. Hicks, M. Behr, C. U. Meteyer, B. M. Berlowski-Zier, E. L. Buckles, J. T. H. Coleman, S. R. Darling, A. Gargas, R. Niver, J. C. Okoniewski, R. J. Rudd, and W. B. Stone. 2009. Bat White-Nose Syndrome: An Emerging Fungal Pathogen? Science **323**:227.

Bolger, A. M., M. Lohse, and B. Usadel. 2014. Trimmomatic: A flexible trimmer for Illumina Sequence Data. Bioinformatics **btu170**.

Bonneville, C. D., S. Derville, J. A. Luksenburg, M. Oremus, and C. Garrigue. 2021. Social Structure, Habitat Use and Injuries of Indo-Pacific Bottlenose Dolphins (*Tursiops aduncus*) Reveal Isolated, Coastal, and Threatened Communities in the South Pacific. Frontiers in Marine Science **8**.

Borena, B. M., A. Martens, S. Y. Broeckx, E. Meyer, K. Chiers, L. Duchateau, and J. H. Spaas. 2015. Regenerative Skin Wound Healing in Mammals: State-of-the-Art on Growth Factor and Stem Cell Based Treatments. Cell Physiol Biochem **36**:1-23.

Bosi, E., B. Donati, M. Galardini, S. Brunetti, M. F. Sagot, P. Lio, P. Crescenzi, R. Fani, and M. Fondi. 2015. MeDuSa: a multi-draft based scaffolder. Bioinformatics **31**:2443-2451.

Bossart, G. D., T. A. Romano, M. M. Peden-Adams, A. Schaefer, S. McCulloch, J. D. Goldstein, C. D. Rice, J. T. Saliki, P. A. Fair, and J. S. Reif. 2011. Clinicoimmunopathologic findings in Atlantic bottlenose dolphins Tursiops truncatus with positive cetacean morbillivirus antibody titers. Diseases of Aquatic Organisms **97**:103-112.

Bossart, G. D., T. A. Romano, M. M. Peden-Adams, A. M. Schaefer, C. D. Rice, P. A. Fair, and J. S. Reif. 2019. Comparative Innate and Adaptive Immune Responses in Atlantic Bottlenose Dolphins (*Tursiops truncatus*) With Viral, Bacterial, and Fungal Infections. Frontiers in Immunology **10**.

Bradshaw, C. J. A., P. R. Ehrlich, A. Beattie, G. Ceballos, E. Crist, J. Diamond, R. Dirzo, A. H. Ehrlich, J. Harte, M. E. Harte, G. Pyke, P. H. Raven, W. J. Ripple, F. Saltré, C. Turnbull, M. Wackernagel, and D. T. Blumstein. 2021. Underestimating the Challenges of Avoiding a Ghastly Future. Frontiers in Conservation Science **1**.

Brandies, P., E. Peel, C. J. Hogg, and K. Belov. 2019. The Value of Reference Genomes in the Conservation of Threatened Species. Genes **10**.

Brauer, C. J., M. P. Hammer, and L. B. Beheregaray. 2016. Riverscape genomics of a threatened fish across a hydroclimatically heterogeneous river basin. Molecular Ecology **25**:5093-5113.

Braulik, G., A. Natoli, J. Kiszka, G. Parra, S. Plön, and B. D. Smith. 2019. *Tursiops aduncus*. The IUCN Red List of Threatened Species.

Browning, S. R., and B. L. Browning. 2015. Accurate Non-parametric Estimation of Recent Effective Population Size from Segments of Identity by Descent. Am J Hum Genet **97**:404-418.

Brüniche-Olsen, A., K. F. Kellner, C. J. Anderson, and J. A. DeWoody. 2018. Runs of homozygosity have utility in mammalian conservation and evolutionary studies. Conservation genetics **19**:1295-1307.

Bruniche-Olsen, A., R. Westerman, Z. Kazmierczyk, V. V. Vertyankin, C. Godard-Codding, J. W. Bickham, and J. A. DeWoody. 2018. The inference of gray whale (Eschrichtius robustus) historical population attributes from whole-genome sequences. BMC Evol Biol **18**:87.

Bu, G., H. J. Geuze, G. J. Strous, and A. L. Schwartz. 1995. 39 kDa receptor-associated protein is an ER resident protein and molecular chaperone for LDL receptor-related protein. The EMBO journal **14**:2269-2280.

Burge, C. A., C. Mark Eakin, C. S. Friedman, B. Froelich, P. K. Hershberger, E. E. Hofmann, L. E. Petes, K. C. Prager, E. Weil, B. L. Willis, S. E. Ford, and C. D. Harvell. 2014. Climate change influences on marine infectious diseases: implications for management and society. Annual Review of Marine Science **6**:249-277.

Cammen, K. M., K. R. Andrews, E. L. Carroll, A. D. Foote, E. Humble, J. I. Khudyakov, M. Louis, M. R. McGowen, M. T. Olsen, and A. M. Van Cise. 2016. Genomic Methods Take the Plunge: Recent Advances in High-Throughput Sequencing of Marine Mammals. Journal of Heredity **107**:481-495.

Cammen, K. M., T. F. Schultz, W. Don Bowen, M. O. Hammill, W. B. Puryear, J. Runstadler, F. W. Wenzel, S. A. Wood, and M. Kinnison. 2018. Genomic signatures of population bottleneck and recovery in Northwest Atlantic pinnipeds. Ecology and Evolution **8**:6599-6614.

Cammen, K. M., T. F. Schultz, P. E. Rosel, R. S. Wells, and A. J. Read. 2015a. Genomewide investigation of adaptation to harmful algal blooms in common bottlenose dolphins (*Tursiops truncatus*). Molecular Ecology **24**:4697-4710.

Cammen, K. M., L. A. Wilcox, P. E. Rosel, R. S. Wells, and A. J. Read. 2015b. From genome-wide to candidate gene: an investigation of variation at the major histocompatibility complex in common bottlenose dolphins exposed to harmful algal blooms. Immunogenetics **67**:125-133.

Ceballos, F. C., S. Hazelhurst, and M. Ramsay. 2019. Runs of homozygosity in sub-Saharan African populations provide insights into complex demographic histories. Hum Genet **138**:1123-1142.

Ceballos, F. C., P. K. Joshi, D. W. Clark, M. Ramsay, and J. F. Wilson. 2018. Runs of homozygosity: windows into population history and trait architecture. Nat Rev Genet **19**:220-234.

Ceballos, G., and P. R. Ehrlich. 2002. Mammal Population Losses and the Extinction Crisis. Science **296**:904-907.

Ceballos, G., P. R. Ehrlich, A. D. Barnosky, A. García, R. M. Pringle, and T. M. Palmer. 2015. Accelerated modern human–induced species losses: Entering the sixth mass extinction. Science Advances **1**:e1400253.

Ceballos, G., P. R. Ehrlich, and R. Dirzo. 2017. Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. PNAS **114**:E6089-E6096.

Ceballos, G., P. R. Ehrlich, and P. H. Raven. 2020. Vertebrates on the brink as indicators of biological annihilation and the sixth mass extinction. PNAS **117**:13596-13602.

Chapin, F. S., E. S. Zavaleta, V. T. Eviner, R. L. Naylor, P. M. Vitousek, H. L. Reynolds, D. U. Hooper, S. Lavorel, O. E. Sala, S. E. Hobbie, M. C. Mack, and S. Díaz. 2000. Consequences of changing biodiversity. Nature **405**:234-242.

Charlton-Robb, K., L. Gershwin, R. M. Thompson, J. Austin, K. Owen, and S. McKechnie. 2011a. A new dolphin species, the Burrunan dolphin *Tursiops australis* sp. nov., endemic to southern Australian coastal waters. PLoS ONE **6**:p.e24047.

Charlton-Robb, K., L. A. Gershwin, R. Thompson, J. Austin, K. Owen, and S. McKechnie. 2011b. A new dolphin species, the Burrunan Dolphin Tursiops australis sp. nov., endemic to southern Australian coastal waters. PLoS ONE **6**:e24047.

Charlton-Robb, K., A. C. Taylor, and S. W. McKechnie. 2014. Population genetic structure of the Burrunan dolphin (*Tursiops australis*) in coastal waters of south-eastern Australia: conservation implications. Conservation genetics **16**:195-207.

Chatterjee, N., and G. C. Walker. 2017. Mechanisms of DNA damage, repair, and mutagenesis. Environmental and Molecular Mutagenesis **58**:235-263.

Chen, I. C., J. K. Hill, R. Ohlemüller, D. B. Roy, and C. D. Thomas. 2011. Rapid Range Shifts of Species Associated with High Levels of Climate Warming. Science **333**:1024.

Cheng, Y., and K. Belov. 2011. Isolation and characterisation of 11 MHC-linked microsatellite loci in the Tasmanian devil (*Sarcophilus harrisii*). Conservation Genetics Resources **4**:463-465.

Chikina, M., J. D. Robinson, and N. L. Clark. 2016. Hundreds of Genes Experienced Convergent Shifts in Selective Pressure in Marine Mammals. Mol Biol Evol **33**:2182-2192.

Cho, Y. S., L. Hu, H. Hou, H. Lee, J. Xu, S. Kwon, S. Oh, H. M. Kim, S. Jho, S. Kim, Y. A. Shin, B. C. Kim, H. Kim, C. U. Kim, S. J. Luo, W. E. Johnson, K. P. Koepfli, A. Schmidt-Kuntzel, J. A. Turner, L. Marker, C. Harper, S. M. Miller, W. Jacobs, L. D. Bertola, T. H. Kim, S. Lee, Q. Zhou, H. J. Jung, X. Xu, P. Gadhvi, P. Xu, Y. Xiong, Y. Luo, S. Pan, C. Gou, X. Chu, J. Zhang, S. Liu, J. He, Y. Chen, L. Yang, Y. Yang, J. He, S. Liu, J. Wang, C. H. Kim, H. Kwak, J. S. Kim, S. Hwang, J. Ko, C. B. Kim, S. Kim, D. Bayarlkhagva, W. K. Paek, S. J. Kim, S. J. O'Brien, J. Wang, and J. Bhak. 2013. The tiger genome and comparative analysis with lion and snow leopard genomes. Nature Communications **4**:2433.

Chorev, M., A. Joseph Bekker, J. Goldberger, and L. Carmel. 2017. Identification of introns harboring functional sequence elements through positional conservation. Scientific Reports **7**:4201.

Cingolani, P., A. Platts, L. Wang le, M. Coon, T. Nguyen, L. Wang, S. J. Land, X. Lu, and D. M. Ruden. 2012. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly **6**:80-92.

Committee on Taxonomy of the Society for Marine Mammalogy. 2020. List of marine mammal species and subspecies. Society for Marine Mammalogy, www.marinemammalscience.org, consulted on 07/12/2020.

Costa, A. P. B., P. F. Fruet, E. R. Secchi, F. G. Daura-Jorge, P. C. Simoes-Lopes, J. C. Di Tullio, and P. E. Rosel. 2019. Ecological divergence and speciation in common bottlenose dolphins in the western South Atlantic. Journal of Evolutionary Biology.

Costa, A. P. B., P. E. Rosel, F. G. Daura-Jorge, and P. C. Simões-Lopes. 2016. Offshore and coastal common bottlenose dolphins of the western South Atlantic face-to-face: What the skull and the spine can tell us. Marine Mammal Science **32**:1433-1457.

Cunningham, A. A., P. Daszak, and J. L. N. Wood. 2017. One Health, emerging infectious diseases and wildlife: two decades of progress? Philosophical Transactions of the Royal Society B **372**:10.1098/rstb.2016.0167.

da Fontoura Budaszewski, R., and V. von Messling. 2016. Morbillivirus Experimental Animal Models: Measles Virus Pathogenesis Insights from Canine Distemper Virus. Viruses **8**:10.3390/v8100274.

Danecek, P., A. Auton, G. Abecasis, C. A. Albers, E. Banks, M. A. DePristo, R. E. Handsaker, G. Lunter, G. T. Marth, S. T. Sherry, G. McVean, R. Durbin, and 1000 Genomes Project Analysis Group. 2011. The variant call format and VCFtools. Bioinformatics **27**:2156-2158.

Davidson, A. D., A. G. Boyer, H. Kim, S. Pompa-Mansilla, M. J. Hamilton, D. P. Costa, G. Ceballos, and J. H. Brown. 2012. Drivers and hotspots of extinction risk in marine mammals. PNAS **109**:3395-3400.

Davies, K. T. J., N. C. Bennett, C. G. Faulkes, and S. J. Rossiter. 2018. Limited Evidence for Parallel Molecular Adaptations Associated with the Subterranean Niche in Mammals: A Comparative Study of Three Superorders. Mol Biol Evol **35**:2544-2559.

Dawson, M. N. 2005. Incipient speciation of Catostylus mosaicus (Scyphozoa, Rhizostomeae, Catostylidae), comparative phylogeography and biogeography in south-east Australia. Journal of Biogeography **32**:515-533.

de Jong, M. J., Z. Li, Y. Qin, E. Quemere, K. Baker, W. Wang, and A. R. Hoelzel. 2020. Demography and adaptation promoting evolutionary transitions in a mammalian genus that diversified during the Pleistocene. Molecular Ecology **29**:2777-2792.

de Sa, A. L. A., B. Breaux, T. C. T. Burlamaqui, T. C. Deiss, L. Sena, M. F. Criscitiello, and M. P. C. Schneider. 2019. The Marine Mammal Class II Major Histocompatibility Complex Organization. Frontiers in Immunology **10**:696.

Desforges, J.-P., A. Hall, B. McConnell, A. Rosing-Asvid, J. L. Barber, A. Brownlow, S. De Guise, I. Eulaers, P. D. Jepson, R. J. Letcher, M. Levin, P. S. Ross, F. Samarra, G. Víkingson, C. Sonne, and R. Dietz. 2018. Predicting global killer whale population collapse from PCB pollution. Science **361**:1373.

Dhiman, N., I. G. Ovsyannikova, R. A. Vierkant, J. E. Ryan, V. S. Pankratz, R. M. Jacobson, and G. A. Poland. 2008. Associations between SNPs in toll-like receptors and related intracellular signaling molecules and immune responses to measles vaccine: preliminary results. Vaccine **26**:1731-1736.

Di Guardo, G., G. Marruchella, U. Agrimi, and S. Kennedy. 2005. Morbillivirus Infections in Aquatic Mammals: A Brief Overview. Journal of Veterinary Medicine Series A **52**:88-93.

Diaz-Delgado, J., K. R. Groch, E. Sierra, S. Sacchini, D. Zucca, O. Quesada-Canales, M. Arbelo, A. Fernandez, E. Santos, J. Ikeda, R. Carvalho, A. F. Azevedo, J. Lailson-Brito, Jr., L. Flach, R. Ressio, C. T. Kanamura, M. Sansone, C. Favero, B. F. Porter, C. Centelleghe, S. Mazzariol, L. Di Renzo, G. Di Francesco, G. Di Guardo, and J. L. Catao-Dias. 2019. Comparative histopathologic and viral immunohistochemical studies on CeMV infection among Western Mediterranean, Northeast-Central, and Southwestern Atlantic cetaceans. PLoS ONE **14**:e0213363.

Diaz-Delgado, J., E. Sierra, A. I. Vela, M. Arbelo, D. Zucca, K. R. Groch, and A. Fernandez. 2017. Coinfection by Streptococcus phocae and cetacean morbillivirus in a short-beaked common dolphin *Delphinus delphis*. Diseases of Aquatic Organisms **124**:247-252.

Díaz-Gamboa, R. E., D. Gendron, and G. Busquets-Vass. 2018. Isotopic niche width differentiation between common bottlenose dolphin ecotypes and sperm whales in the Gulf of California. Marine Mammal Science **34**:440-457.

Dobrynin, P., S. Liu, G. Tamazian, Z. Xiong, A. A. Yurchenko, K. Krasheninnikova, S. Kliver, A. Schmidt-Kuntzel, K. P. Koepfli, W. Johnson, L. F. Kuderna, R. Garcia-Perez, M. Manuel, R. Godinez, A. Komissarov, A. Makunin, V. Brukhin, W. Qiu, L. Zhou, F. Li, J. Yi, C. Driscoll, A. Antunes, T. K. Oleksyk, E. Eizirik, P. Perelman, M. Roelke, D. Wildt, M. Diekhans, T. Marques-Bonet, L. Marker, J. Bhak, J. Wang, G. Zhang, and S. J. O'Brien. 2015. Genomic legacy of the African cheetah, *Acinonyx jubatus*. Genome Biology **16**:277.

Donaldson, R., H. Finn, and M. Calver. 2010. Illegal feeding increases risk of boat-strike and entanglement in Bottlenose Dolphins in Perth, Western Australia. Pacific Conservation Biology **16**:157-161.

Dong, C., R. J. Davis, and R. A. Flavell. 2002. MAP kinases in the immune response. Annual Review of Immunology **20**:55-72.

Dooley, C. T., T. Ferrer, H. Pagan, and G. M. O'Corry-Crowe. 2018. Bridging immunogenetics and immunoproteomics: Model positional scanning library analysis for Major Histocompatibility Complex class II DQ in *Tursiops truncatus*. PLoS ONE **13**:e0201299.

Doughty, C. E., J. Roman, S. Faurby, A. Wolf, A. Haque, E. S. Bakker, Y. Malhi, J. B. Dunning, Jr., and J. C. Svenning. 2016. Global nutrient transport in a world of giants. PNAS **113**:868-873.

Drew Harvell, C., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate Warming and Disease Risks for Terrestrial and Marine Biota. Science **292**:2158-2162.

Dudchenko, O., S. S. Batra, A. D. Omer, S. K. Nyquist, M. Hoeger, N. C. Durand, M. S. Shamim, I. Machol, E. S. Lander, A. P. Aiden, and E. L. Aiden. 2017. De novo assembly of the *Aedes aegypti* genome using Hi-C yields chromosome-length scaffolds. Science **356**:92-95.

Dudchenko, O., M. S. Shamim, S. Batra, N. C. Durand, N. T. Musial, R. Mostofa, M. Pham, B. G. S. Hilaire, W. Yao, E. Stamenova, M. Hoeger, S. K. Nyquist, V. Korchina, K. Pletch, J. P. Flanagan, A. Tomaszewicz, D. McAloose, C. P. Estrada, B. J. Novak, A. D. Omer, and E. L. Aiden. 2018. The Juicebox Assembly Tools module facilitates de novo assembly of mammalian genomes with chromosome-length scaffolds for under $1000. bioRxiv 254797.

Duignan, P. J., J. R. Geraci, J. A. Raga, and N. Calzada. 1992. Pathology of Morbillivirus Infection in Striped Dolphins (*Stenella coeruleoalba*) from Valencia and Murcia, Spain. Canadian Journal of Veterinary Research **56**:242-248.

Eddy, S. R. 2011. Accelerated Profile HMM Searches. PLoS Comput Biol **7**:e1002195.

Elbers, J. P., M. B. Brown, and S. S. Taylor. 2018. Identifying genome-wide immune gene variation underlying infectious disease in wildlife populations - a next generation sequencing approach in the gopher tortoise. BMC Genomics **19**:64.

Ellegren, H., and N. Galtier. 2016. Determinants of genetic diversity. Nature Reviews Genetics **17**:422-433.

Elmer, K. R., and A. Meyer. 2011. Adaptation in the age of ecological genomics: insights from parallelism and convergence. Trends in Ecology & Evolution **26**:298-306.

Epstein, B., M. Jones, R. Hamede, S. Hendricks, H. McCallum, E. P. Murchison, B. Schönfeld, C. Wiench, P. Hohenlohe, and A. Storfer. 2016. Rapid evolutionary response to a transmissible cancer in Tasmanian devils. Nature Communications **7**.

Estes, J. A., A. Burdin, and D. F. Doak. 2016a. Sea otters, kelp forests, and the extinction of Steller's sea cow. PNAS **113**:880-885.

Estes, J. A., M. Heithaus, D. J. McCauley, D. B. Rasher, and B. Worm. 2016b. Megafaunal Impacts on Structure and Function of Ocean Ecosystems. Annual Review of Environment and Resources **41**:83-116.

Estes, J. A., and J. F. Palmisano. 1974. Sea Otters: Their Role in Structuring Nearshore Communities. Science **185**:1058.

Fabregat, A., S. Jupe, L. Matthews, K. Sidiropoulos, M. Gillespie, P. Garapati, R. Haw, B. Jassal, F. Korninger, B. May, M. Milacic, C. D. Roca, K. Rothfels, C. Sevilla, V. Shamovsky, S. Shorser, T. Varusai, G. Viteri, J. Weiser, G. Wu, L. Stein, H. Hermjakob, and P. D'Eustachio. 2018. The Reactome Pathway Knowledgebase. Nucleic Acids Research **46**:D649-D655.

Fair, P. A., and P. R. Becker. 2000. Review of stress in marine mammals. Journal of Aquatic Ecosystem Stress and Recovery **7**:335–354.

Fair, P. A., A. M. Schaefer, D. S. Houser, G. D. Bossart, T. A. Romano, C. D. Champagne, J. L. Stott, C. D. Rice, N. White, and J. S. Reif. 2017. The environment as a driver of immune and endocrine responses in dolphins (*Tursiops truncatus*). PLoS ONE **12**:e0176202.

Fan, G., Y. Zhang, X. Liu, J. Wang, Z. Sun, S. Sun, H. Zhang, J. Chen, M. Lv, K. Han, X. Tan, J. Hu, R. Guan, Y. Fu, S. Liu, X. Chen, Q. Xu, Y. Qin, L. Liu, J. Bai, O. Wang, J. Tang, H. Lu, Z. Shang, B. Wang, G. Hu, X. Zhao, Y. Zou, A. Chen, M. Gong, W. Zhang, S. M. Lee, S. Li, J. Liu, Z. Li, Y. Lu, J. S. M. Sabir, M. J. Sabir, M. Khan, N. H. Hajrah, Y. Yin, K. Kristiansen, H. Yang, J. Wang, X. Xu, and X. Liu. 2019. The first chromosome-level genome for a marine mammal as a resource to study ecology and evolution. Molecular Ecology Resources.

Fenner, F. 2000. Adventures with poxviruses of vertebrates. FEMS Microbiology Reviews **24**:123-133.

Fish, R., A. Church, and M. Winter. 2016. Conceptualising cultural ecosystem services: A novel framework for research and critical engagement. Ecosystem Services **21**:208-217.

Fitzpatrick, B. M., J. A. Fordyce, M. L. Niemiller, and R. G. Reynolds. 2011. What can DNA tell us about biological invasions? Biological Invasions **14**:245-253.

Foden, W. B., S. H. Butchart, S. N. Stuart, J. C. Vie, H. R. Akcakaya, A. Angulo, L. M. DeVantier, A. Gutsche, E. Turak, L. Cao, S. D. Donner, V. Katariya, R. Bernard, R. A. Holland, A. F. Hughes, S. E. O'Hanlon, S. T. Garnett, C. H. Sekercioglu, and G. M. Mace. 2013. Identifying the world's most climate change vulnerable species: a systematic trait-based assessment of all birds, amphibians and corals. PLoS ONE **8**:e65427.

Foote, A. D., Y. Liu, G. W. C. Thomas, T. Vinař, J. Alföldi, J. Deng, S. Dugan, C. E. Van Elk, M. E. Hunter, V. Joshi, Z. Khan, C. Kovar, S. L. Lee, K. Lindblad-Toh, A. Mancia, R. Nielsen, X. Qin, J. Qu, B. J. Raney, N. Vijay, J. B. W. Wolf, M. W. Hahn, D. M. Muzny, K. C. Worley, M. T. P. Gilbert, and R. A. Gibbs. 2015. Convergent evolution of the genomes of marine mammals. Nature Genetics **47**:272-275.

Foote, A. D., M. D. Martin, M. Louis, G. Pacheco, K. M. Robertson, M. S. Sinding, A. R. Amaral, R. W. Baird, C. S. Baker, L. Ballance, J. Barlow, A. Brownlow, T. Collins, R. Constantine, W. Dabin, L. Dalla Rosa, N. J. Davison, J. W. Durban, R. Esteban, S. H. Ferguson, T. Gerrodette, C. Guinet, M. B. Hanson, W. Hoggard, C. J. D. Matthews, F. I. P. Samarra, R. de Stephanis, S. B. Tavares, P. Tixier, J. A. Totterdell, P. Wade, L. Excoffier, M. T. P. Gilbert, J. B. W. Wolf, and P. A. Morin. 2019. Killer whale genomes

reveal a complex history of recurrent admixture and vicariance. Molecular Ecology **28**:3427-3444.

Foote, A. D., and P. A. Morin. 2016. Genome-wide SNP data suggest complex ancestry of sympatric North Pacific killer whale ecotypes. Heredity **117**:316-325.

Foote, A. D., N. Vijay, M. C. Avila-Arcos, R. W. Baird, J. W. Durban, M. Fumagalli, R. A. Gibbs, M. B. Hanson, T. S. Korneliussen, M. D. Martin, K. M. Robertson, V. C. Sousa, F. G. Vieira, T. Vinar, P. Wade, K. C. Worley, L. Excoffier, P. A. Morin, M. T. P. Gilbert, and J. B. W. Wolf. 2016. Genome-culture coevolution promotes rapid divergence of killer whale ecotypes. Nature Communications **7**:11693.

Fraik, A. K., M. J. Margres, B. Epstein, S. Barbosa, M. Jones, S. Hendricks, B. Schonfeld, A. R. Stahlke, A. Veillet, R. Hamede, H. McCallum, E. Lopez-Contreras, S. J. Kallinen, P. A. Hohenlohe, J. L. Kelley, and A. Storfer. 2020. Disease swamps molecular signatures of genetic-environmental associations to abiotic factors in Tasmanian devil (*Sarcophilus harrisii*) populations. Evolution **74**:1392-1408.

Frere, C. H., M. Krutzen, A. M. Kopps, P. Ward, J. Mann, and W. B. Sherwin. 2010. Inbreeding tolerance and fitness costs in wild bottlenose dolphins. Proceedings of the Royal Society B **277**:2667-2673.

Fric, J., T. Zelante, and P. Ricciardi-Castagnoli. 2014. Phagocytosis of Particulate Antigens - All Roads Lead to Calcineurin/NFAT Signaling Pathway. Frontiers in Immunology **4**:513.

Frick, W. F., J. F. Pollock, A. C. Hicks, K. E. Langwig, D. S. Reynolds, G. G. Turner, C. M. Butchkoski, and T. H. Kunz. 2010. An Emerging Disease Causes Regional Population Collapse of a Common North American Bat Species. Science **329**:679.

Fruet, P. F., P. G. Kinas, K. G. da Silva, J. C. Di Tullio, D. S. Monteiro, L. D. Rosa, S. C. Estima, and E. R. Secchi. 2012. Temporal trends in mortality and effects of by-catch on common bottlenose dolphins, Tursiops truncatus, in southern Brazil. Journal of the Marine Biological Association of the United Kingdom **92**:1865-1876.

Fruet, P. F., L. M. Möller, and E. R. Secchi. 2021. Dynamics and Viability of a Small, Estuarine-Resident Population of Lahille's Bottlenose Dolphins From Southern Brazil. Frontiers in Marine Science **7**.

Fruet, P. F., E. R. Secchi, F. Daura-Jorge, E. Vermeulen, P. A. C. Flores, P. C. Simões-Lopes, R. C. Genoves, P. Laporta, J. C. Di Tullio, T. R. O. Freitas, L. D. Rosa, V. H. Valiati, L. B. Beheregaray, and L. M. Möller. 2014. Remarkably low genetic diversity and strong population structure in common bottlenose dolphins (Tursiops truncatus) from coastal waters of the Southwestern Atlantic Ocean. Conservation genetics.

Fruet, P. F., E. R. Secchi, J. C. Di Tullio, P. C. Simoes-Lopes, F. Daura-Jorge, A. P. B. Costa, E. Vermeulen, P. A. C. Flores, R. C. Genoves, P. Laporta, L. B. Beheregaray, and L. M. Moller. 2017. Genetic divergence between two phenotypically distinct bottlenose dolphin ecotypes suggests separate evolutionary trajectories. Ecology and Evolution **7**:9131-9143.

Fury, C. A., and J. S. Reif. 2012. Incidence of poxvirus-like lesions in two estuarine dolphin populations in Australia: links to flood events. Science of the Total Environment **416**:536-540.

Garner, B. A., S. Hoban, and G. Luikart. 2020. IUCN Red List and the value of integrating genetics. Conservation genetics **21**:795-801.

Gaspari, S., A. Scheinin, D. Holcer, C. Fortuna, C. Natali, T. Genov, A. Frantzis, G. Chelazzi, and A. E. Moura. 2015. Drivers of Population Structure of the Bottlenose Dolphin (Tursiops truncatus) in the Eastern Mediterranean Sea. Evolutionary Biology **42**:177-190.

Gaylard, S. 2017. Per and polyfluorinated alkyl substances (PFAS) in the marine environment – Preliminary ecological findings. Environment Protection Authority.

Gelain, M. E., and F. Bonsembiante. 2019. Acute Phase Proteins in Marine Mammals: State of Art, Perspectives and Challenges. Frontiers in Immunology **10**:1220.

Genoves, R. C., P. F. Fruet, S. Botta, L. B. Beheregaray, L. M. Möller, and E. R. Secchi. 2020. Fine-scale genetic structure in Lahille's bottlenose dolphins (*Tursiops truncatus gephyreus*) is associated with social structure and feeding ecology. Marine Biology **167**.

Gray, H. W. I., S. Nishida, A. J. Welch, A. E. Moura, S. Tanabe, M. S. Kiani, R. Culloch, L. Moller, A. Natoli, L. S. Ponnampalam, G. Minton, M. Gore, T. Collins, A. Willson, R. Baldwin, and A. R. Hoelzel. 2018. Cryptic lineage differentiation among Indo-Pacific bottlenose dolphins (*Tursiops aduncus*) in the northwest Indian Ocean. Mol Phylogenet Evol **122**:1-14.

Gridley, T., S. H. Elwen, G. Harris, D. M. Moore, A. R. Hoelzel, and F. Lampen. 2018. Hybridization in bottlenose dolphins-A case study of Tursiops aduncus x T. truncatus hybrids and successful backcross hybridization events. PLoS ONE **13**:e0201722.

Grummer, J. A., L. B. Beheregaray, L. Bernatchez, B. K. Hand, G. Luikart, S. R. Narum, and E. B. Taylor. 2019. Aquatic Landscape Genomics and Environmental Effects on Genetic Variation. Trends in Ecology & Evolution **34**:641-654.

Gui, D., K. Jia, J. Xia, L. Yang, J. Chen, Y. Wu, and M. Yi. 2013. De novo assembly of the Indo-Pacific humpback dolphin leucocyte transcriptome to identify putative genes involved in the aquatic adaptation and immune response. PLoS ONE **8**:e72417.

Guigó, R., and S. Ullrich. 2020. Dynamic changes in intron retention are tightly associated with regulation of splicing factors and proliferative activity during B-cell development. Nucleic Acids Research **48**:1327-1340.

Guindon, S., J. F. Dufayard, V. Lefort, M. Anisimova, W. Hordijk, and O. Gascuel. 2010. New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. Systematic Biology **59**:307-231.

Gulland, F. M. D., and A. J. Hall. 2007. Is Marine Mammal Health Deteriorating? Trends in the Global Reporting of Marine Mammal Disease. EcoHealth **4**:135-150.

Gurevich, A., V. Saveliev, N. Vyahhi, and G. Tesler. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics **29**:1072-1075.

Hagen, W., G. Kattner, and C. Friedrich. 2000. The lipid compositions of high-Antarctic notothenioid fish species with different life strategies. Polar Biology **23**:785-791.

Hamilton, C. D., J. Vacquie-Garcia, K. M. Kovacs, R. A. Ims, J. Kohler, and C. Lydersen. 2019. Contrasting changes in space use induced by climate change in two Arctic marine mammal species. Biol Lett **15**:20180834.

Hammond, P. S., G. Bearzi, A. Bjørge, K. Forney, L. Karczmarski, T. Kasuya, W. F. Perrin, M. D. Scott, J. Y. Wang, R. S. Wells, and B. Wilson. 2008. *Delphinus delphis*. The IUCN Red List of Threatened Species.

Haralambieva, I. H., R. B. Kennedy, I. G. Ovsyannikova, J. A. Whitaker, and G. A. Poland. 2015. Variability in Humoral Immunity to Measles Vaccine: New Developments. Trends in Molecular Medicine **21**:789-801.

Hashiguchi, T., T. Ose, M. Kubota, N. Maita, J. Kamishikiryo, K. Maenaka, and Y. Yanagi. 2011. Structure of the measles virus hemagglutinin bound to its cellular receptor SLAM. Nature Structural & Molecular Biology **18**:135-141.

Hazen, E. L., B. Abrahms, S. Brodie, G. Carroll, M. G. Jacox, M. S. Savoca, K. L. Scales, W. J. Sydeman, and S. J. Bograd. 2019. Marine top predators as climate and ecosystem sentinels. Frontiers in Ecology and the Environment **17**:565-574.

Hendricks, S., B. Epstein, B. Schonfeld, C. Wiench, R. Hamede, M. Jones, A. Storfer, and P. Hohenlohe. 2017. Conservation implications of limited genetic diversity and population structure in Tasmanian devils (*Sarcophilus harrisii*). Conservation genetics **18**:977-982.

Hershkovitz, P. 1966. Catalog of Living Whales. Bulletin of the United States National Museum.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature **405**:907–913.

Hindle, A. G. 2020. Diving deep: understanding the genetic components of hypoxia tolerance in marine mammals. Journal of Applied Physiology **128**:1439-1446.

Hoban, S., M. Bruford, J. D'Urban Jackson, M. Lopes-Fernandes, M. Heuertz, P. A. Hohenlohe, I. Paz-Vinas, P. Sjögren-Gulve, G. Segelbacher, C. Vernesi, S. Aitken, L. D. Bertola, P. Bloomer, M. Breed, H. Rodríguez-Correa, W. C. Funk, C. E. Grueber, M. E. Hunter, R. Jaffe, L. Liggins, J. Mergeay, F. Moharrek, D. O'Brien, R. Ogden, C. Palma-Silva, J. Pierson, U. Ramakrishnan, M. Simo-Droissart, N. Tani, L. Waits, and L. Laikre. 2020a. Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. Biological Conservation **248**.

Hoban, S., C. Campbell, J. da Silva, R. Ekblom, W. C. Funk, B. Garner, J. A. Godoy, F. Kershaw, A. MacDonald, J. Mergeay, M. Minter, D. O'Brien, I. Paz-Vinas, S. K. Pearson, S. Perez-Espona, K. Potter, I.-R. Russo, G. Segelbacher, C. Vernesi, and M. E. Hunter. 2020b. An analysis of genetic diversity actions, indicators and targets in 114 National Reports to the Convention on Biological Diversity. bioRxiv.

Hoegh-Guldberg, O., and J. F. Bruno. 2010. The Impact of Climate Change on the World's Marine Ecosystems. Science **328**:1523-1528.

Hoelzel, R. A., C. W. Potter, and P. B. Best. 1998. Genetic differentiation between parapatric `nearshore' and `offshore' populations of the bottlenose dolphin. Proceedings of the Royal Society B **265**:1177–1183.

Hoffman, J. I., F. Simpson, P. David, J. M. Rijks, T. Kuiken, M. A. Thorne, R. C. Lacy, and K. K. Dasmahapatra. 2014. High-throughput sequencing reveals inbreeding depression in a natural population. PNAS **111**:3775-3780.

Hogg, C. J., A. V. Lee, C. Srb, and C. Hibbard. 2017. Metapopulation management of an Endangered species with limited genetic diversity in the presence of disease: the Tasmanian devilSarcophilus harrisii. International Zoo Yearbook **51**:137-153.

Hogg, C. J., E. A. McLennan, P. Wise, A. V. Lee, D. Pemberton, S. Fox, K. Belov, and C. E. Grueber. 2020. Preserving the demographic and genetic integrity of a single source population during multiple translocations. Biological Conservation **241**.

Höglund, J. 2009. Evolutionary Conservation Genetics. Oxford University Press, Oxford.

Hohl, L. S. L., F. L. Sicuro, J. C. Wickert, I. B. Moreno, O. Rocha-Barbosa, and A. S. Barreto. 2020. Skull morphology of bottlenose dolphins from different ocean populations with emphasis on South America. Journal of Morphology **281**:564-577.

Hooper, D. U., F. S. Chapin III, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. **75**:3-35.

Hooper, R., L. Excoffier, K. A. Forney, M. T. P. Gilbert, M. D. Martin, P. A. Morin, J. B. W. Wolf, and A. D. Foote. 2020. Runs of homozygosity in killer whale genomes provide a global record of demographic histories. bioRxiv.

Hostetler, S., N. Pisias, and A. Mix. 2006. Sensitivity of Last Glacial Maximum climate to uncertainties in tropical and subtropical ocean temperatures. Quaternary Science Reviews **25**:1168-1185.

Howrigan, D. P., M. A. Simonson, and M. C. Keller. 2011. Detecting autozygosity through runs of homozygosity: A comparison of three autozygosity detection algorithms. BMC Genomics **12**:460.

Huelsmann, M., N. Hecker, M. S. Springer, J. Gatesy, V. Sharma, and M. Hiller. 2019. Genes lost during the transition from land to water in cetaceans highlight genomic changes associated with aquatic adaptations. Evolutionary Biology **5**:eaaw6671.

International HapMap, C., K. A. Frazer, D. G. Ballinger, D. R. Cox, D. A. Hinds, L. L. Stuve, R. A. Gibbs, J. W. Belmont, A. Boudreau, P. Hardenbol, S. M. Leal, S. Pasternak, D. A. Wheeler, T. D. Willis, F. Yu, H. Yang, C. Zeng, Y. Gao, H. Hu, W. Hu, C. Li, W. Lin, S. Liu, H. Pan, X. Tang, J. Wang, W. Wang, J. Yu, B. Zhang, Q. Zhang, H. Zhao, H. Zhao, J. Zhou, S. B. Gabriel, R. Barry, B. Blumenstiel, A. Camargo, M. Defelice, M. Faggart, M. Goyette, S. Gupta, J. Moore, H. Nguyen, R. C. Onofrio, M. Parkin, J. Roy, E. Stahl, E. Winchester, L. Ziaugra, D. Altshuler, Y. Shen, Z. Yao, W. Huang, X. Chu, Y. He, L. Jin, Y. Liu, Y. Shen, W. Sun, H. Wang, Y. Wang, Y. Wang, X. Xiong, L. Xu, M. M. Waye, S. K. Tsui, H. Xue, J. T. Wong, L. M. Galver, J. B. Fan, K. Gunderson, S. S. Murray, A. R. Oliphant, M. S. Chee, A. Montpetit, F. Chagnon, V. Ferretti, M. Leboeuf, J. F. Olivier, M. S. Phillips, S. Roumy, C. Sallee, A. Verner, T. J. Hudson, P. Y. Kwok, D. Cai, D. C. Koboldt, R. D. Miller, L. Pawlikowska, P. Taillon-Miller, M. Xiao, L. C. Tsui, W. Mak, Y. Q. Song, P. K. Tam, Y. Nakamura, T. Kawaguchi, T. Kitamoto, T. Morizono, A. Nagashima, Y. Ohnishi, A. Sekine, T. Tanaka, T. Tsunoda, P. Deloukas, C. P. Bird, M. Delgado, E. T. Dermitzakis, R. Gwilliam, S. Hunt, J. Morrison, D. Powell, B. E. Stranger, P. Whittaker, D. R. Bentley, M. J. Daly, P. I. de Bakker, J. Barrett, Y. R. Chretien, J. Maller, S. McCarroll, N. Patterson, I. Pe'er, A. Price, S. Purcell, D. J. Richter, P. Sabeti, R. Saxena, S. F. Schaffner, P. C. Sham, P. Varilly, D. Altshuler, L. D. Stein, L. Krishnan, A. V. Smith, M. K. Tello-Ruiz, G. A. Thorisson, A. Chakravarti, P. E. Chen, D. J. Cutler, C. S. Kashuk, S. Lin, G. R. Abecasis, W. Guan, Y. Li, H. M. Munro, Z. S. Qin, D. J. Thomas, G. McVean, A. Auton, L. Bottolo, N. Cardin, S. Eyheramendy, C. Freeman, J. Marchini, S. Myers, C. Spencer, M. Stephens, P. Donnelly, L. R. Cardon, G. Clarke, D. M. Evans, A. P. Morris, B. S. Weir, T. Tsunoda, J. C. Mullikin, S. T. Sherry, M. Feolo, A. Skol, H. Zhang, C. Zeng, H. Zhao, I. Matsuda, Y. Fukushima, D. R. Macer, E. Suda, C. N. Rotimi, C. A. Adebamowo, I. Ajayi, T. Aniagwu, P. A. Marshall, C. Nkwodimmah, C. D. Royal, M. F. Leppert, M. Dixon, A. Peiffer, R. Qiu, A. Kent, K. Kato, N. Niikawa, I. F. Adewole, B. M. Knoppers, M. W. Foster, E. W. Clayton, J. Watkin, R. A. Gibbs, J. W. Belmont, D. Muzny, L. Nazareth, E. Sodergren, G. M. Weinstock, D. A. Wheeler, I. Yakub, S. B. Gabriel, R. C. Onofrio, D. J. Richter, L. Ziaugra, B. W. Birren, M. J. Daly, D. Altshuler, R. K. Wilson, L. L. Fulton, J. Rogers, J. Burton, N. P. Carter, C. M. Clee, M. Griffiths, M. C. Jones, K. McLay, R. W. Plumb, M. T. Ross, S. K. Sims, D. L. Willey, Z. Chen, H. Han, L. Kang, M. Godbout, J. C. Wallenburg, P. L'Archeveque, G. Bellemare, K. Saeki, H. Wang, D. An, H. Fu, Q. Li, Z. Wang, R. Wang, A. L. Holden, L. D. Brooks, J. E. McEwen, M. S. Guyer, V. O. Wang, J. L. Peterson, M. Shi, J. Spiegel, L. M. Sung, L. F. Zacharia, F. S. Collins, K. Kennedy, R. Jamieson, and J. Stewart. 2007. A second generation human haplotype map of over 3.1 million SNPs. Nature **449**:851-861.

IUCN. 2020. The IUCN Red List of Threatened Species. Version 2020-3. https://www.iucnredlist.org. Downloaded on 28/12/2020.

Jaramillo-Legorreta, A. M., G. Cardenas-Hinojosa, E. Nieto-Garcia, L. Rojas-Bracho, L. Thomas, J. M. Ver Hoef, J. Moore, B. Taylor, J. Barlow, and N. Tregenza. 2019. Decline towards extinction of Mexico's vaquita porpoise (Phocoena sinus). Royal Society Open Science **6**:190598.

Jedensjö, M., C. M. Kemper, and M. Krützen. 2017. Cranial morphology and taxonomic resolution of some dolphin taxa (Delphinidae) in Australian waters, with a focus on the genusTursiops. Marine Mammal Science **33**:187-205.

Jedensjö, M., C. M. Kemper, M. Milella, E. P. Willems, and M. Krützen. 2020. Taxonomy and distribution of bottlenose dolphins (genus *Tursiops*) in Australian waters: an osteological clarification. Canadian Journal of Zoology **98**:461-479.

Jo, B. S., and S. S. Choi. 2015. Introns: The Functional Benefits of Introns in Genomes. Genomics Informatics **13**:112-118.

Jo, W. K., J. Kruppa, A. Habierski, M. van de Bildt, S. Mazzariol, G. Di Guardo, U. Siebert, T. Kuiken, K. Jung, A. Osterhaus, and M. Ludlow. 2018. Evolutionary evidence for multi-host transmission of cetacean morbillivirus. Emerging Microbes & Infections **7**:201.

Johnson, R. N., D. O'Meally, Z. Chen, G. J. Etherington, S. Y. W. Ho, W. J. Nash, C. E. Grueber, Y. Cheng, C. M. Whittington, S. Dennison, E. Peel, W. Haerty, R. J. O'Neill, D. Colgan, T. L. Russell, D. E. Alquezar-Planas, V. Attenbrow, J. G. Bragg, P. A. Brandies, A. Y. Chong, J. E. Deakin, F. Di Palma, Z. Duda, M. D. B. Eldridge, K. M. Ewart, C. J. Hogg, G. J. Frankham, A. Georges, A. K. Gillett, M. Govendir, A. D. Greenwood, T. Hayakawa, K. M. Helgen, M. Hobbs, C. E. Holleley, T. N. Heider, E. A. Jones, A. King, D. Madden, J. A. M. Graves, K. M. Morris, L. E. Neaves, H. R. Patel, A. Polkinghorne, M. B. Renfree, C. Robin, R. Salinas, K. Tsangaras, P. D. Waters, S. A. Waters, B. Wright, M. R. Wilkins, P. Timms, and K. Belov. 2018. Adaptation and conservation insights from the koala genome. Nature Genetics **50**:1102-1111.

Johnstone, C. P., A. Lill, and R. D. Reina. 2014. Habitat loss, fragmentation and degradation effects on small mammals: Analysis with conditional inference tree statistical modelling. Biological Conservation **176**:80-98.

Jones, F. C., M. G. Grabherr, Y. F. Chan, P. Russell, E. Mauceli, J. Johnson, R. Swofford, M. Pirun, M. C. Zody, S. White, E. Birney, S. Searle, J. Schmutz, J. Grimwood, M. C. Dickson, R. M. Myers, C. T. Miller, B. R. Summers, A. K. Knecht, S. D. Brady, H. Zhang, A. A. Pollen, T. Howes, C. Amemiya, P. Broad Institute Genome Sequencing, T. Whole Genome Assembly, J. Baldwin, T. Bloom, D. B. Jaffe, R. Nicol, J. Wilkinson, E. S. Lander, F. Di Palma, K. Lindblad-Toh, and D. M. Kingsley. 2012. The genomic basis of adaptive evolution in threespine sticklebacks. Nature **484**:55-61.

Jones, M. E., D. Paetkau, E. Geffen, and C. Moritz. 2003. Microsatellites for the Tasmanian devil (*Sarcophilus laniarius*). Molecular Ecology Notes **3**:277-279.

Kardos, M., G. Luikart, R. Bunch, S. Dewey, W. Edwards, S. McWilliam, J. Stephenson, F. W. Allendorf, J. T. Hogg, and J. Kijas. 2015. Whole-genome resequencing uncovers molecular signatures of natural and sexual selection in wild bighorn sheep. Molecular Ecology **24**:5616-5632.

Karlsson, E. K., D. P. Kwiatkowski, and P. C. Sabeti. 2014. Natural selection and infectious disease in human populations. Nature Reviews Genetics **15**:379-393.

Keane, M., J. Semeiks, A. E. Webb, Y. I. Li, V. Quesada, T. Craig, L. B. Madsen, S. van Dam, D. Brawand, P. I. Marques, P. Michalak, L. Kang, J. Bhak, H. S. Yim, N. V. Grishin, N. H. Nielsen, M. P. Heide-Jorgensen, E. M. Oziolor, C. W. Matson, G. M. Church, G. W. Stuart, J. C. Patton, J. C. George, R. Suydam, K. Larsen, C. Lopez-Otin, M. J. O'Connell, J. W. Bickham, B. Thomsen, and J. P. de Magalhaes. 2015. Insights into the evolution of longevity from the bowhead whale genome. Cell Reports **10**:112-122.

Keller, O., M. Kollmar, M. Stanke, and S. Waack. 2011. A novel hybrid gene prediction method employing protein multiple sequence alignments. Bioinformatics **27**.

Kelley, J. L., A. P. Brown, N. O. Therkildsen, and A. D. Foote. 2016. The life aquatic: advances in marine vertebrate genomics. Nature Reviews Genetics **17**:523-534.

Kemper, C. M., M. Bossley, and P. Shaughnessy. 2008. Marine mammals of Gulf St Vincent, investigator strait and backstairs passage, In *Natural history of GulfSt Vincent* (eds S Shepherd, S Bryars, IR Kirkegaard, P Harbison, JT Jennings).339–352.

Kemper, C. M., I. Tomo, J. Bingham, S. S. Bastianello, J. Wang, S. E. Gibbs, L. Woolford, C. Dickason, and D. Kelly. 2016. Morbillivirus-associated unusual mortality event in South Australian bottlenose dolphins is largest reported for the Southern Hemisphere. Royal Society Open Science **3**:160838.

Kerosky, S. M., A. Širović, L. K. Roche, S. Baumann-Pickering, S. M. Wiggins, and J. A. Hildebrand. 2012. Bryde's whale seasonal range expansion and increasing presence in the Southern California Bight from 2000 to 2010. Deep Sea Research Part I: Oceanographic Research Papers **65**:125-132.

Kishida, T., J. Thewissen, T. Hayakawa, H. Imai, and K. Agata. 2015. Aquatic adaptation and the evolution of smell and taste in whales. Zoological Letters **1**:9.

Klein, M., S. Klein-Hessling, A. Palmetshofer, E. Serfling, C. Tertilt, T. Bopp, V. Heib, M. Becker, C. Taube, H. Schild, E. Schmitt, and M. Stassen. 2006. Specific and redundant roles for NFAT transcription factors in the expression of mast cell-derived cytokines. The Journal of Immunology **177**:6667-6674.

Koepfli, K. P., B. Paten, K. C. o. S. Genome, and S. J. O'Brien. 2015. The Genome 10K Project: a way forward. Annual Review of Animal Biosciences **3**:57-111.

Kolmogorov, M., J. Armstrong, B. J. Raney, I. Streeter, M. Dunn, F. Yang, D. Odom, P. Flicek, T. M. Keane, D. Thybert, B. Paten, and S. Pham. 2018. Chromosome assembly of large and complex genomes using multiple references. Genome Res **28**:1720-1732.

Korneliussen, T. S., A. Albrechtsen, and R. Nielsen. 2014. ANGSD: Analysis of Next Generation Sequencing Data. BMC Bioinformatics **15**:356.

Kosiol, C., T. Vinar, R. R. da Fonseca, M. J. Hubisz, C. D. Bustamante, R. Nielsen, and A. Siepel. 2008. Patterns of positive selection in six Mammalian genomes. PLoS Genet **4**:e1000144.

Kriventseva, E. V., D. Kuznetsov, F. Tegenfeldt, M. Manni, R. Dias, F. A. Simao, and E. M. Zdobnov. 2019. OrthoDB v10: sampling the diversity of animal, plant, fungal, protist, bacterial and viral genomes for evolutionary and functional annotations of orthologs. Nucleic Acids Research **47**:D807-D811.

Krützen, M., L. Barre, L. M. Möller, M. Heithaus, C. Simms, and W. Sherwin. 2002. A biopsy system for small cetaceans: Darting success and wound healing in *Tursiops* spp. Marine Mammal Science **18**:863-878.

Krutzen, M., W. B. Sherwin, R. C. Connor, L. M. Barre, T. Van de Casteele, J. Mann, and R. Brooks. 2003. Contrasting relatedness patterns in bottlenose dolphins (*Tursiops* sp.) with different alliance strategies. Proceedings of the Royal Society B **270**:497-502.

Kunstner, A., J. B. Wolf, N. Backstrom, O. Whitney, C. N. Balakrishnan, L. Day, S. V. Edwards, D. E. Janes, B. A. Schlinger, R. K. Wilson, E. D. Jarvis, W. C. Warren, and H. Ellegren. 2010. Comparative genomics based on massive parallel transcriptome sequencing reveals patterns of substitution and selection across 10 bird species. Molecular Ecology **19 Suppl 1**:266-276.

Lacy, P. 2006. Mechanisms of Degranulation in Neutrophils. Allergy, Asthma & Clinical Immunology **2**.

Lah, L., D. Trense, H. Benke, P. Berggren, T. Gunnlaugsson, C. Lockyer, A. Ozturk, B. Ozturk, I. Pawliczka, A. Roos, U. Siebert, K. Skora, G. Vikingsson, and R. Tiedemann. 2016. Spatially Explicit Analysis of Genome-Wide SNPs Detects Subtle Population Structure in a Mobile Marine Mammal, the Harbor Porpoise. PLoS ONE **11**:e0162792.

Laikre, L. 2010. Genetic diversity is overlooked in international conservation policy implementation. Conservation genetics **11**:349-354.

Laikre, L., S. Hoban, M. W. Bruford, G. Segelbacher, F. W. Allendorf, G. Gajardo, A. G. Rodríguez, P. W. Hedrick, M. Heuertz, P. A. Hohenlohe, R. Jaffé, K. Johannesson, L. Liggins, A. J. MacDonald, P. OrozcoterWengel, T. B. H. Reusch, H. Rodríguez-Correa, I. M. Russo, N. Ryman, and C. Vernesi. 2020. Post-2020 goals overlook genetic diversity. Science **367**:1083-1085.

Lambeck, K., and J. Chappell. 2001. Sea level change through the last glacial cycle. Science **292**:679–686.

Lammers, F., M. Blumer, C. Rücklé, and M. A. Nilsson. 2019. Retrophylogenomics in rorquals indicate large ancestral population sizes and a rapid radiation. Mobile DNA **10**.

Langmead, B., and S. Salzberg. 2012. Fast gapped-read alignment with bowtie 2. Nature Methods **9**:357-359.

Larsen, P. A., and M. D. Matocq. 2019. Emerging genomic applications in mammalian ecology, evolution, and conservation. Journal of Mammalogy **100**:786-801.

Lavery, T. J., N. Butterfield, C. M. Kemper, R. J. Reid, and K. Sanderson. 2008. Metals and selenium in the liver and bone of three dolphin species from South Australia, 1988-2004. Science of the Total Environment **390**:77-85.

Lawrence, K. T., and T. D. Herbert. 2005. Late quaternary sea-surface temperatures in the western Coral Sea: implications for the growth of the Australian Great Barrier Reef. Geology **33**:677-680.

Lazenby, B. T., M. W. Tobler, W. E. Brown, C. E. Hawkins, G. J. Hocking, F. Hume, S. Huxtable, P. Iles, M. E. Jones, C. Lawrence, S. Thalmann, P. Wise, H. Williams, S. Fox, and D. Pemberton. 2018. Density trends and demographic signals uncover the long-term impact of transmissible cancer in Tasmanian devils. Journal of Applied Ecology **55**:1368-1379.

Lê, S., J. Josse, and F. Husson. 2008. FactoMineR: A Package for Multivariate Analysis. Journal of Statistical Software **25**:1-18.

Learmonth, J., C. MacLeod, M. Santos, G. Pierce, H. Crick, and R. Robinson. 2006. Potential Effects Of Climate Change On Marine Mammals. Pages 431-464 Oceanography and Marine Biology.

Lee, H. H., M. M. Wallen, E. Krzyszczyk, and J. Mann. 2019. Every scar has a story: age and sex-specific conflict rates in wild bottlenose dolphins. Behavioral Ecology and Sociobiology **73**.

Leigh, D. M., A. P. Hendry, E. Vazquez-Dominguez, and V. L. Friesen. 2019. Estimated six per cent loss of genetic variation in wild populations since the industrial revolution. Evolutionary Applications **12**:1505-1512.

Li, H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics **27**:2987-2993.

Li, H. 2018. Minimap2: pairwise alignment for nucleotide sequences. Bioinformatics **34**:3094-3100.

Li, H., B. Handsaker, A. Wysoker, T. Fennell, J. Ruan, N. Homer, G. Marth, G. Abecasis, R. Durbin, and Genome Project Data Processing Subgroup. 2009. The Sequence Alignment/Map format and SAMtools. Bioinformatics **25**:2078-2079.

Li, M. O., Y. Y. Wan, S. Sanjabi, A. K. Robertson, and R. A. Flavell. 2006. Transforming growth factor-beta regulation of immune responses. Annual Review of Immunology **24**:99-146.

Lindblad-Toh, K., M. Garber, O. Zuk, M. F. Lin, B. J. Parker, S. Washietl, P. Kheradpour, J. Ernst, G. Jordan, E. Mauceli, L. D. Ward, C. B. Lowe, A. K. Holloway, M. Clamp, S.

Gnerre, J. Alfoldi, K. Beal, J. Chang, H. Clawson, J. Cuff, F. Di Palma, S. Fitzgerald, P. Flicek, M. Guttman, M. J. Hubisz, D. B. Jaffe, I. Jungreis, W. J. Kent, D. Kostka, M. Lara, A. L. Martins, T. Massingham, I. Moltke, B. J. Raney, M. D. Rasmussen, J. Robinson, A. Stark, A. J. Vilella, J. Wen, X. Xie, M. C. Zody, P. Broad Institute Sequencing, T. Whole Genome Assembly, J. Baldwin, T. Bloom, C. W. Chin, D. Heiman, R. Nicol, C. Nusbaum, S. Young, J. Wilkinson, K. C. Worley, C. L. Kovar, D. M. Muzny, R. A. Gibbs, T. Baylor College of Medicine Human Genome Sequencing Center Sequencing, A. Cree, H. H. Dihn, G. Fowler, S. Jhangiani, V. Joshi, S. Lee, L. R. Lewis, L. V. Nazareth, G. Okwuonu, J. Santibanez, W. C. Warren, E. R. Mardis, G. M. Weinstock, R. K. Wilson, U. Genome Institute at Washington, K. Delehaunty, D. Dooling, C. Fronik, L. Fulton, B. Fulton, T. Graves, P. Minx, E. Sodergren, E. Birney, E. H. Margulies, J. Herrero, E. D. Green, D. Haussler, A. Siepel, N. Goldman, K. S. Pollard, J. S. Pedersen, E. S. Lander, and M. Kellis. 2011. A high-resolution map of human evolutionary constraint using 29 mammals. Nature **478**:476-482.

Lischer, H. E. L., and K. K. Shimizu. 2017. Reference-guided de novo assembly approach improves genome reconstruction for related species. BMC Bioinformatics **18**:474.

Liu, S., E. D. Lorenzen, M. Fumagalli, B. Li, K. Harris, Z. Xiong, L. Zhou, T. S. Korneliussen, M. Somel, C. Babbitt, G. Wray, J. Li, W. He, Z. Wang, W. Fu, X. Xiang, C. C. Morgan, A. Doherty, M. J. O'Connell, J. O. McInerney, E. W. Born, L. Dalen, R. Dietz, L. Orlando, C. Sonne, G. Zhang, R. Nielsen, E. Willerslev, and J. Wang. 2014. Population genomics reveal recent speciation and rapid evolutionary adaptation in polar bears. Cell **157**:785-794.

Liwanag, H. E. M., A. Berta, D. P. Costa, S. M. Budge, and T. M. Williams. 2012. Morphological and thermal properties of mammalian insulation: the evolutionary transition to blubber in pinnipeds Biological Journal of the Linnean Society **107**:774-787.

Lopes-Marques, M., A. M. Machado, S. Barbosa, M. M. Fonseca, R. Ruivo, and L. F. C. Castro. 2018. Cetacea are natural knockouts for IL20. Immunogenetics **70**:681-687.

Lotze, H. K., M. Coll, A. M. Magera, C. Ward-Paige, and L. Airoldi. 2011. Recovery of marine animal populations and ecosystems. Trends in Ecology & Evolution **26**:595-605.

Lotze, H. K., H. S. Lenihan, B. J. Bourque, R. H. Bradbury, R. G. Cooke, M. C. Kay, S. M. Kidwell, M. X. Kirby, C. H. Peterson, and J. B. C. Jackson. 2006. Depletion, Degradation, and Recovery Potential of Estuaries and Coastal Seas. Science **312**:1806-1809.

Louis, M., M. C. Fontaine, J. Spitz, E. Schlund, W. Dabin, R. Deaville, F. Caurant, Y. Cherel, C. Guinet, and B. Simon-Bouhet. 2014a. Ecological opportunities and specializations shaped genetic divergence in a highly mobile marine top predator. Proceedings of the Royal Society B **281**.

Louis, M., M. Galimberti, F. Archer, S. Berrow, A. Brownlow, R. Fallon, M. Nykänen, J. O'Brien, K. M. Roberston, P. E. Rosel, B. Simon-Bouhet, D. Wegmann, M. C. Fontaine, A. D. Foote, and O. E. Gaggiotti. 2020. Selection on ancestral genetic variation fuels parallel ecotype formation in bottlenose dolphins. bioRxiv.

Louis, M., A. Viricel, T. Lucas, H. Peltier, E. Alfonsi, S. Berrow, A. Brownlow, P. Covelo, W. Dabin, R. Deaville, R. de Stephanis, F. Gally, P. Gauffier, R. Penrose, M. A. Silva, C. Guinet, and B. Simon-Bouhet. 2014b. Habitat-driven population structure of bottlenose dolphins, T*ursiops truncatus*, in the North-East Atlantic. Molecular Ecology **23**:857-874.

Lowther-Thieleking, J. L., F. I. Archer, A. R. Lang, and D. W. Weller. 2015. Genetic differentiation among coastal and offshore common bottlenose dolphins,Tursiops truncatus, in the eastern North Pacific Ocean. Marine Mammal Science **31**:1-20.

Löytynoja, A. 2014. Phylogeny-aware alignment with PRANK. Pages 155-170 *in* D. J. Russell, editor. Multiple Sequence Alignment Methods. Humana Press, Totowa, NJ.

Ludt, W. B., L. A. Rocha, and J. Ali. 2015. Shifting seas: the impacts of Pleistocene sea-level fluctuations on the evolution of tropical marine taxa. Journal of Biogeography **42**:25-38.

Mace, G. M., K. Norris, and A. H. Fitter. 2012. Biodiversity and ecosystem services: a multilayered relationship. Trends in Ecology & Evolution **27**:19-26.

Magera, A. M., J. E. Mills Flemming, K. Kaschner, L. B. Christensen, and H. K. Lotze. 2013. Recovery trends in marine mammal populations. PLoS ONE **8**:e77908.

Manlik, O., M. Krützen, A. M. Kopps, J. Mann, L. Bejder, S. J. Allen, C. Frère, R. C. Connor, and W. B. Sherwin. 2019. Is MHC diversity a better marker for conservation than neutral genetic diversity? A case study of two contrasting dolphin populations. Ecology and Evolution.

Margres, M. J., M. E. Jones, B. Epstein, D. H. Kerlin, S. Comte, S. Fox, A. K. Fraik, S. A. Hendricks, S. Huxtable, S. Lachish, B. Lazenby, S. M. O'Rourke, A. R. Stahlke, C. G. Wiench, R. Hamede, B. Schonfeld, H. McCallum, M. R. Miller, P. A. Hohenlohe, and A. Storfer. 2018. Large-effect loci affect survival in Tasmanian devils (*Sarcophilus harrisii*) infected with a transmissible cancer. Molecular Ecology **27**:4189-4199.

Marie, J. C., J. Kehren, M. C. Trescol-Biemont, A. Evlashev, H. Valentin, T. Walzer, R. Tedone, B. Loveland, J. F. Nicolas, C. Rabourdin-Combe, and B. Horvat. 2001. Mechanism of Measles Virus–Induced Suppression of Inflammatory Immune Responses. Immunity **14**.

Marino, L., D. W. McShea, and M. D. Uhen. 2004. Origin and evolution of large brains in toothed whales. Anat Rec A Discov Mol Cell Evol Biol **281**:1247-1255.

Maroso, F., J. E. J. Hillen, B. G. Pardo, K. Gkagkavouzis, I. Coscia, M. Hermida, R. Franch, B. Hellemans, J. Van Houdt, B. Simionati, J. B. Taggart, E. E. Nielsen, G. Maes, S. A. Ciavaglia, L. M. I. Webster, F. A. M. Volckaert, P. Martinez, L. Bargelloni, R. Ogden, and C. AquaTrace. 2018. Performance and precision of double digestion RAD (ddRAD) genotyping in large multiplexed datasets of marine fish species. Marine Genomics **39**:64-72.

Marra, N. J., M. J. Stanhope, N. K. Jue, M. Wang, Q. Sun, P. Pavinski Bitar, V. P. Richards, A. Komissarov, M. Rayko, S. Kliver, B. J. Stanhope, C. Winkler, S. J. O'Brien, A. Antunes, S. Jorgensen, and M. S. Shivji. 2019. White shark genome reveals ancient elasmobranch adaptations associated with wound healing and the maintenance of genome stability. Proc Natl Acad Sci U S A **116**:4446-4455.

Martin, M. P., and M. Carrington. 2005. Immunogenetics of viral infections. Current Opinion in Immunology **17**:510-516.

Martineau, D., K. Lemberger, A. Dallaire, P. Labelle, T. P. Lipscomb, P. Michel, and I. Mikaelian. 2002. Cancer in wildlife, a case study: beluga from the St. Lawrence estuary, Québec, Canada. Environmental Health Perspectives **110**:285–292.

Maxwell, S. L., R. A. Fuller, T. M. Brooks, and J. E. Watson. 2016. Biodiversity: The ravages of guns, nets and bulldozers. Nature **536**:143-145.

Mazor, T., C. Doropoulos, F. Schwarzmueller, D. W. Gladish, N. Kumaran, K. Merkel, M. Di Marco, and V. Gagic. 2018. Global mismatch of policy and research on drivers of biodiversity loss. Nature Ecology & Evolution **2**:1071-1074.

McCarthy, A. J., M. A. Shaw, P. D. Jepson, S. M. J. M. Brasseur, P. J. H. Reijnders, and S. J. Goodman. 2011. Variation in European harbour seal immune response genes and susceptibility to phocine distemper virus (PDV). Infection, Genetics and Evolution **11**:1616-1623.

McGowen, M. R. 2011. Toward the resolution of an explosive radiation--a multilocus phylogeny of oceanic dolphins (Delphinidae). Mol Phylogenet Evol **60**:345-357.
McGowen, M. R., J. Gatesy, and D. E. Wildman. 2014. Molecular evolution tracks macroevolutionary transitions in Cetacea. Trends in Ecology & Evolution **29**:336-346.
McGowen, M. R., L. I. Grossman, and D. E. Wildman. 2012. Dolphin genome provides evidence for adaptive evolution of nervous system genes and a molecular rate slowdown. Proceedings of the Royal Society B: Biological Sciences **279**:3643-3651.
McGowen, M. R., M. Spaulding, and J. Gatesy. 2009. Divergence date estimation and a comprehensive molecular tree of extant cetaceans. Molecular Phylogenetics and Evolution **53**:891-906.
McGowen, M. R., G. Tsagkogeorga, J. Williamson, P. A. Morin, and A. S. J. Rossiter. 2020. Positive Selection and Inactivation in the Vision and Hearing Genes of Cetaceans. Molecular Biology and Evolution **37**:2069-2083.
McKee, J. K., P. W. Sciulli, C. D. Fooce, and T. A. Waite. 2004. Forecasting global biodiversity threats associated with human population growth. Biological Conservation **115**:161-164.
McMahon, B. J., E. C. Teeling, and J. Hoglund. 2014. How and why should we implement genomics into conservation? Evolutionary Applications **7**:999-1007.
Meager, J. J., and C. Limpus. 2014. Mortality of inshore marine mammals in eastern Australia is predicted by freshwater discharge and air temperature. PLoS ONE **9**:e94849.
Melia, M. M., J. P. Earle, H. Abdullah, K. Reaney, F. Tangy, and S. L. Cosby. 2014. Use of SLAM and PVRL4 and identification of pro-HB-EGF as cell entry receptors for wild type phocine distemper virus. PLoS ONE **9**:e106281.
Miller, W., V. M. Hayes, A. Ratan, D. C. Petersen, N. E. Wittekindt, J. Miller, B. Walenz, J. Knight, J. Qi, F. Zhao, Q. Wang, O. C. Bedoya-Reina, N. Katiyar, L. P. Tomsho, L. M. Kasson, R. A. Hardie, P. Woodbridge, E. A. Tindall, M. F. Bertelsen, D. Dixon, S. Pyecroft, K. M. Helgen, A. M. Lesk, T. H. Pringle, N. Patterson, Y. Zhang, A. Kreiss, G. M. Woods, M. E. Jones, and S. C. Schuster. 2011. Genetic diversity and population structure of the endangered marsupial *Sarcophilus harrisii* (Tasmanian devil). PNAS **108**:12348-12353.
Miller, W., S. C. Schuster, A. J. Welch, A. Ratan, O. C. Bedoya-Reina, F. Zhao, H. L. Kim, R. C. Burhans, D. I. Drautz, N. E. Wittekindt, L. P. Tomsho, E. Ibarra-Laclette, L. Herrera-Estrella, E. Peacock, S. Farley, G. K. Sage, K. Rode, M. Obbard, R. Montiel, L. Bachmann, O. Ingolfsson, J. Aars, T. Mailund, O. Wiig, S. L. Talbot, and C. Lindqvist. 2012. Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. PNAS **109**:E2382-2390.
Ming, Y., J. Jian, X. Yu, J. Wang, and W. Liu. 2019. The genome resources for conservation of Indo-Pacific humpback dolphin, Sousa chinensis. Scientific Data **6**:68.
Minkin, I., and P. Medvedev. 2019. Scalable multiple whole-genome alignment and locally collinear block construction with SibeliaZ.
MMPA. 1972. Marine Mammal Unusual Mortality Events. See https://www.fisheries.noaa.gov/national/marine-mammal-protection/marine-mammal-unusual-mortality-events (updated 9 September 2020; accessed 12 January 2021).
Mollenhauer, M. A., B. J. Carter, M. M. Peden-Adams, G. D. Bossart, and P. A. Fair. 2009. Gene expression changes in bottlenose dolphin, Tursiops truncatus, skin cells following exposure to methylmercury (MeHg) or perfluorooctane sulfonate (PFOS). Aquatic Toxicology **91**:10-18.
Möller, L. M. 2012. Sociogenetic structure, kin associations and bonding in delphinids. Molecular Ecology **21**:745-764.

Möller, L. M., and L. B. Beheregaray. 2001. Coastal bottlenose dolphins from southeastern Australia are Tursiops aduncus according to sequences of the mitochondrial DNA control region. Marine Mammal Science **17**:249-263.

Möller, L. M., and L. B. Beheregaray. 2004. Genetic evidence for sex-biased dispersal in resident bottlenose dolphins (*Tursiops aduncus*). Mol Ecol **13**:1607-1612.

Möller, L. M., K. Bilgmann, K. Charlton-Robb, and L. Beheregaray. 2008. Multi-gene evidence for a new bottlenose dolphin species in southern Australia. Molecular Phylogenetics and Evolution **49**:674-681.

Möller, L. M., J. Wiszniewski, S. J. Allen, and L. B. Beheregaray. 2007. Habitat type promotes rapid and extremely localised genetic differentiation in dolphins. Marine and Freshwater Research **58**:640-648.

Morán-Ordóñez, A., A. L. Whitehead, G. W. Luck, G. D. Cook, R. Maggini, J. A. Fitzsimons, and B. A. Wintle. 2017. Analysis of Trade-Offs Between Biodiversity, Carbon Farming and Agricultural Development in Northern Australia Reveals the Benefits of Strategic Planning. Conservation Letters **10**:94-104.

Moreno-Santillan, D. D., E. A. Lacey, D. Gendron, and J. Ortega. 2016. Genetic Variation at Exon 2 of the MHC Class II DQB Locus in Blue Whale (*Balaenoptera musculus*) from the Gulf of California. PLoS ONE **11**:e0141296.

Morens, D. M., G. K. Folkers, and A. S. Fauci. 2004. The challenge of emerging and re-emerging infectious diseases. Nature **430**:242-249.

Morin, P. A., A. Alexander, M. Blaxter, S. Caballero, O. Fedrigo, M. C. Fontaine, A. D. Foote, S. Kuraku, B. Maloney, M. L. McCarthy, M. R. McGowen, J. Mountcastle, M. F. Nery, M. T. Olsen, P. E. Rosel, and E. D. Jarvis. 2020a. Building genomic infrastructure: Sequencing platinum-standard reference-quality genomes of all cetacean species. Marine Mammal Science **36**:1356-1366.

Morin, P. A., F. I. Archer, C. D. Avila, J. R. Balacco, Y. V. Bukhman, W. Chow, O. Fedrigo, G. Formenti, J. A. Fronczek, A. Fungtammasan, F. M. D. Gulland, B. Haase, M. Peter Heide-Jorgensen, M. L. Houck, K. Howe, A. C. Misuraca, J. Mountcastle, W. Musser, S. Paez, S. Pelan, A. Phillippy, A. Rhie, J. Robinson, L. Rojas-Bracho, T. K. Rowles, O. A. Ryder, C. R. Smith, S. Stevenson, B. L. Taylor, J. Teilmann, J. Torrance, R. S. Wells, A. J. Westgate, and E. D. Jarvis. 2020b. Reference genome and demographic history of the most endangered marine mammal, the vaquita. Molecular Ecology Resources.

Morris, K. M., B. Wright, C. E. Grueber, C. Hogg, and K. Belov. 2015. Lack of genetic diversity across diverse immune genes in an endangered mammal, the Tasmanian devil (*Sarcophilus harrisii*). Molecular Ecology **24**:3860-3872.

Moshkovits, I., D. Karo-Atar, M. Itan, H. Reichman, P. Rozenberg, N. Morgenstern-Ben-Baruch, D. Shik, A. Ejarque-Ortiz, A. Y. Hershko, L. Tian, J. E. Coligan, J. Sayos, and A. Munitz. 2015. CD300f associates with IL-4 receptor alpha and amplifies IL-4-induced immune cell responses. PNAS **112**:8708-8713.

Moura, A. E., C. Janse van Rensburg, M. Pilot, A. Tehrani, P. B. Best, M. Thornton, S. Plon, P. J. de Bruyn, K. C. Worley, R. A. Gibbs, M. E. Dahlheim, and A. R. Hoelzel. 2014a. Killer whale nuclear genome and mtDNA reveal widespread population bottleneck during the last glacial maximum. Molecular Biology and Evolution **31**:1121-1131.

Moura, A. E., J. G. Kenny, R. Chaudhuri, M. A. Hughes, J. W. A, R. R. Reisinger, P. J. de Bruyn, M. E. Dahlheim, N. Hall, and A. R. Hoelzel. 2014b. Population genomics of the killer whale indicates ecotype evolution in sympatry involving both selection and drift. Molecular Ecology **23**:5179-5192.

Moura, A. E., J. G. Kenny, R. R. Chaudhuri, M. A. Hughes, R. R. Reisinger, P. J. de Bruyn, M. E. Dahlheim, N. Hall, and A. R. Hoelzel. 2015. Phylogenomics of the killer whale indicates ecotype divergence in sympatry. Heredity **114**:48-55.

Moura, A. E., S. C. A. Nielsen, J. T. Vilstrup, J. V. Moreno-Mayar, M. T. P. Gilbert, H. W. I. Gray, A. Natoli, L. Moller, and A. R. Hoelzel. 2013. Recent Diversification of a Marine Genus (*Tursiops* spp.) Tracks Habitat Preference and Environmental Change. Systematic Biology **62**:867-877.

Moura, A. E., K. Shreves, M. Pilot, K. R. Andrews, D. M. Moore, T. Kishida, L. Möller, A. Natoli, S. Gaspari, M. McGowen, I. Chen, H. Gray, M. Gore, R. M. Culloch, M. S. Kiani, M. Sarrouf Willson, A. Bulushi, T. Collins, R. Baldwin, A. Willson, G. Minton, L. Ponnampalam, and A. Rus Hoelzel. 2020. Phylogenomics of the genus Tursiops and closely related Delphininae reveals extensive reticulation among lineages and provides inference about eco-evolutionary drivers. Molecular Phylogenetics and Evolution.

Myers, R. A., J. K. Baum, T. D. Shepherd, S. P. Powers, and C. H. Peterson. 2007. Cascading Effects of the Loss of Apex Predatory Sharks from a Coastal Ocean. Science **315**:1846.

Naegelen, I., N. Beaume, S. Plancon, V. Schenten, E. J. Tschirhart, and S. Brechard. 2015. Regulation of Neutrophil Degranulation and Cytokine Secretion: A Novel Model Approach Based on Linear Fitting. The Journal of Immunology Research **2015**:817038.

Nair, A., P. Chauhan, B. Saha, and K. F. Kubatzky. 2019. Conceptual Evolution of Cell Signaling. International Journal of Molecular Sciences **20**.

Natoli, A., A. Birkun, A. Aguilar, A. Lopez, and A. R. Hoelzel. 2005. Habitat structure and the dispersal of male and female bottlenose dolphins (Tursiops truncatus). Proceedings of the Royal Society B **272**:1217-1226.

Natoli, A., V. M. Peddemors, and A. Rus Hoelzel. 2004. Population structure and speciation in the genus Tursiops based on microsatellite and mitochondrial DNA analyses. Journal of Evolutionary Biology **17**:363-375.

Nery, M. F., D. J. Gonzalez, and J. C. Opazo. 2013. How to Make a Dolphin: Molecular Signature of Positive Selection in Cetacean Genome. PLoS ONE **8**:e65491.

Nguyen, L. T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution **32**:268-274.

Noren, D. P., and J. A. Mocklin. 2012. Review of cetacean biopsy techniques: Factors contributing to successful sample collection and physiological and behavioral impacts. Marine Mammal Science **28**:154-199.

Normandeau, E. 2020. Genome Annotation Without Nightmares.

Nykanen, M., E. Dillane, A. Englund, A. D. Foote, S. N. Ingram, M. Louis, L. Mirimin, M. Oudejans, and E. Rogan. 2018. Quantifying dispersal between marine protected areas by a highly mobile species, the bottlenose dolphin, *Tursiops truncatus*. Ecology and Evolution **8**:9241-9258.

Nykanen, M., K. Kaschner, W. Dabin, A. Brownlow, N. J. Davison, R. Deaville, C. Garilao, K. Kesner-Reyes, T. P. Gilbert, R. Penrose, V. Islas-Villanueva, N. Wales, S. N. Ingram, E. Rogan, M. Louis, and A. D. Foote. 2019. Post-glacial colonization of northern coastal habitat by bottlenose dolphins: A marine leading-edge expansion? Journal of Heredity.

O'Connor, S., R. Campbell, H. Cortez, and T. Knowles. 2009. Whale Watching Worldwide: tourism numbers, expenditures and expanding economic benefits a special report from the International Fund for Animal Welfare. Yarmouth MA, USA, prepared by Economists at Large.

O'Loughlin, P. M., J. M. Waters, and M. S. Roy. 2003. A molecular and morphological review of the asterinid, *Patiriella gunnii* (Gray) (Echinodermata: Asteroidea). Memoirs of Museum Victoria **60**:181–195.

O'Brien, S. M., V. F. Gallucci, and L. Hauser. 2012. Effects of species biology on the historical demography of sharks and their implications for likely consequences of contemporary climate change. Conservation genetics **14**:125-144.

Ohishi, K., T. Maruyama, F. Seki, and M. Takeda. 2019. Marine Morbilliviruses: Diversity and Interaction with Signaling Lymphocyte Activation Molecules. Viruses **11**.

Ohishi, K., R. Shishido, Y. Iwata, M. Saitoh, R. Takenaka, D. Ohtsu, K. Okutsu, and T. Maruyama. 2011. Lipopolysaccharide-induced innate immune factors in the bottlenose dolphin (*Tursiops truncatus*) detected in expression sequence tag analysis. Microbiology and Immunology **55**:790-797.

Oliveira, L. R. d., L. D. Fraga, P. H. Ott, S. Siciliano, F. Lopes, R. Almeida, J. C. Wickert, L. Milmann, D. Danilewicz, N. R. Emin-Lima, A. C. Meirelles, V. Luz, L. F. Do Nascimento, B. De Thoisy, M. Tavares, A. N. Zerbini, M. Baumgarten, V. H. Valiati, and S. L. Bonatto. 2019. Population structure, phylogeography, and genetic diversity of the common bottlenose dolphin in the tropical and subtropical southwestern Atlantic Ocean. Journal of Mammalogy **100**:564-577.

Ouborg, N. J., C. Pertoldi, V. Loeschcke, R. K. Bijlsma, and P. W. Hedrick. 2010. Conservation genetics in transition to conservation genomics. Trends in Genetics **26**:177-187.

Pacifici, M., W. B. Foden, P. Visconti, J. E. M. Watson, S. H. M. Butchart, K. M. Kovacs, B. R. Scheffers, D. G. Hole, T. G. Martin, H. R. Akçakaya, R. T. Corlett, B. Huntley, D. Bickford, J. A. Carr, A. A. Hoffmann, G. F. Midgley, P. Pearce-Kelly, R. G. Pearson, S. E. Williams, S. G. Willis, B. Young, and C. Rondinini. 2015. Assessing species vulnerability to climate change. Nature Climate Change **5**:215-224.

Padalino, I., G. Di Guardo, A. Carbone, P. Troiano, A. Parisi, D. Galante, M. A. Cafiero, M. Caruso, L. Palazzo, L. Guarino, L. De Riso, C. Centelleghe, S. Mazzariol, and A. Petrella. 2019. Dolphin Morbillivirus in Eurasian Otters, Italy. Emerging Infectious Diseases **25**:372-374.

Pagan, H. J. T., T. Ferrer, and G. O'Corry-Crowe. 2018. Positive selection in coding regions and motif duplication in regulatory regions of bottlenose dolphin MHC class II genes. PLoS ONE **13**:e0203450.

Park, J. Y., Y. R. An, N. Kanda, C. M. An, H. S. An, J. H. Kang, E. M. Kim, D. H. An, H. Jung, M. Joung, M. H. Park, S. H. Yoon, B. Y. Lee, T. Lee, K. W. Kim, W. C. Park, D. H. Shin, Y. S. Lee, J. Kim, W. Kwak, H. J. Kim, Y. J. Kwon, S. Moon, Y. Kim, D. W. Burt, S. Cho, and H. Kim. 2015. Cetaceans evolution: insights from the genome sequences of common minke whales. BMC Genomics **16**:13.

Park, J. Y., K. Kim, H. Sohn, H. W. Kim, Y. R. An, J. H. Kang, E. M. Kim, W. Kwak, C. Lee, D. Yoo, J. Jung, S. Sung, J. Yoon, and H. Kim. 2018. Deciphering the evolutionary signatures of pinnipeds using novel genome sequences: The first genomes of *Phoca largha*, *Callorhinus ursinus*, and *Eumetopias jubatus*. Scientific Reports **8**:16877.

Parmesan, C. 2006. Ecological and Evolutionary Responses to Recent Climate Change. Annual Review of Ecology, Evolution, and Systematics **37**:637-669.

Parra, G., K. Bilgmann, K. Peters, and L. Moller. in review. Abundance and potential biological removal of common dolphins subject to fishery-impacts in South Australian Waters.

Partridge, F. A., M. J. Gravato-Nobre, and J. Hodgkin. 2010. Signal transduction pathways that function in both development and innate immunity. Developmental Dynamics **239**:1330-1336.

Patton, A. H., M. J. Margres, A. R. Stahlke, S. Hendricks, K. Lewallen, R. K. Hamede, M. Ruiz-Aravena, O. Ryder, H. I. McCallum, M. E. Jones, P. A. Hohenlohe, and A. Storfer.

2019. Contemporary Demographic Reconstruction Methods Are Robust to Genome Assembly Quality: A Case Study in Tasmanian Devils. Molecular Biology and Evolution **36**:2906-2921.

Peart, C. R., S. Tusso, S. D. Pophaly, F. Botero-Castro, C. C. Wu, D. Aurioles-Gamboa, A. B. Baird, J. W. Bickham, J. Forcada, F. Galimberti, N. J. Gemmell, J. I. Hoffman, K. M. Kovacs, M. Kunnasranta, C. Lydersen, T. Nyman, L. R. de Oliveira, A. J. Orr, S. Sanvito, M. Valtonen, A. B. A. Shafer, and J. B. W. Wolf. 2020. Determinants of genetic variation across eco-evolutionary scales in pinnipeds. Nature Ecology & Evolution **4**:1095-1104.

Perrin, W. F. 2009. Common dolphins. Pages 255–259 *in* W. F. Perrin, B. Wursig, and J. G. M. Thewissen, editors. Encylopedia of marine mammals, San Diego: Academic Press.

Perrin, W. F., K. M. Robertson, P. J. H. van Bree, and J. G. Mead. 2007. Cranial Description and Genetic Identity of the Holotype Specimen of *Tursiops aduncus* (Ehrenberg, 1832). Marine Mammal Science **23**:343-357.

Perrin, W. F., P. E. Rosel, and F. Cipriano. 2013. How to contend with paraphyly in the taxonomy of the delphinine cetaceans? Marine Mammal Science:n/a-n/a.

Peters, K. J., G. J. Parra, P. P. Skuza, and L. M. Möller. 2012. First insights into the effects of swim-with-dolphin tourism on the behavior, response, and group structure of southern Australian bottlenose dolphins. Marine Mammal Science:n/a-n/a.

Picard Toolkit. 2019. Picard Toolkit. Broad Institute, GitHub Repository. http://broadinstitute.github.io/picard/; Broad Institute.

Pimm, S. L., C. N. Jenkins, R. Abell, T. M. Brooks, J. L. Gittleman, L. N. Joppa, P. H. Raven, C. M. Roberts, and J. O. Sexton. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. Science **344**:1246752.

Potter, S., and M. Eldridge. 2017. Oz Mammal Genomics. Pages 19–21 Australasian Science.

Pratt, E. A. 2020. The Genomic Basis of Adaptation in Bottlenose Dolphins (genus *Tursiops*). PhD. Flinders University, Adelaide, South Australia, Australia.

Pratt, E. A. L., L. B. Beheregaray, K. Bilgmann, N. Zanardo, F. Diaz-Aguirre, and L. M. Möller. 2018. Hierarchical metapopulation structure in a highly mobile marine predator: the southern Australian coastal bottlenose dolphin (*Tursiops* cf. *australis*). Conservation genetics **19**:637-654.

Purcell, S., B. Neale, K. Todd-Brown, L. Thomas, M. A. Ferreira, D. Bender, J. Maller, P. Sklar, P. I. de Bakker, M. J. Daly, and P. C. Sham. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. The American Journal of Human Genetics **81**:559-575.

Pye, R., A. Patchett, E. McLennan, R. Thomson, S. Carver, S. Fox, D. Pemberton, A. Kreiss, A. Baz Morelli, A. Silva, M. J. Pearse, L. M. Corcoran, K. Belov, C. J. Hogg, G. M. Woods, and A. B. Lyons. 2018. Immunization Strategies Producing a Humoral IgG Immune Response against Devil Facial Tumor Disease in the Majority of Tasmanian Devils Destined for Wild Release. Frontiers in Immunology **9**:259.

Qiu, Q., G. Zhang, T. Ma, W. Qian, J. Wang, Z. Ye, C. Cao, Q. Hu, J. Kim, D. M. Larkin, L. Auvil, B. Capitanu, J. Ma, H. A. Lewin, X. Qian, Y. Lang, R. Zhou, L. Wang, K. Wang, J. Xia, S. Liao, S. Pan, X. Lu, H. Hou, Y. Wang, X. Zang, Y. Yin, H. Ma, J. Zhang, Z. Wang, Y. Zhang, D. Zhang, T. Yonezawa, M. Hasegawa, Y. Zhong, W. Liu, Y. Zhang, Z. Huang, S. Zhang, R. Long, H. Yang, J. Wang, J. A. Lenstra, D. N. Cooper, Y. Wu, J. Wang, P. Shi, J. Wang, and J. Liu. 2012. The yak genome and adaptation to life at high altitude. Nature Genetics **44**:946-949.

Quail, M. A., H. Swerdlow, and D. J. Turner. 2009. Improved protocols for the illumina genome analyzer sequencing system. Curr Protoc Hum Genet **Chapter 18**:Unit 18 12.

Quérouil, S., M. A. Silva, L. Freitas, R. Prieto, S. Magalhães, A. Dinis, F. Alves, J. A. Matos, D. Mendonça, P. S. Hammond, and R. S. Santos. 2007. High gene flow in oceanic bottlenose dolphins (*Tursiops truncatus*) of the North Atlantic. Conservation genetics **8**:1405-1419.

Quesada, V., S. Freitas-Rodriguez, J. Miller, J. G. Perez-Silva, Z. F. Jiang, W. Tapia, O. Santiago-Fernandez, D. Campos-Iglesias, L. F. K. Kuderna, M. Quinzin, M. G. Alvarez, D. Carrero, L. B. Beheregaray, J. P. Gibbs, Y. Chiari, S. Glaberman, C. Ciofi, M. Araujo-Voces, P. Mayoral, J. R. Arango, I. Tamargo-Gomez, D. Roiz-Valle, M. Pascual-Torner, B. R. Evans, D. L. Edwards, R. C. Garrick, M. A. Russello, N. Poulakakis, S. J. Gaughran, D. O. Rueda, G. Bretones, T. Marques-Bonet, K. P. White, A. Caccone, and C. Lopez-Otin. 2019. Giant tortoise genomes provide insights into longevity and age-related disease. Nature Ecology & Evolution **3**:87-95.

Quinlan, A. R., and I. M. Hall. 2010. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics **26**:841-842.

Rambaut, A. 2014. FigTree v.1.4.3: tree figure drawing tool.

Randhawa, N., F. Gulland, G. M. Ylitalo, R. DeLong, and J. A. K. Mazet. 2015. Sentinel California sea lions provide insight into legacy organochlorine exposure trends and their association with cancer and infectious disease. One Health **1**:37-43.

Read, A. J. 2008. The Looming Crisis: Interactions between Marine Mammals and Fisheries. Journal of Mammalogy **89**:541–548.

Reed, D. H., and R. Frankham. 2003. Correlation between Fitness and Genetic Diversity. Conservation Biology **17**:230-237.

Reed, J., R. Harcourt, L. New, and K. Bilgmann. 2020. Extreme Effects of Extreme Disturbances: A Simulation Approach to Assess Population Specific Responses. Frontiers in Marine Science **7**.

Resistance. 2021. In Oxford Online Dictionary. Retrieved from https://en.oxforddictionaries.com/definition/resistance.

Rhie, A., S. A. McCarthy, O. Fedrigo, J. Damas, G. Formenti, S. Koren, M. Uliano-Silva, W. Chow, A. Fungtammasan, G. L. Gedman, L. J. Cantin, F. Thibaud-Nissen, L. Haggerty, C. Lee, B. J. Ko, J. Kim, I. Bista, M. Smith, B. Haase, J. Mountcastle, S. Winkler, S. Paez, J. Howard, S. C. Vernes, T. M. Lama, F. Grutzner, W. C. Warren, C. Balakrishnan, D. Burt, J. M. George, M. Biegler, D. Iorns, A. Digby, D. Eason, T. Edwards, M. Wilkinson, G. Turner, A. Meyer, A. F. Kautt, P. Franchini, H. W. Detrich, H. Svardal, M. Wagner, G. J. P. Naylor, M. Pippel, M. Malinsky, M. Mooney, M. Simbirsky, B. T. Hannigan, T. Pesout, M. Houck, A. Misuraca, S. B. Kingan, R. Hall, Z. Kronenberg, J. Korlach, I. Sović, C. Dunn, Z. Ning, A. Hastie, J. Lee, S. Selvaraj, R. E. Green, N. H. Putnam, J. Ghurye, E. Garrison, Y. Sims, J. Collins, S. Pelan, J. Torrance, A. Tracey, J. Wood, D. Guan, S. E. London, D. F. Clayton, C. V. Mello, S. R. Friedrich, P. V. Lovell, E. Osipova, F. O. Al-Ajli, S. Secomandi, H. Kim, C. Theofanopoulou, Y. Zhou, R. S. Harris, K. D. Makova, P. Medvedev, J. Hoffman, P. Masterson, K. Clark, F. Martin, K. Howe, P. Flicek, B. P. Walenz, W. Kwak, H. Clawson, M. Diekhans, L. Nassar, B. Paten, R. H. S. Kraus, H. Lewin, A. J. Crawford, M. T. P. Gilbert, G. Zhang, B. Venkatesh, R. W. Murphy, K.-P. Koepfli, B. Shapiro, W. E. Johnson, F. Di Palma, T. Margues-Bonet, E. C. Teeling, T. Warnow, J. M. Graves, O. A. Ryder, D. Hausler, S. J. O'Brien, K. Howe, E. W. Myers, R. Durbin, A. M. Phillippy, and E. D. Jarvis. 2020. Towards complete and error-free genome assemblies of all vertebrate species. bioRxiv.

Ribeiro, A. M., A. D. Foote, A. Kupczok, B. Frazao, M. T. Limborg, R. Pineiro, S. Abalde, S. Rocha, and R. R. da Fonseca. 2017. Marine genomics: News and views. Marine Genomics **31**:1-8.

Robinson, J. A., D. Ortega-Del Vecchyo, Z. Fan, B. Y. Kim, B. M. vonHoldt, C. D. Marsden, K. E. Lohmueller, and R. K. Wayne. 2016. Genomic Flatlining in the Endangered Island Fox. Curr Biol **26**:1183-1189.

Robinson, J. A., J. Räikkönen, L. M. Vucetich, J. A. Vucetich, R. O. Peterson, K. E. Lohmueller, and R. K. Wayne. 2019. Genomic signatures of extensive inbreeding in Isle Royale wolves, a population on the threshold of extinction. Science Advances **5**:eaau0757.

Rojas-Bracho, L., and R. R. Reeves. 2013. Vaquitas and gillnets: Mexico's ultimate cetacean conservation challenge. Endangered Species Research **21**:77-87.

Roman, J., J. A. Estes, L. Morissette, C. Smith, D. Costa, J. McCarthy, J. B. Nation, S. Nicol, A. Pershing, and V. Smetacek. 2014. Whales as marine ecosystem engineers. Frontiers in Ecology and the Environment **12**:377-385.

Roman, J., and J. J. McCarthy. 2010. The whale pump: marine mammals enhance primary productivity in a coastal basin. PLoS ONE **5**:e13255.

Roos, A. M., B. M. Backlin, B. O. Helander, F. F. Riget, and U. C. Eriksson. 2012. Improved reproductive success in otters (*Lutra lutra*), grey seals (*Halichoerus grypus*) and sea eagles (*Haliaeetus albicilla*) from Sweden in relation to concentrations of organochlorine contaminants. Environmental Pollution **170**:268-275.

Ross, P. S. 2000. Marine Mammals as Sentinels in Ecological Risk Assessment. Human and Ecological Risk Assessment: An International Journal **6**:29-46.

Rossi, A. C., C. Mammucari, C. Argentini, C. Reggiani, and S. Schiaffino. 2010. Two novel/ancient myosins in mammalian skeletal muscles: MYH14/7b and MYH15 are expressed in extraocular muscles and muscle spindles. J Physiol **588**:353-364.

Ruan, R., J. Ruan, X. L. Wan, Y. Zheng, M. M. Chen, J. S. Zheng, and D. Wang. 2016. Organization and characteristics of the major histocompatibility complex class II region in the Yangtze finless porpoise (Neophocaena asiaeorientalis asiaeorientalis). Scientific Reports **6**:22471.

Rycyk, A. M., C. J. Deutsch, M. E. Barlas, S. K. Hardy, K. Frisch, E. H. Leone, and D. P. Nowacek. 2018. Manatee behavioral response to boats. Marine Mammal Science **34**:924-962.

Sacristán, C., M. Carballo, M. J. Muñoz, E. N. Bellière, E. Neves, V. Nogal, and F. Esperón. 2015. Diagnosis of Cetacean morbillivirus: A sensitive one step real time RT fast-PCR method based on SYBR® Green. Journal of Virological Methods **226**:25-30.

Sanderson, C. E., and K. A. Alexander. 2020. Unchartered waters: Climate change likely to intensify infectious disease outbreaks causing mass mortality events in marine mammals. Global Change Biology **26**:4284-4301.

Sandoval-Castillo, J., and L. B. Beheregaray. 2020. Oceanographic heterogeneity influences an ecological radiation in elasmobranchs. Journal of Biogeography **47**:1599-1611.

Santos, S. G., E. C. Campbell, S. Lynch, V. Wong, A. N. Antoniou, and S. J. Powis. 2007. Major histocompatibility complex class I-ERp57-tapasin interactions within the peptide-loading complex. The Journal of Biological Chemistry **282**:17587-17593.

Santure, A. W., and D. Garant. 2018. Wild GWAS-association mapping in natural populations. Molecular Ecology Resources **18**:729-738.

Sato, H., M. Yoneda, T. Honda, and C. Kai. 2012. Morbillivirus receptors and tropism: multiple pathways for infection. Frontiers in Microbiology **3**:75.

Schipper, J., J. S. Chanson, F. Chiozza, N. A. Cox, M. Hoffmann, V. Katariya, J. Lamoreux, A. S. L. Rodrigues, S. N. Stuart, H. J. Temple, J. Baillie, L. Boitani, T. E. Lacher, R. A. Mittermeier, A. T. Smith, D. Absolon, J. M. Aguiar, G. Amori, N. Bakkour, R. Baldi, R. J. Berridge, J. Bielby, P. A. Black, J. J. Blanc, T. M. Brooks, J. A. Burton, T. M. Butynski, G. Catullo, R. Chapman, Z. Cokeliss, B. Collen, J. Conroy, J. G. Cooke, G.

A. B. da Fonseca, A. E. Derocher, H. T. Dublin, J. W. Duckworth, L. Emmons, R. H. Emslie, M. Festa-Bianchet, M. Foster, S. Foster, D. L. Garshelis, C. Gates, M. Gimenez-Dixon, S. Gonzalez, J. F. Gonzalez-Maya, T. C. Good, G. Hammerson, P. S. Hammond, D. Happold, M. Happold, J. Hare, R. B. Harris, C. E. Hawkins, M. Haywood, L. R. Heaney, S. Hedges, K. M. Helgen, C. Hilton-Taylor, S. A. Hussain, N. Ishii, T. A. Jefferson, R. K. B. Jenkins, C. H. Johnston, M. Keith, J. Kingdon, D. H. Knox, K. M. Kovacs, P. Langhammer, K. Leus, R. Lewison, G. Lichtenstein, L. F. Lowry, Z. Macavoy, G. M. Mace, D. P. Mallon, M. Masi, M. W. McKnight, R. A. Medellín, P. Medici, G. Mills, P. D. Moehlman, S. Molur, A. Mora, K. Nowell, J. F. Oates, W. Olech, W. R. L. Oliver, M. Oprea, B. D. Patterson, W. F. Perrin, B. A. Polidoro, C. Pollock, A. Powel, Y. Protas, P. Racey, J. Ragle, P. Ramani, G. Rathbun, R. R. Reeves, S. B. Reilly, J. E. Reynolds, C. Rondinini, R. G. Rosell-Ambal, M. Rulli, A. B. Rylands, S. Savini, C. J. Schank, W. Sechrest, C. Self-Sullivan, A. Shoemaker, C. Sillero-Zubiri, N. De Silva, D. E. Smith, C. Srinivasulu, P. J. Stephenson, N. van Strien, B. K. Talukdar, B. L. Taylor, R. Timmins, D. G. Tirira, M. F. Tognelli, K. Tsytsulina, L. M. Veiga, J.-C. Vié, E. A. Williamson, S. A. Wyatt, Y. Xie, and B. E. Young. 2008. The Status of the World's Land and Marine Mammals: Diversity, Threat, and Knowledge. Science **322**:225-230.

Schluter, D. 2009. Evidence for Ecological Speciation and Its Alternative. Science **323**:737-741.

Scholz, C., and R. Tampe. 2009. The peptide-loading complex--antigen translocation and MHC class I loading. Biological Chemistry **390**:783-794.

Schumann, N., N. J. Gales, R. G. Harcourt, and J. P. Y. Arnould. 2013. Impacts of climate change on Australian marine mammals. Australian Journal of Zoology **61**:146.

Scott, E. M., J. Mann, J. J. Watson-Capps, B. L. Sargeant, and R. C. Connor. 2005. Aggression in Bottlenose Dolphins: Evidence for Sexual Coercion, Male-Male Competition, and Female Tolerance through Analysis of Tooth-Rake Marks and Behaviour. Behaviour **142**:21-44.

Secchi, E. R. 2007. Population Viability Analysis of a small resident population of bottlenose dolphins, *Tursiops truncatus*, in southern Brazil. Rio Grande: Yaqupacha/Laboratório Mamíferos Marinhos, Museu Oceanográfico.

Secchi, E. R., S. Botta, M. M. Wiegand, L. A. Lopez, P. F. Fruet, R. C. Genoves, and J. C. Di Tullio. 2016. Long-term and gender-related variation in the feeding ecology of common bottlenose dolphins inhabiting a subtropical estuary and the adjacent marine coast in the western South Atlantic. Marine Biology Research **13**:121-134.

Sellinger, T., D. A. Awad, and A. Tellier. 2020. Limits and Convergence properties of the Sequentially Markovian Coalescent. bioRxiv.

Seoighe, C., and P. K. Korir. 2011. Evidence for intron length conservation in a set of mammalian genes associated with embryonic development. BMC Bioinformatics **12 Suppl 9**:S16.

Seppey, M., M. Manni, and E. M. Zdobnov. 2019. BUSCO: Assessing Genome Assembly and Annotation Completeness. Pages 227-245 *in* M. Kollmar, editor. Gene Prediction: Methods and Protocols. Springer New York, New York, NY.

Shafer, A. B., C. W. Fan, S. D. Cote, and D. W. Coltman. 2012. (Lack of) genetic diversity in immune genes predates glacial isolation in the North American mountain goat (*Oreamnos americanus*). Journal of Heredity **103**:371-379.

Shafer, A. B., L. M. Gattepaille, R. E. Stewart, and J. B. Wolf. 2015a. Demographic inferences using short-read genomic data in an approximate Bayesian computation framework: in silico evaluation of power, biases and proof of concept in Atlantic walrus. Molecular Ecology **24**:328-345.

Shafer, A. B., J. B. Wolf, P. C. Alves, L. Bergstrom, M. W. Bruford, I. Brannstrom, G. Colling, L. Dalen, L. De Meester, R. Ekblom, K. D. Fawcett, S. Fior, M. Hajibabaei, J. A. Hill, A. R. Hoezel, J. Hoglund, E. L. Jensen, J. Krause, T. N. Kristensen, M. Krutzen, J. K. McKay, A. J. Norman, R. Ogden, E. M. Osterling, N. J. Ouborg, J. Piccolo, D. Popovic, C. R. Primmer, F. A. Reed, M. Roumet, J. Salmona, T. Schenekar, M. K. Schwartz, G. Segelbacher, H. Senn, J. Thaulow, M. Valtonen, A. Veale, P. Vergeer, N. Vijay, C. Vila, M. Weissensteiner, L. Wennerstrom, C. W. Wheat, and P. Zielinski. 2015b. Genomics and the challenging translation into conservation practice. Trends in Ecology & Evolution **30**:78-87.

Shen, Y. Y., W. P. Zhou, T. C. Zhou, Y. N. Zeng, G. M. Li, D. M. Irwin, and Y. P. Zhang. 2012. Genome-wide scan for bats and dolphin to detect their genetic basis for new locomotive styles. PLoS ONE **7**:e46455.

Shimizu, Y., K. Ohishi, R. Suzuki, Y. Tajima, T. Yamada, Y. Kakizoe, T. Bando, Y. Fujise, H. Taru, T. Murayama, and T. Maruyama. 2013. Amino acid sequence variations of signaling lymphocyte activation molecule and mortality caused by morbillivirus infection in cetaceans. Microbiology and Immunology **57**:624-632.

Shultz, A. J., and T. B. Sackton. 2019. Immune genes are hotspots of shared positive selection across birds and mammals. Elife **8**.

Silber, G. K., M. D. Lettrich, P. O. Thomas, J. D. Baker, M. Baumgartner, E. A. Becker, P. Boveng, D. M. Dick, J. Fiechter, J. Forcada, K. A. Forney, R. B. Griffis, J. A. Hare, A. J. Hobday, D. Howell, K. L. Laidre, N. Mantua, L. Quakenbush, J. A. Santora, K. M. Stafford, P. Spencer, C. Stock, W. Sydeman, K. Van Houtan, and R. S. Waples. 2017. Projecting Marine Mammal Distribution in a Changing Climate. Frontiers in Marine Science **4**.

Silva, M. A., R. Prieto, S. Magalhães, M. I. Seabra, R. S. Santos, and P. S. Hammond. 2008. Ranging patterns of bottlenose dolphins living in oceanic waters: implications for population structure. Marine Biology **156**:179-192.

Simao, F. A., R. M. Waterhouse, P. Ioannidis, E. V. Kriventseva, and E. M. Zdobnov. 2015. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics **31**:3210-3212.

Singh, I., S.-H. Lee, A. S. Sperling, M. K. Samur, Y.-T. Tai, M. Fulciniti, N. C. Munshi, C. Mayr, and C. S. Leslie. 2018. Widespread intronic polyadenylation diversifies immune cell transcriptomes. Nature Communications **9**.

Slatkin, M. 2008. Linkage disequilibrium--understanding the evolutionary past and mapping the medical future. Nature Reviews Genetics **9**:477-485.

Smith, K. F., K. Acevedo-Whitehouse, and A. B. Pedersen. 2009. The role of infectious diseases in biological conservation. Animal Conservation **12**:1-12.

Springer, A. M., J. A. Estes, G. B. van Vliet, T. M. Williams, D. F. Doak, E. M. Danner, K. A. Forney, and B. Pfister. 2003. Sequential megafaunal collapse in the North Pacific Ocean: An ongoing legacy of industrial whaling? PNAS **100**:12223.

Staal, F. J., T. C. Luis, and M. M. Tiemessen. 2008. WNT signalling in the immune system: WNT is spreading its wings. Nature Reviews Immunology **8**:581-593.

Steiner, C. C., A. S. Putnam, P. E. Hoeck, and O. A. Ryder. 2013. Conservation genomics of threatened animal species. Annual Review of Animal Biosciences **1**:261-281.

Stejskalova, K., Z. Bayerova, J. Futas, K. Hrazdilova, M. Klumplerova, J. Oppelt, P. Splichalova, G. Di Guardo, S. Mazzariol, C. E. Di Francesco, G. Di Francesco, G. Terracciano, R. M. Paiu, T. D. Ursache, D. Modry, and P. Horin. 2017. Candidate gene molecular markers as tools for analyzing genetic susceptibility to Morbillivirus infection in stranded Cetaceans. HLA:10.1111/tan.13146.

Stephens, N., P. J. Duignan, J. Wang, J. Bingham, H. Finn, L. Bejder, I. A. P. Patterson, and C. Holyoake. 2014. Cetacean morbillivirus in coastal indo-pacific bottlenose dolphins, Western Australia. Emerging Infectious Diseases **20**:666-670.

Stock, A., L. B. Crowder, B. S. Halpern, and F. Micheli. 2018. Uncertainty analysis and robust areas of high and low modeled human impact on the global oceans. Conserv Biol **32**:1368-1379.

Stone, B. M., D. J. Blyde, J. T. Saliki, U. Blas-Machado, J. Bingham, A. Hyatt, J. Wang, J. Payne, and S. Crameri. 2011. Fatal cetacean morbillivirus infection in an Australian offshore bottlenose dolphin (*Tursiops truncatus*). Australian Veterinary Journal **89**:452-457.

Stone, B. M., D. J. Blyde, J. T. Saliki, and J. M. Morton. 2012. Morbillivirus infection in live stranded, injured, trapped, and captive cetaceans in southeastern Queensland and Northern New South Wales, Australia. Journal of Wildlife Diseases **48**:47-55.

Strehl, B., U. Seifert, E. Krüger, S. Heink, U. Kuckelkorn, and P. M. Kloetzel. 2005. Interferon-g, the functional plasticity of the ubiquitin–proteasome system, and MHC class I antigen processing. Immunological Reviews **207**:19-30.

Sun, Y. B., W. P. Zhou, H. Q. Liu, D. M. Irwin, Y. Y. Shen, and Y. P. Zhang. 2013. Genome-wide scans for candidate genes involved in the aquatic adaptation of dolphins. Genome Biology and Evolution **5**:130-139.

Sunnucks, P., and D. F. Hales. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). Molecular Biology and Evolution **13**:510-524.

Susceptibility. 2021. In Oxford Online Dictionary. Retrieved from https://en.oxforddictionaries.com/definition/susceptibility.

Tagliani, P. R. A., M. L. Asmus, C. R. A. Tagliani, M. Polette, C. S. B. Costa, and E. Salas. 2007. Integrated coastal zone management in the Patos Lagoon Estuary (South Brazil): state of art. Pages 679-686 Water Resources Management IV.

Takai, T. 2002. Roles of Fc receptors in autoimmunity. Nature Reviews Immunology **2**:580–592.

Takai, T. 2005. Fc Receptors and Their Role in Immune Regulation and Autoimmunity. Journal of Clinical Immunology **25**.

Tavares, D. C., J. F. Moura, E. Acevedo-Trejos, and A. Merico. 2019. Traits Shared by Marine Megafauna and Their Relationships With Ecosystem Functions and Services. Frontiers in Marine Science **6**.

Taylor, B. L., S. J. Chivers, J. P. Larese, and W. F. Perrin. 2007. Generation length and percent mature estimates for IUCN assessments of cetaceans. Adm. Rep. LJ-07-01, NOAA, NMFS, Southwest Fisheries Science Centre.

Tejada-Martinez, D., J. P. de Magalhaes, and J. C. Opazo. 2021. Positive selection and gene duplications in tumour suppressor genes reveal clues about how cetaceans resist cancer. Proceedings of the Royal Society B **288**:20202592.

Terhorst, J., J. A. Kamm, and Y. S. Song. 2017. Robust and scalable inference of population history from hundreds of unphased whole genomes. Nat Genet **49**:303-309.

Thomas, C. D., B. J. Anderson, A. Moilanen, F. Eigenbrod, A. Heinemeyer, T. Quaife, D. B. Roy, S. Gillings, P. R. Armsworth, and K. J. Gaston. 2013. Reconciling biodiversity and carbon conservation. Ecology Letters **16 Suppl 1**:39-47.

Tian, R., D. Yin, Y. Liu, I. Seim, S. Xu, and G. Yang. 2017. Adaptive Evolution of Energy Metabolism-Related Genes in Hypoxia-Tolerant Mammals. Frontiers in Genetics **8**:205.

Tilman, D., M. Clark, D. R. Williams, K. Kimmel, S. Polasky, and C. Packer. 2017. Future threats to biodiversity and pathways to their prevention. Nature **546**:73-81.

Titcomb, G. C., C. L. Jerde, and H. S. Young. 2019. High-Throughput Sequencing for Understanding the Ecology of Emerging Infectious Diseases at the Wildlife-Human Interface. Frontiers in Ecology and Evolution **7**.

Turner, H., M. Gomez, E. McKenzie, A. Kirchem, A. Lennard, and D. A. Cantrell. 1998. Rac-1 Regulates Nuclear Factor of Activated T Cells (NFAT) C1 Nuclear Translocation in Response to Fc ⑀ Receptor Type 1 Stimulation of Mast Cells. Journal of Experimental Medicine **188**:527-537.

Turner, H., and J. P. Kinet. 1999. Signalling through the high-afinity IgE receptor FceRI. Nature **402**.

Turvey, S. T., R. L. Pitman, B. L. Taylor, J. Barlow, T. Akamatsu, L. A. Barrett, X. Zhao, R. R. Reeves, B. S. Stewart, K. Wang, Z. Wei, X. Zhang, L. T. Pusser, M. Richlen, J. R. Brandon, and D. Wang. 2007. First human-caused extinction of a cetacean species? Biology Letters **3**:537-540.

Tyack, P. L., W. M. Zimmer, D. Moretti, B. L. Southall, D. E. Claridge, J. W. Durban, C. W. Clark, A. D'Amico, N. DiMarzio, S. Jarvis, E. McCarthy, R. Morrissey, J. Ward, and I. L. Boyd. 2011. Beaked whales respond to simulated and actual navy sonar. PLoS ONE **6**:e17009.

Uhen, M. D. 2010. The Origin(s) of Whales. Annual Review of Earth and Planetary Sciences **38**:189-219.

UniProt, C. 2019. UniProt: a worldwide hub of protein knowledge. Nucleic Acids Research **47**:D506-D515.

Valsecchi, E., W. Amos, J. A. Raga, M. Podestà, and W. Sherwin. 2004. The effects of inbreeding on mortality during a morbillivirus outbreak in the Mediterranean striped dolphin (Stenella coeruleoalba). Animal Conservation **7**:139-146.

Van Bressem, M. F., P. J. Duignan, A. Banyard, M. Barbieri, K. M. Colegrove, S. de Guise, G. di Guardo, A. Dobson, M. Domingo, D. Fauquier, A. Fernandez, T. Goldstein, B. Grenfell, K. R. Groch, F. Gulland, B. A. Jensen, P. D. Jepson, A. Hall, T. Kuiken, S. Mazzariol, S. E. Morris, O. Nielsen, J. A. Raga, T. K. Rowles, J. Saliki, E. Sierra, N. Stephens, B. Stone, I. Tomo, J. Wang, T. Waltzek, and J. F. X. Wellehan. 2014. Cetacean morbillivirus: Current knowledge and future directions. Viruses **6**:5145-5181.

Van Bressem, M. F., J. A. Raga, G. Di Guardo, P. D. Jepson, P. J. Duignan, U. Siebert, T. Barrett, M. C. De Oliveira Santos, I. B. Moreno, S. Siciliano, A. Aguilar, and K. Van Waerebeek. 2009a. Emerging infectious diseases in cetaceans worldwide and the possible role of environmental stressors. Diseases of Aquatic Organisms **86**:143-157.

Van Bressem, M. F., J. C. Reyes, F. Félix, M. Echegaray, S. Siciliano, A. P. Di Beneditto, L. Flach, F. Viddi, I. C. Avila, J. C. Herrera, I. C. Tobón, J. Bolaños-Jiménez, I. B. Moreno, P. H. Ott, G. P. Sanino, E. Castineira, D. Montes, E. Crespo, P. A. C. Flores, B. Haase, S. M. F. M. Souza, M. Laeta, and A. B. Fragoso. 2007. A preliminary overview of skin and skeletal diseases and traumata in small cetaceans from South American waters. Latin American Journal of Aquatic Mammals **6**.

Van Bressem, M. F., P. C. Simoes-Lopes, F. Felix, J. J. Kiszka, F. G. Daura-Jorge, I. C. Avila, E. R. Secchi, L. Flach, P. F. Fruet, K. du Toit, P. H. Ott, S. Elwen, A. B. Di Giacomo, J. Wagner, A. Banks, and K. Van Waerebeek. 2015. Epidemiology of lobomycosis-like disease in bottlenose dolphins Tursiops spp. from South America and southern Africa. Dis Aquat Organ **117**:59-75.

Van Bressem, M. F., K. Van Waerebeek, F. J. Aznar, J. A. Raga, P. D. Jepson, P. Duignan, R. Deaville, L. Flach, F. Viddi, J. R. Baker, A. P. Di Beneditto, M. Echegaray, T. Genovo, J. Reyes, F. Felix, R. Gaspar, R. Ramos, V. Peddemors, G. P. Sanino, and U. Siebert. 2009b. Epidemiological pattern of tattoo skin disease: a potential general health indicator for cetaceans. Diseases of Aquatic Organisms **85**:225-237.

Van Bressem, M. F., K. Van Waerebeek, and J. A. Raga. 1999. A review of virus infections of cetaceans and the potential impact of morbilliviruses, poxviruses and papillomaviruses on host population dynamics. Diseases of Aquatic Organisms **38**:53-65.

Van der Auwera, G. A., M. O. Carneiro, C. Hartl, R. Poplin, G. Del Angel, A. Levy-Moonshine, T. Jordan, K. Shakir, D. Roazen, J. Thibault, E. Banks, K. V. Garimella, D. Altshuler, S. Gabriel, and M. A. DePristo. 2013. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. Current Protocols in Bioinformatics **43**:11 10 11-11 10 33.

Venuto, R., S. Botta, A. S. Barreto, E. R. Secchi, and P. F. Fruet. 2020. Age structure of strandings and growth of Lahille's bottlenose dolphin (*Tursiops truncatus gephyreus*). Marine Mammal Science **36**:813-827.

Vermeulen, E., P. Fruet, A. Costa, M. Coscarella, and P. Laporta. 2019. *Tursiops truncatus ssp. gephyreus*. The IUCN Red List of Threatened Species

Vianna, J. A., F. A. N. Fernandes, M. J. Frugone, H. V. Figueiro, L. R. Pertierra, D. Noll, K. Bi, C. Y. Wang-Claypool, A. Lowther, P. Parker, C. Le Bohec, F. Bonadonna, B. Wienecke, P. Pistorius, A. Steinfurth, C. P. Burridge, G. P. M. Dantas, E. Poulin, W. B. Simison, J. Henderson, E. Eizirik, M. F. Nery, and R. C. K. Bowie. 2020. Genome-wide analyses reveal drivers of penguin diversification. Proc Natl Acad Sci U S A **117**:22303-22310.

Viaud-Martinez, K. A., R. L. Brownell, A. Komnenou, and A. J. Bohonak. 2008. Genetic isolation and morphological divergence of Black Sea bottlenose dolphins. Biological Conservation **141**:1600-1611.

Vijay, N., C. Park, J. Oh, S. Jin, E. Kern, H. W. Kim, J. Zhang, and J. K. Park. 2018. Population Genomic Analysis Reveals Contrasting Demographic Changes of Two Closely Related Dolphin Species in the Last Glacial. Molecular Biology and Evolution **35**:2026-2033.

Viricel, A., E. Pante, W. Dabin, and B. Simon-Bouhet. 2014. Applicability of RAD-tag genotyping for interfamilial comparisons: empirical data from two cetaceans. Molecular Ecology Resources **14**:597-605.

Wang, J. Y. J. 2001. DNA damage and apoptosis. Cell Death & Differentiation **8**:1047-1048.

Ward, J. R., and K. D. Lafferty. 2004. The elusive baseline of marine disease: are diseases in ocean ecosystems increasing? PLoS Biol **2**:E120.

Warren, W. C., L. Kuderna, A. Alexander, J. Catchen, J. G. Perez-Silva, C. Lopez-Otin, V. Quesada, P. Minx, C. Tomlinson, M. J. Montague, F. H. G. Farias, R. B. Walter, T. Marques-Bonet, T. Glenn, T. J. Kieran, S. S. Wise, J. P. Wise, Jr., R. M. Waterhouse, and J. P. Wise, Sr. 2017. The Novel Evolution of the Sperm Whale Genome. Genome Biology and Evolution **9**:3260-3264.

Waterhouse, R. M., M. Seppey, F. A. Simao, M. Manni, P. Ioannidis, G. Klioutchnikov, E. V. Kriventseva, and E. M. Zdobnov. 2017. BUSCO applications from quality assessments to gene prediction and phylogenomics. Molecular Biology and Evolution.

Waters, J. M. 2008. Marine biogeographical disjunction in temperate Australia: historical landbridge, contemporary currents, or both? Diversity and Distributions **14**:692-700.

Waters, J. M., T. M. King, P. M. O'Loughlin, and H. G. Spencer. 2005. Phylogeographical disjunction in abundant high-dispersal littoral gastropods. Molecular Ecology **14**:2789-2802.

Waters, J. M., P. M. O'Loughlin, and M. S. Roy. 2004. Cladogenesis in a starfish species complex from southern Australia: evidence for vicariant speciation? Molecular Phylogenetics and Evolution **32**:236-245.

Weir, B. S., and C. C. Cockerham. 1984. Estimating F-Statistics for the Analysis of Population Structure. Evolution **38**:1358-1370.

Weiss, M. N., D. W. Franks, K. C. Balcomb, D. K. Ellifrit, M. J. Silk, M. A. Cant, and D. P. Croft. 2020. Modelling cetacean morbillivirus outbreaks in an endangered killer whale population. Biological Conservation **242**.

Wells, R., H. Rhinehart, L. Hansen, J. Sweeney, F. Townsend, R. Stone, D. R. Casper, M. Scott, A. Hohn, and T. Rowles. 2004. Bottlenose Dolphins as Marine Ecosystem Sentinels: Developing a Health Monitoring System. EcoHealth **1**.

Wells, R. S., A. Natoli, and G. Braulik. 2019. *Tursiops truncatus*. The IUCN Red List of Threatened Species.

Wells, R. S., M. D. Scott, and A. B. Irvine. 1987. The Social Structure of Free-Ranging Bottlenose Dolphins. Pages 247-305 *in* H. H. Genoways, editor. Current Mammalogy. Springer US, Boston, MA.

Werling, D., and T. W. Jungi. 2003. TOLL-like receptors linking innate and adaptive immune response. Veterinary Immunology and Immunopathology **91**:1-12.

Wernberg, T., M. A. Coleman, S. Bennett, M. S. Thomsen, F. Tuya, and B. P. Kelaher. 2018. Genetic diversity and kelp forest vulnerability to climatic stress. Scientific Reports **8**:1851.

Westbury, M. V., B. Petersen, E. Garde, M. P. Heide-Jorgensen, and E. D. Lorenzen. 2019. Narwhal Genome Reveals Long-Term Low Genetic Diversity despite Current Large Abundance Size. iScience **15**:592-599.

Wickert, J. C., S. M. von Eye, L. R. Oliveira, and I. B. Moreno. 2016. Revalidation ofTursiops gephyreusLahille, 1908 (Cetartiodactyla: Delphinidae) from the southwestern Atlantic Ocean. Journal of Mammalogy **97**:1728-1737.

Wild, S., M. Krutzen, R. W. Rankin, W. J. E. Hoppitt, L. Gerber, and S. J. Allen. 2019. Long-term decline in survival and reproduction of dolphins following a marine heatwave. Current Biology **29**:R239-R240.

Williams, E. S., T. Yuill, M. Artois, J. Fischer, and S. A. Haigh. 2002. Emerging infectious diseases in wildlife. Revue Scientifique et Technique de l'OIE **21**:139-157.

Wilson, N. G., J. Stiller, and G. W. Rouse. 2016. Barriers to gene flow in common seadragons (Syngnathidae: Phyllopteryx taeniolatus). Conservation genetics **18**:53-66.

Wiszniewski, J., L. B. Beheregaray, S. J. Allen, and L. M. Möller. 2009. Environmental and social influences on the genetic structure of bottlenose dolphins (*Tursiops aduncus*) in Southeastern Australia. Conservation genetics **11**:1405-1419.

Worm, B., E. B. Barbier, N. Beaumont, J. E. Duffy, C. Folke, B. S. Halpern, J. B. C. Jackson, H. K. Lotze, F. Micheli, S. R. Palumbi, E. Sala, K. A. Selkoe, J. J. Stachowicz, and R. Watson. 2006. Impacts of Biodiversity Loss on Ocean Ecosystem Services. Science **314**:787-790.

Wright, B., K. Morris, C. E. Grueber, C. E. Willet, R. Gooley, C. J. Hogg, D. O'Meally, R. Hamede, M. Jones, C. Wade, and K. Belov. 2015. Development of a SNP-based assay for measuring genetic diversity in the Tasmanian devil insurance population. BMC Genomics **16**:791.

Wright, B., C. E. Willet, R. Hamede, M. Jones, K. Belov, and C. M. Wade. 2017. Variants in the host genome may inhibit tumour growth in devil facial tumours: evidence from genome-wide association. Scientific Reports **7**:423.

Wright, B. R., K. A. Farquharson, E. A. McLennan, K. Belov, C. J. Hogg, and C. E. Grueber. 2020. A demonstration of conservation genomics for threatened species management. Molecular Ecology Resources.

WWF. 2020. Living Planet Report 2020. Gland: WWF.

Yang, J., B. Benyamin, B. P. McEvoy, S. Gordon, A. K. Henders, D. R. Nyholt, P. A. Madden, A. C. Heath, N. G. Martin, G. W. Montgomery, M. E. Goddard, and P. M. Visscher.

2010. Common SNPs explain a large proportion of the heritability for human height. Nature Genetics **42**:565-569.

Yang, Z. 2007. PAML 4: Phylogenetic Analysis by Maximum Likelihood. Molecular Biology and Evolution **24**:1586-1591.

Yim, H. S., Y. S. Cho, X. Guang, S. G. Kang, J. Y. Jeong, S. S. Cha, H. M. Oh, J. H. Lee, E. C. Yang, K. K. Kwon, Y. J. Kim, T. W. Kim, W. Kim, J. H. Jeon, S. J. Kim, D. H. Choi, S. Jho, H. M. Kim, J. Ko, H. Kim, Y. A. Shin, H. J. Jung, Y. Zheng, Z. Wang, Y. Chen, M. Chen, A. Jiang, E. Li, S. Zhang, H. Hou, T. H. Kim, L. Yu, S. Liu, K. Ahn, J. Cooper, S. G. Park, C. P. Hong, W. Jin, H. S. Kim, C. Park, K. Lee, S. Chun, P. A. Morin, S. J. O'Brien, H. Lee, J. Kimura, D. Y. Moon, A. Manica, J. Edwards, B. C. Kim, S. Kim, J. Wang, J. Bhak, H. S. Lee, and J. H. Lee. 2014. Minke whale genome and aquatic adaptation in cetaceans. Nature Genetics **46**:88-92.

Zanardo, N., K. Bilgmann, G. J. Parra, and L. M. Möller. 2016a. Socio-genetic structure of short-beaked common dolphins in southern Australia. Journal of Zoology **299**:89-97.

Zanardo, N., G. J. Parra, and L. M. Möller. 2016b. Site fidelity, residency, and abundance of bottlenose dolphins (*Tursiops* sp.) in Adelaide's coastal waters, South Australia. Marine Mammal Science **32**:1381-1401.

Supplementary Figures

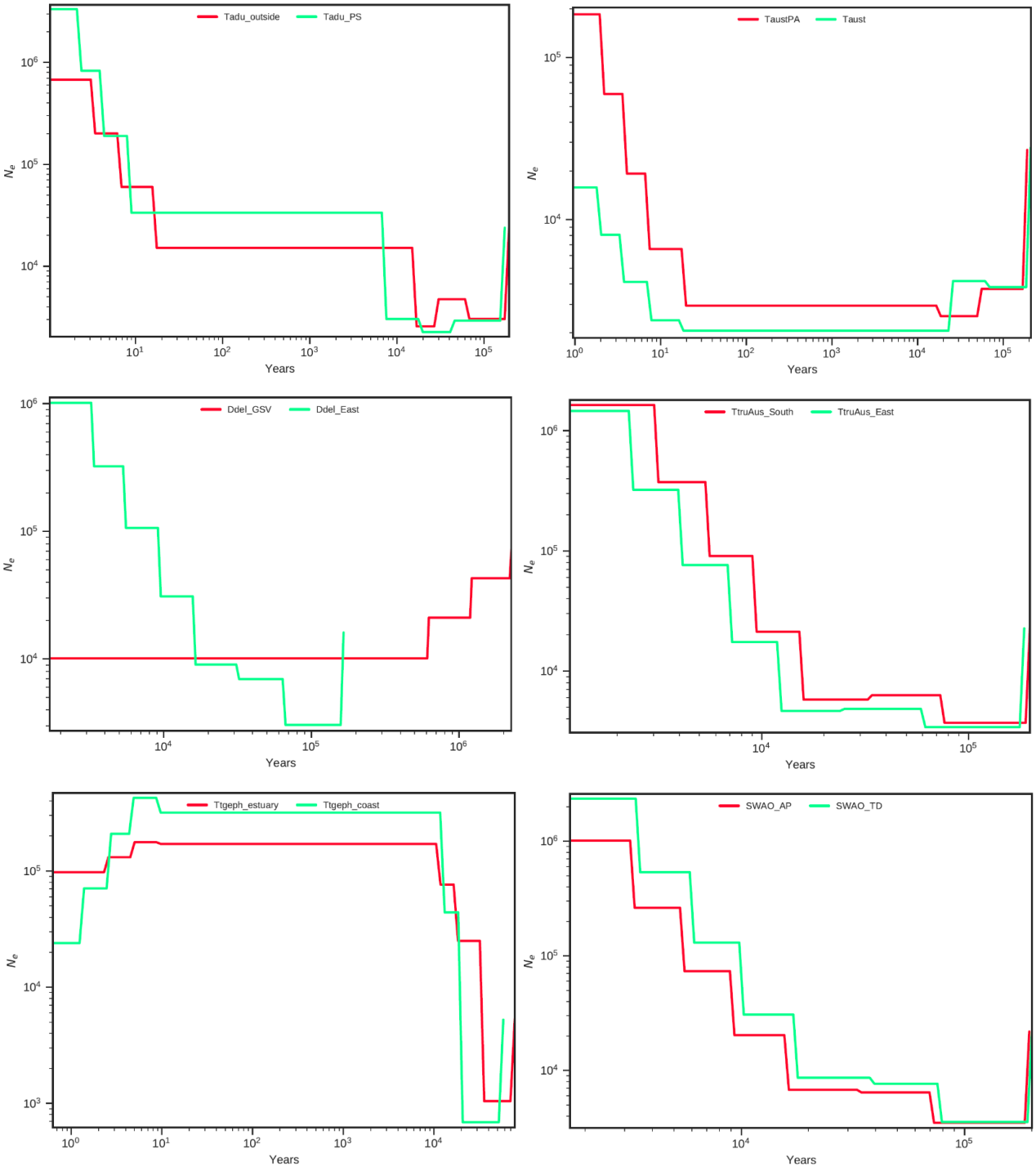

Figure S2.1: Demographic histories between populations within each dolphin lineage (*Tursiops* and *D. delphis*) examined from the Southern Hemisphere.

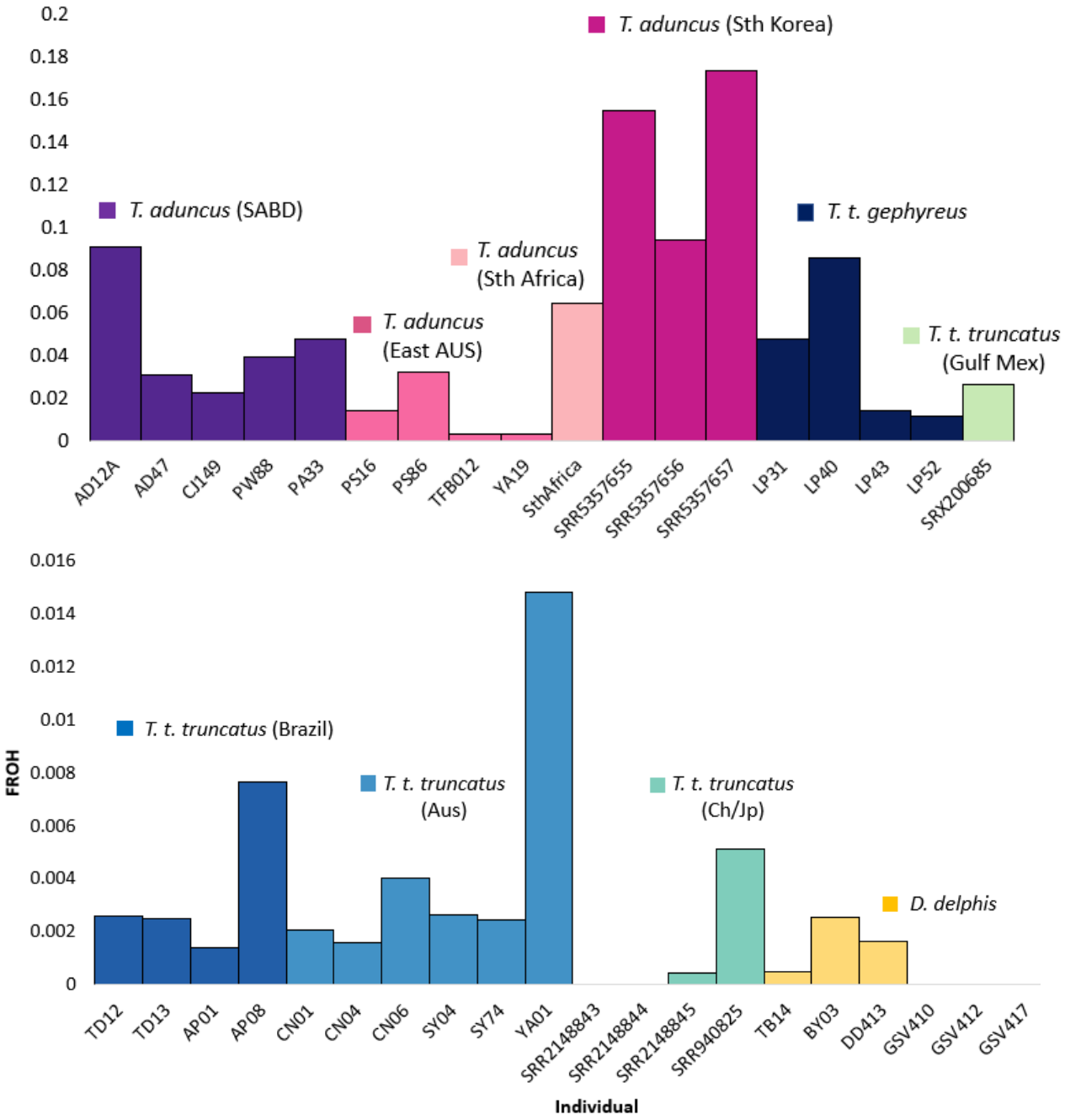

Figure S3.2: FROH (total sum of ROH > 1MB divided by the total length of autosomes) for the 38 individual dolphins analysed from the inshore ecotype (top), nearshore *D. delphis* and offshore ecotype (bottom). For sampling locations see Table S1.

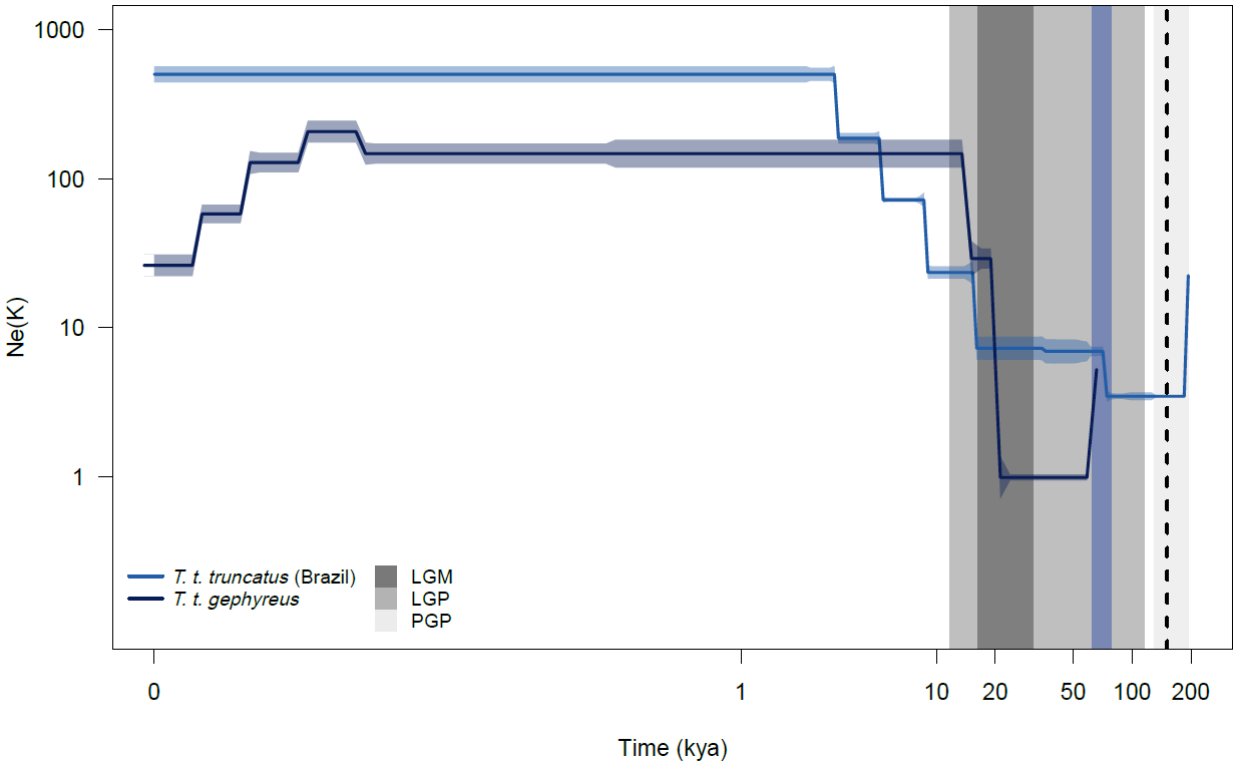

Figure S3.3: Estimated time of divergence between the offshore *T. t. truncatus* and the inshore *T. t. gephyreus* from the Southwest Atlantic Ocean, estimated using SMC++ (~151 kya, 95% CI 62,798-77,987).

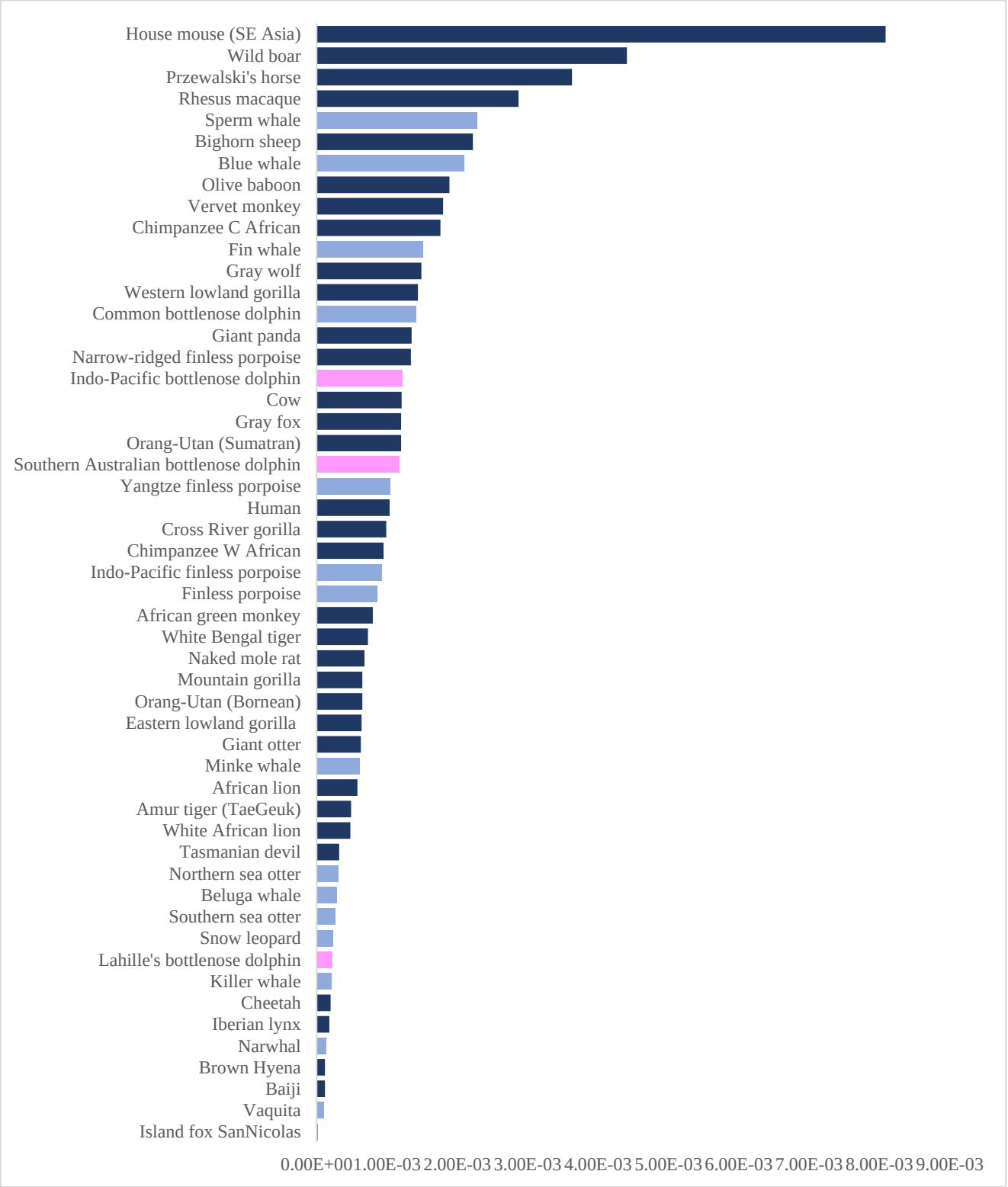

Figure S3.4: Comparison of genome-wide heterozygosity among mammals. Heterozygosity values are downloaded from Morin et al. (2020b) and based on (Robinson et al. 2016). Dark blue bards indicate terrestrial mammals; light blue bars indicate marine mammals (including the common bottlenose dolphin), and pink bars represent data generated in this study (Lahille's bottlenose dolphin; *T. t. gephyreus*, the Southern Australian bottlenose dolphin; *T. aduncus* (SABD), and the Indo-Pacific bottlenose dolphin; *T. aduncus*). See Table S4 for heterozygosity information.

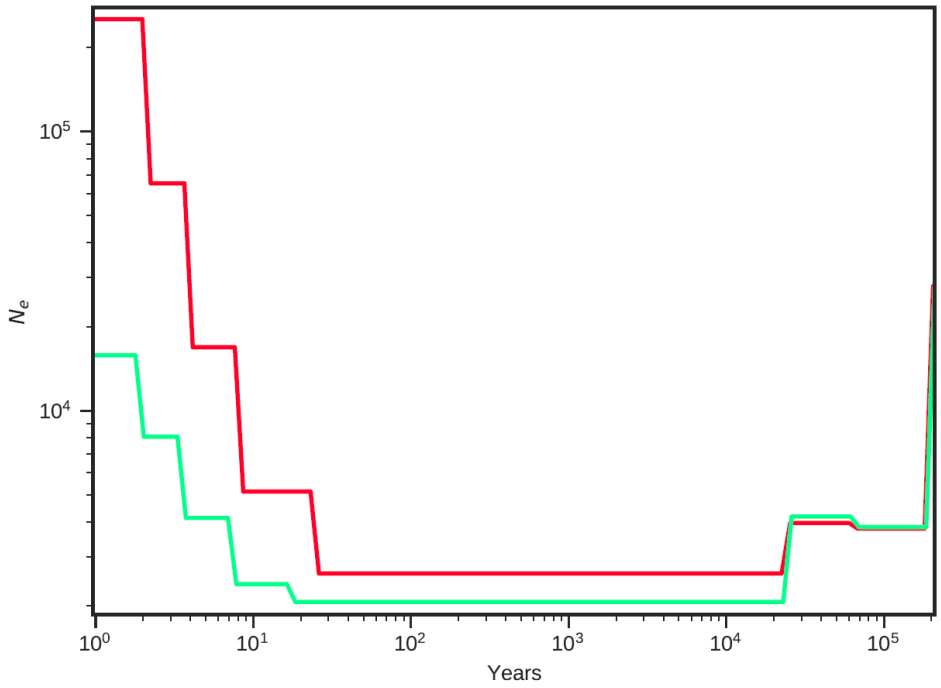

Figure S3.5: Comparison of demographic histories for *T. aduncus* (SABD) using all available genomes from this lineage (n = 30) (red line) and the five genomes used in the main text of this chapter (n = 5).

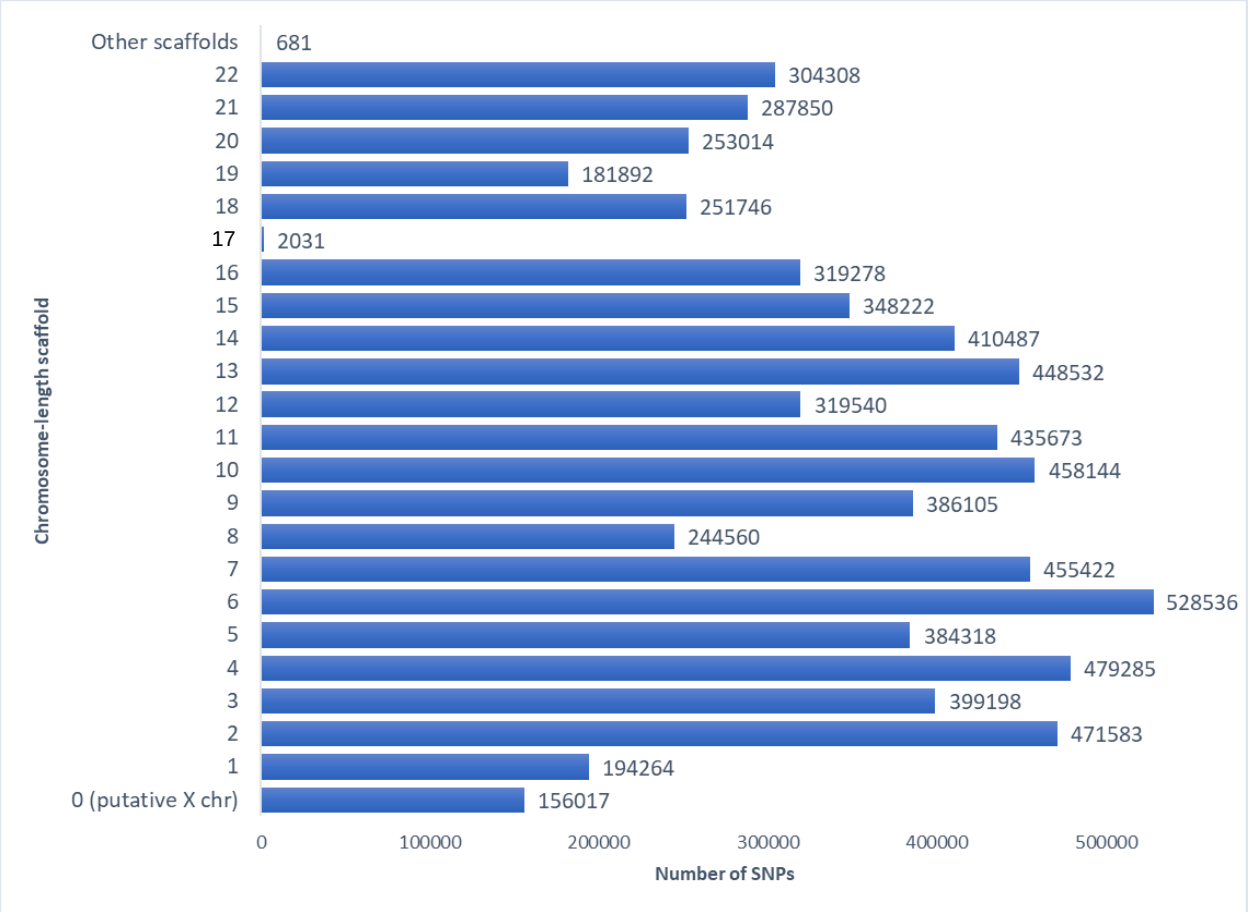

Figure S4.1: The number of SNPs anchored onto 22 chromosome-length scaffolds and 77 short scaffolds, used in a whole genome study of susceptibility and resistance of *Tursiops aduncus* to cetacean morbillivirus in Gulf Saint Vincent, South Australia.