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.