GENETIC STRUCTURE AND DIVERSITY OF SOUTH AFRICA'S INSHORE BRYDE'S WHALE (*BALAENOPTERA EDENI BRYDEI,* OLSEN 1913) POPULATION

by

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SUMMARY

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by

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South Africa's inshore Bryde's whale (*Balaenoptera edeni brydei*) is the largest, resident baleen whale species occurring in our coastal waters. However, due to being one of the most elusive and shy marine mammals, there are significant gaps in knowledge relating to their phylogeny, ecology, and demography. The few studies done on this population provided important, broadscale information but also highlighted the more detailed research needed to improve and develop the understanding of South Africa's inshore Bryde's whales. It is generally accepted that these whales form a small population (< 1,000 mature individuals) that shows a restricted coastal distribution along the coastline of South Africa. Their movements along the coast are largely dictated by the movements of their main food sources, sardine (*Sardinops sagax)* and anchovy (*Engraulis capensis*). However, their population structure, movement patterns, and habitat use across the diverse oceanic system surrounding South Africa, as well as the level of genetic diversity remained unclear. Within the broader, evolutionary perspective the connectivity of the inshore population to other Bryde's whale populations worldwide is unknown.

This dissertation uses conservation genetic approaches combined with photo-identification methods to build our understanding of South Africa's inshore Bryde's whale population in two main areas. Microsatellite and mitochondrial DNA (mtDNA) markers were used to detect any evidence of population structure and connectivity while assessing current levels of genetic diversity on both local and global scales. Photo-identification data was then used to assess the movement patterns of individuals and formalize a national catalogue for the population. Genetic results show that these whales form one population with low, but significant, levels of genetic differentiation between the west and south coast of South Africa. Neutral nuclear genetic diversity was high which may be indicative of a larger effective population size whereas mtDNA diversity was low, suggesting potential sex-biased dispersal. Results also indicated genetic distinction between South African, Namibian, and Madagascan individuals but highlighted the need for stronger sampling within these regions. The lack of a stronger population structure was also reflected in photo-identification data, with identified individuals moving across different coastal regions, seemingly forming one population. However, many individuals were also re-sighted in the same general area which may relate to the low levels of genetic differentiation still evident within the population. On a global scale, mtDNA results indicated that South Africa's offshore and inshore populations show connections to different oceanic regions. In addition, inshore individuals share a haplotype with an individual from the East Indian Ocean. These results provide preliminary insight into the origins of the South African Bryde's whale populations and highlight the overall need for more representative sampling globally. Overall, the results of this study provide important insights into the demography and ecology of the South African inshore population which is key in the formulation of effective conservation strategies for the species.

RESEARCH OUTPUTS

Conference presentations

Oral presentations:

- South African Marine Science Symposium 2022

Poster presentations

- International Conference of Zoology, Virtual poster presentation, 2021
- Society of Marine Mammalogy, Virtual poster presentation, 2022

LIST OF ABBREVIATIONS

mtDNA	Mitochondrial DNA
ESU	Evolutionary Significant Unit
GOMx	Gulf of Mexico
Photo-ID	Photo-identification
Rpm	Rounds per minute
PCR	Polymerase chain reaction
$MgCl_2$	Magnesium Chloride
TAE	Tris, Acetic acid, EDTA
HWE	Hardy-Weinberg Equilibrium
LD	Linkage disequilibrium
PCoA	Principal Coordinate's Analysis
FCA	Factorial Correspondence Analysis

DECLARATION

I, Dominique Kelsi Paynee, declare that the dissertation, which I hereby submit for the degree Master of Science (MSc) Zoology at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

ETHICS STATEMENT

I, Dominique Kelsi Paynee, whose name appears on the title page of this dissertation, has obtained, for the research described in this work, the applicable research ethics approval.

I, Dominique Kelsi Paynee have observed the ethical standards required in terms of the University of Pretoria's Code of Ethics for Researchers and the Policy guidelines for responsible research.

DISCLAIMER

Chapters 2 and 3 in this MSc dissertation were structured according to the author guidelines of *Molecular Ecology*. Therefore, it is inevitable for some overlap and repetition between chapters to exist.

Dominique Paynee
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#TEAMBRYDE'S

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Dominique Paynee

CONTENTS

CHAPTER 1

South Africa’s secret: the inshore Bryde’s whale (*Balaenoptera edeni brydei*)

1.1 |THE BRYDE'S WHALE

1.1.1 | History and taxonomy

The Bryde's whale (*Balaenoptera edeni)* is an elusive and rare baleen whale species with little information available regarding its taxonomy, phylogeny, and global population connectivity. They are classified within the family Balaenopteridae (Committee on Taxonomy, 2020), and, to date, a unanimous agreement on the number of species and subspecies of Bryde's whale has not been established. This is a common challenge presented throughout cetacean taxa, which has direct effects on the success of conservation and management practices of many populations, and is particularly prevalent in Bryde's whales (Martien et al., 2017; Rosel et al., 2017; Taylor et al., 2017; Taylor et al., 2017). Bryde's whales have complex and, to a large extent, unresolved phylogenetic relationships and taxonomy since their history of discovery is associated with much confusion and uncertainty (Kershaw et al., 2013; Penry et al., 2011; Sasaki et al., 2006). The basis of their unresolved taxonomy stems from several complications encountered in the initial description of the species. Bryde's whales were first described by Anderson (1878) from a specimen in Burma which was initially named Eden's whale (*B. edeni)* (Sasaki et al., 2006). After this discovery, a new baleen whale species (often confused with the sei whale) was described in South Africa by Olsen (1913). As it was newly classified, this specimen was named the Bryde's whale (*B. brydei)* after Johan Bryde a Norwegian national who started up the first whaling station in Durban (Kato, 2002; Penry et al., 2018). Upon initial inspection, the two specimens seemed extremely similar which directed the process of skeletal comparisons of both *B. edeni* and *B.brydei* to the sei whale (*B. borealis)* by Junge (1950). Based on this comparison, it was concluded that *B. edeni* and *B .brydei* were conspecific and should be synonymized under *B .edeni* (Junge, 1950). However, subsequent studies revealed that this conclusion was an oversimplification as several morphological, ecological, and behavioural differences were recorded between global populations of Bryde's whales (Best, 1977, 2001; Penry et al., 2018; Wiseman, 2008; Yoshida & Kato, 1999). These differences, which are evident between large-scale and small, coastal populations of Bryde's whales, could potentially justify separate species or subspecies assignment (Penry et al., 2018).

In efforts to clarify their taxonomy, three potential subspecies represented the "Bryde's whales complex": the larger *B. brydei* and two smaller forms: *B. edeni* and *B. omurai* (Luksenburg et al., 2015; Sasaki et al., 2006; Wada et al., 2003). But subsequent molecular analyses revealed that *B.omurai* should be classified as a separate species, which is now known as Omura's whale (Sasaki et al., 2006). This reclassification clarified some of the confusion associated with the taxonomy of Bryde's whales (Penry et al., 2018; Sasaki et al., 2006), and thus, the Society of Marine Mammalogy recognizes a single Bryde's whale species (*B. edeni*) with two provisionally accepted subspecies (*B. e.edeni* and *B. e.brydei*) (Committee on Taxonomy, 2020). Although there are multiple lines of evidence that these subspecies could be separated at the species level; there are gaps in key information needed for species assignment (Constantine et al. 2018). The process is halted by a lack of genetic analyses and confirmation on the type specimen of *B. edeni* coupled with an undesignated holotype specimen of *B. brydei* (Constantine et al., 2018; Penry et al., 2018; Rosel et al., 2021). Later studies also suggest that Olsen's description of the Bryde's whale was not entirely accurate, given that the description included features of both inshore and offshore forms in the southern African region (Best 1977, 2001; Kanda et al. 2007; Penry, 2010; Constantine et al. 2018). Nonetheless, these proposed subspecies show several key ecological differences that can be illustrated effectively by comparison of their distribution, reproductive and foraging behaviours.

1.1.2 | Distribution

Bryde's whales are distributed globally throughout most tropical, subtropical, and temperate oceanic regions between approximate latitudes of 40° N and 40° S (Best, 2007; Kato & Perrin, 2009; Penry, 2010) (Figure 1). The different subspecies of Bryde's whales correlate with differing distributions where, often, individuals of *B. e. edeni* represent a small coastal form of Bryde's whales distributed in the western Pacific and Indian oceans, and *B. e .brydei* represents the larger, globally distributed pelagic form (Sasaki *et al.* 2006; Committee on Taxonomy, 2020). In some cases, evidence has indicated the subspecies *B .e.brydei* is possibly associated with coastal distributions as indicated in genetic analyses of the South African inshore population completed by Penry (2018). These whales generally do not undertake long-distance migrations (Omura, 1962) which sets them apart from other baleen whale species, such as the humpback whale (*Megaptera novaeangliae)* and the southern right whale (*Eubalaena autralis*), in the Southern hemisphere that are often characterized by routine feeding migrations to the Antarctic (Best, 2007).

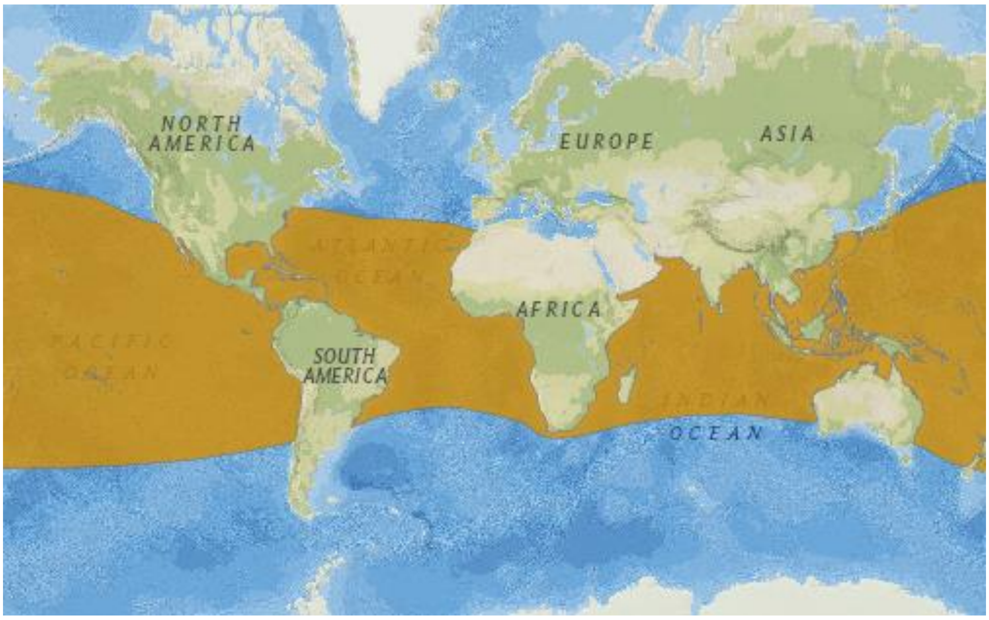

FIGURE 1 The global distribution of the Bryde's whale (IUCN, 2021)

Along the southern African coastline, two forms of Bryde's whales occur with differing distributions as seen worldwide, one is an offshore form, and the second an inshore form (Best, 1977). Later investigations proposed that three subpopulations reside within the area (Best, 2001). The first subpopulation is an inshore population that is found year-round along the coastline and undertakes small-scale movements along the west and east coasts of the country following their main prey sources(Figure 2A). The remaining two are both separate offshore populations found in different areas of the greater southern African region. Evidence shows that the southeast Atlantic offshore subpopulation is found to the west of southern Africa (Figure 2B) which makes seasonal movements north in summer and further south in winter (25°–28°S) (Best, 2007). The third subpopulation of the region is the southwest Indian Ocean subpopulation (Figure 2C); although not much is known about this group, it is thought to occur close to the south of Madagascar in summer and move as far as Seychelles in winter (Best, 2001).

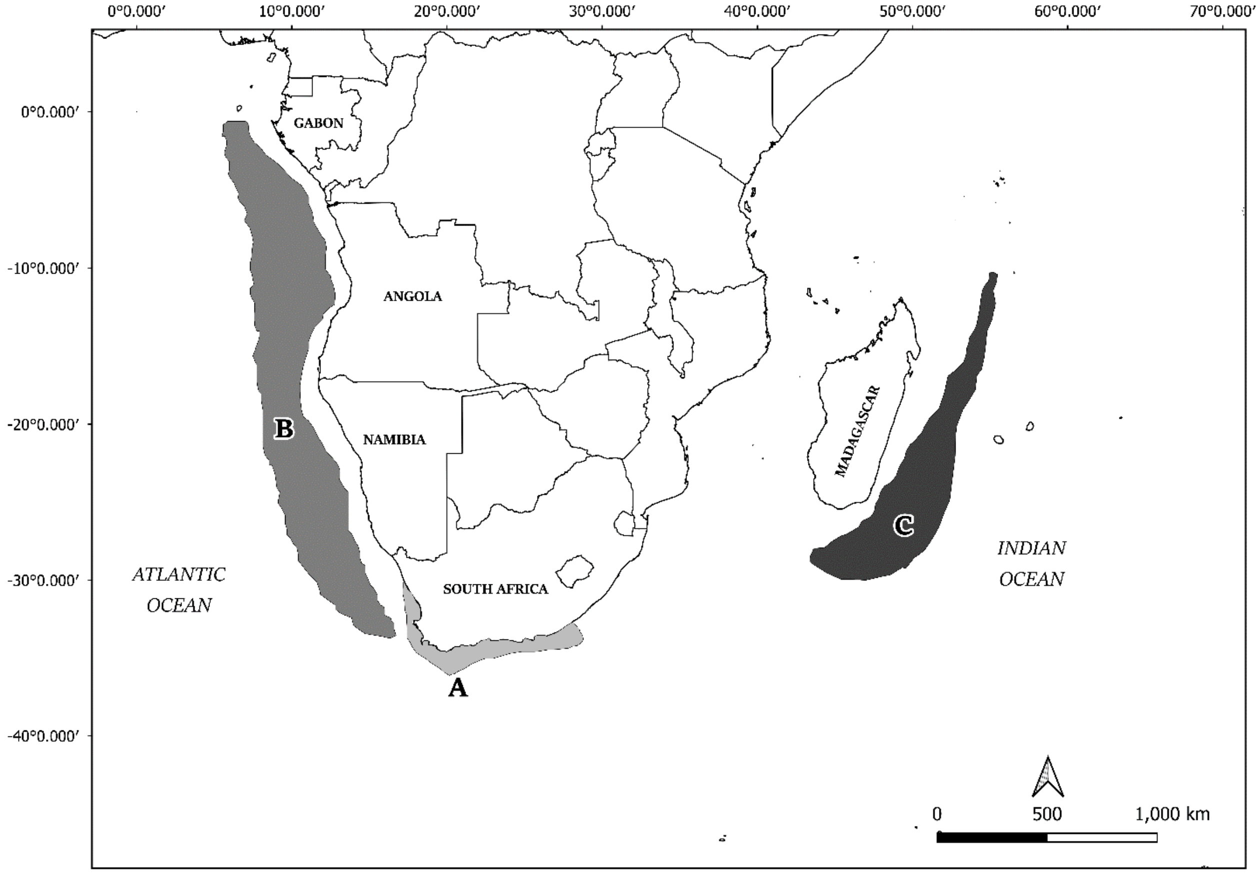

FIGURE 2 Approximate distributions of three subpopulations of the Bryde's whale in the southern African region. **A)** The inshore population, **B)** The southeast Atlantic offshore population, and **C)** The South-West Indian Ocean offshore population

1.1.3 | Reproduction

Since Bryde's whales display several differences among populations, it is difficult to assign generalizations to the species in terms of reproductive behaviours (IUCN, 2021). Thus, only the South African inshore and offshore populations will be discussed and compared in this section. As with most aspects of their ecology, there are some evident differences in reproduction between offshore (Figure 2B) and inshore Bryde's whales (Figure 2A) found on the South African coast. First, size at sexual maturity differs between the inshore and offshore populations. Inshore females and males reach sexual maturity at 11.9–12.5 m and 11.6–12.5 m respectively (Best, 2007). On the other hand, offshore females and males reach sexual maturity at larger sizes of 12.8–13.1 m and 12.8–13.7 m respectively (Best, 2007). Evidence shows that there are also differences in ovulation cycles between the populations. Inshore females seem to ovulate multiple times throughout the reproductive cycle even during lactation (Best, 2007), which could be a result of the inshore population's consistent and year-round food resources (Best, 2001). In contrast, offshore females are similar to most baleen whales and ovulate once in each seasonal cycle (Best, 2007). After a 12–month gestation period (Best, 2007), females give birth to a single calf between 3.81-3.96 m in length, with no distinction in calf size seen between inshore and offshore populations (Best, 2001). The average generation time for Bryde's whales was estimated to be 18 years (Taylor et al., 2007).

1.1.4 | Foraging

Bryde's whales display flexibility in foraging strategies seen by the wide range of different behaviours employed to catch prey, once again, setting them apart from other species of baleen whales (Constantine et al., 2018). Like all members of Balaenopteridae, they use lunge feeding as their primary foraging strategy. This foraging strategy is characterized by the engulfment of a large volume of water at a high speed followed by filtration with the mouth closed (Constantine et al., 2018; Goldbogen et al., 2017). However, Bryde's whale populations in different geographical areas have been observed displaying a range of different foraging behaviours (Constantine et al., 2018). These foraging behaviours include feeding at the sea surface and deeper depths during the daytime or night-time (Alves et al., 2010; Izadi et al., 2018; Tershy, 1992). But these specialized feeding behaviours are specific to one area within a greater region, which means individuals from different areas may not share the same foraging strategy. If a decline in specific prey availability occurs, individuals of a population will need to readapt their foraging strategies to ensure survival (Constantine et al., 2018).

On the South African coast, there are recorded differences in feeding preferences between the inshore and offshore populations. The offshore population generally feeds on euphausiids species such as *Euphausia recurve* and *Euphausia lucens* as well as mesopelagic fish such as lightfish (*Maurolicus spp*) and Atlantic barracudina (*Lestidium spp)* (Best, 2001; Penry et al., 2018), while the inshore population feeds on smaller pelagic fish like sardines (*Sardinops sagax)* or anchovy (*Engraulis capensis*) (Penry et al., 2016). The movements and distributions of these small pelagic fish species influence and dictate the patterns of movements of the inshore population along the coastline (Best, 2001; Penry et al., 2016)

1.1.5 | Threats and conservation status of the South African inshore Bryde's whale

Although Bryde's whales hold a global listing as "Least Concern" according to the IUCN Red List (Cooke & Brownell, 2018); the South African inshore population has a national listing as "Vulnerable" according to the South African Red List Assessment completed in 2016 (Penry et al., 2016). One of the main reasons for this listing is their small population size. The first and most inclusive size estimate of the population was completed in 1984 which indicated that 582 ± 182 individuals made up the inshore population along the entire coastline of South Africa (Best et al., 1984). Abundance estimates calculated in 2010 estimated that 130 to 250 individuals occurred in the restricted area of Plettenberg Bay (Penry, 2010). Based on these two population estimates, it is assumed that fewer than 1,000 mature individuals make up the population, supporting their listing as "Vulnerable" under IUCN D1 criteria (*A taxon with an acute restriction on the number of mature individuals (<1,000) making them particularly vulnerable to stochastic events and other threats;* IUCN 2021). Because of this small population size, additional threats, such as entanglements and indirect effects of climate change, have significant impacts on the inshore Bryde's whale population.

Due to their year-round residency within South African coastal areas, inshore Bryde's whales are frequently exposed to fishing activities and thus extremely prone to entanglements (Findlay, 1989; Meÿer et al., 2011). Entanglement generally occurs in stagnant fishing gear, anchors, or buoy lines (Meÿer et al., 2011). Fatal entanglements are of greatest concern as the repeated removal of individuals from a system will result in population decline. In recent years, the frequency of such fatal entanglements has become a real concern for the inshore Bryde's whale population. Most often entanglements are related to octopus and rock lobster fishing gear (Constantine et al., 2018; Penry et al., 2016). Therefore, as these fisheries expand in South Africa, efficient and effective management will be required to avoid severe population decline for this small inshore Bryde's whale population (Constantine et al., 2018).

As the changing climate poses a serious threat to cetaceans in general (Pendleton et al., 2022; Tulloch et al., 2019) indirect effects of climate change (such as changes in prey availability), likely, impact the South African inshore population of Bryde's whales as well. As such, there has been a significant shift in the ecosystem from the west to the east along the South African coastline

(Blamey et al., 2015). One of the most significant shifts in the ecosystem is in the distributions and movements of pelagic fish such as sardines and anchovy, which are not only of extreme commercial value to the South African fishing industry but are also the main prey source for several marine predators including the inshore Bryde's whales (Blamey et al., 2015). The effects of this shift have cascading effects through the ecosystem and have impacted some of these marine predators, mainly sea birds, which have been investigated and showed bleak results. For example, Crawford *et al.* (2015) documented the effects of the shift in pelagic fish on South African bird species such as the African Penguin (*Spheniscus demersus*), Cape Gannet (*Morus capensis*), Cape Cormorant (*Phalacrocorax capensis*), Bank Cormorant (*P.neglectus*), and Swift Tern (*Thalasseus bergii*) and indicated that the shifts caused significant population declines in several of these species (Crawford et al., 2015). The redistribution of small pelagic fish is expected to have reaching effects throughout trophic levels and affect many predators that rely on these species as food resources (Blamey et al., 2015; Crawford et al., 2015). Since Bryde's whales are the largest marine predator of these pelagic fish it is reasonable to believe that this may also have detrimental effects on the population, although no investigations have been done in this regard. The increasingly serious concern of the observed ecosystem shift in South African coastal waters, adds to the relevance of investigating this small population of inshore Bryde's whales.

Another considerable threat to South African inshore Bryde's whales relates to their small population size and their potential genetic isolation (Penry et al., 2016). In general, smaller populations have low genetic diversity; which has various adverse effects on the species (Hauser & Carvalho, 2008), as it decreases the ability of the species to respond and adapt to various factors such as disease, parasites, or changes in the environment (Amos & Harwood, 1998). Understanding certain aspects of a species within its distributional range, in the context of several threats, will inform effective management strategies and conservation practices that can be implemented for the species (Kanda et al., 2007).

1.2 | THE SOUTH AFRICAN COASTAL REGION

1.2.1 | Oceanography of the South African coastal region

The southern African region is characterized by diverse and complex oceanic circulation systems (Lutjeharms et al., 2001) which, in turn, supports a rich marine diversity. South Africa's coastline is

3,650 km in length (Driver et al., 2005) and is surrounded by the Atlantic Ocean on the west coast and the Indian Ocean on the east coast (Griffiths et al. 2010). These two oceans form two main current systems within the South African coastal region (Griffiths et al., 2010). The warm Agulhas Current, which is an Indian Ocean western boundary current, flows on South Africa's east coast (Findlay et al., 1992), while the cold Benguela Current flows along the Atlantic coast to the west of the country (Griffiths et al., 2010; Lutjeharms et al., 2000) (Figure 3).

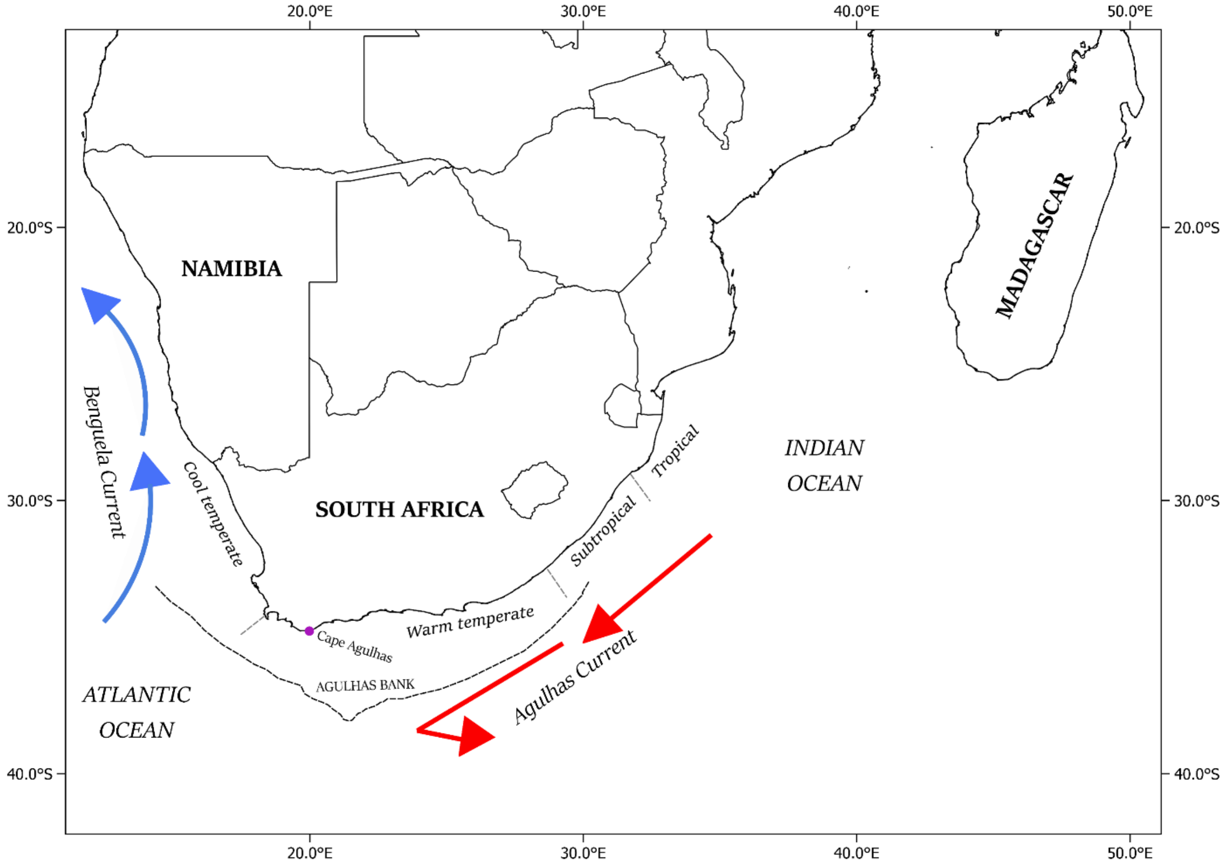

FIGURE 3 Oceanography and the four marine biogeographic provinces along the southern African coast

1.2.1.1 | West coast

The Benguela Current is a complex and important system that spans the coastline of three countries: Angola, Namibia, and South Africa, on the west coast of the African continent (Shannon et al., 2006). This system alone can be further divided into four main "ecosystems" across its range, each with its own set of characteristics (Hutchings et al., 2009). The section of this system that occurs along the South African coast is comprised of two main components, both of which contribute to key characteristics of the west coast of the country. The first component is a coastal element that is regulated by local weather processes while the second is an offshore oceanic flow that forms a part of the eastern stretch of the South Atlantic subtropical gyre (Griffiths et al., 2010; Shannon, 1985). This cold oceanic current along South Africa's coastline is characterized by wide, shallow movements of water toward the equator, with a typical current speed of approximately 0.02 m/sec and temperatures of 8–16 °C (Shannon, 1985). The upwelling processes of the Benguela Current on the west coast of South Africa generate a high productivity zone due to the water being cold and nutrient-rich, which supports a productive system (Best, 2007; Griffiths et al., 2010). These processes involve the removal of surface water offshore by wind action which then causes colder nutrient-rich water to be drawn to the surface (Best, 2007), explaining the high productivity in this zone. This is an important ecological process as it facilitates the increase in primary productivity as a result of the upward movement of nutrients to surface waters (Lutjeharms & Stockton, 1987). Because of the high productivity, this area of the coast serves as a nursery ground for a wide range of marine fish species (Hutchings et al., 2009), which in turn influences the distribution of many marine predators along the South African coast such as the inshore Bryde's whales.

1.2.1.2 | East coast

In contrast to the Benguela Current, the Agulhas Current differs in several aspects. First, it is an intense, warm (20–28 °C; Lutjeharms 2007; Penry 2010), and fast-flowing current, with instances of surface water moving at speeds of 2 m/sec (Best, 2007; Griffiths et al., 2010). Therefore this system plays an important role in facilitating global ocean heat variability (Lutjeharms, 2006). Furthermore, this current system is driven by waters from the tropical and subtropical regions (Figure 3) that are nutrient-poor, resulting in lower biological productivity on the east coast when compared to the west coast of South Africa (Best, 2007; Griffiths et al., 2010). However, the Agulhas Current does display two instances of upwelling processes which increase the productivity in certain areas within the range of this system (Lutjeharms et al., 2000). The first upwelling process takes place between the continental slope and the shoreward side of the Agulhas Current (Lutjeharms et al., 2000). The second occurs where the continental shelf widens along the path of the current and brings in nutrient-rich cold waters throughout the year (Lutjeharms et al., 2000). This process significantly influences the marine biota and productivity in this region of the current's path. The widest part of the continental shelf forms the stretch of the Agulhas Bank which is a significant region in terms of biodiversity and oceanography (Figure 3; Lutjeharms 2007). The waters in this region are colder with a higher salt content allowing for a higher level of productivity in this zone

(Hutchings et al., 2009; Jackson et al., 2012) which, in turn, influences the distribution of many marine organisms.

1.2.2 | Distribution patterns of marine species in relation to oceanographic features

The South African coastline falls within a transition zone between the Indo-Pacific and Atlantic Ocean biome and as a result, marine biodiversity is high within this region (Teske et al., 2011). The South African coastal region can be further divided into four major marine biogeographic provinces namely: the cool temperate, warm temperate, subtropical, and, tropical, each creating specific niches for a range of marine biodiversity (Figure 3 Emanuel et al*.,* 1992; Teske et al., 2011). Such a diversity of marine biota is reflected by the 12,941 species recorded in 2016, representing approximately 15% of the global total (Duncan et al., 2016). The oceanography of the South African coastal region not only supports the high species diversity but also influences the movements and distributions of individuals of marine populations along the coastline.

The west coast is characterized by high phyto- and zooplankton production which supports several populations of pelagic fish in the Benguela region (Verheye et al., 2016). On the east coast of South Africa, there are also notable and important areas for some marine organisms, despite having lower biological productivity in comparison to the west coast. The Agulhas Bank is an important region where pelagic fish species, such as sardines, occur during the autumn and winter months (Coetzee et al., 2018). Here, sardines and other small pelagic fish form spawning aggregations owing to optimal environments being created by upwelling processes(Beckley & Hewitson, 1994; Coetzee et al., 2010). Furthermore, their occurrence here is also determined by a high level of nutrients on the bank that is then used by phytoplankton which, in turn, support these marine fish populations. The distribution of pelagic fish populations such as sardine and anchovy then dictate the movements and distributions of predators that feed on them such as Cape Gannets*,* African Penguins*,* and the inshore Bryde's whale population. This is effectively illustrated by the annual migration of sardines in a well-known event called the "Sardine Run" whereby these pelagic fish move up the east coast of the country in winter. This biological event directs the movements of inshore Bryde's whales along the coastline (Penry et al., 2016) which is a notable characteristic of this small baleen whale population. By accounting for the oceanography and how it influences the movements of specific pelagic fish species, a better understanding of the movements and distribution of marine predators such as the inshore Bryde's whales can be gained.

1.3 | CONSERVATION GENETICS APPROACH

1.3.1 | Population genetics

Population genetics concepts can be applied in the development of effective conservation and management planning of cetacean populations, as they can bring insight into the potential factors obstructing gene flow and highlight genetically distinct populations (von der Heyden, 2009). By identifying potential barriers to gene flow, the dispersal patterns of populations, which is an important part of understanding the life history of species (von der Heyden, 2009), can be better understood. In addition to this, population genetics can also be used to investigate the extent to which demographic changes in populations play a role in altering phenotypic traits and responses to changes in the environment (Hauser & Carvalho, 2008). And lastly, one of the most important aspects of population genetics can estimate is the effective population size and genetic diversity (von der Heyden, 2009), two key concepts in understanding the viability of a population. From a conservation biology perspective, identifying genetically distinct (sub)populations specific conservation measures can be adjusted to conserve the genetic diversity of the population as a whole and thus conserve the species (Rocha et al., 2007). Unfortunately, for many cetacean populations, information on genetic structure and taxonomy is unclear (Kershaw et al., 2013) which is linked to the difficulty in monitoring, studying, and collecting data on marine specimens (Waples, 1998).

Before any attempt at implementation of population genetic approaches is set out, an understanding of what defines a population genetically is fundamental. Waples and Gaggiotti (2006) proposed several quantitative measures that can be used in defining a group of individuals different enough to be considered a "population". These criteria will vary depending on whether the individuals are being looked at from an ecological or evolutionary perspective (Waples & Gaggiotti, 2006). In the evolutionary paradigm, populations are considered as "*A group of individuals of the same species living in close enough proximity that any member of the group can potentially mate with any other member*" (Waples & Gaggiotti, 2006). When an exchange of genes between individuals within populations occurs it homogenizes allele frequencies making individuals within populations more similar (Balloux & Lugon-Moulin, 2002). Thus, population differentiation varies along a continuum from highly structured, genetically distinct populations (Figure 4A) to panmictic populations where mating is random (Figure 4D) (Waples & Gaggiotti, 2006). Most natural populations fall somewhere in the middle (Figure 4B&C) as, usually, not every individual has the same chance of exchanging genes with every other individual in a population (Waples & Gaggiotti, 2006).

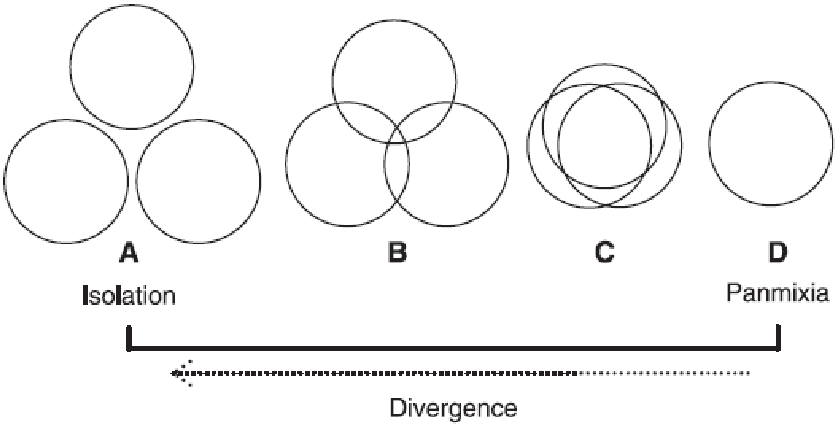

FIGURE 4 Population differentiation continuum, each circle represents a subpopulation. ***A)*** Completely isolated subpopulations, ***B)*** Adequate connectivity between subpopulations, ***C)*** Substantial connectivity between subpopulations, ***D)*** Panmixia (Waples and Gaggiotti 2006)

In recent times, as many wildlife populations are dwindling, conservation biologists have had to find protocols that define distinguishable "units" for conservation planning (Fraser & Bernatchez, 2001). One of the main goals in conservation genetics planning is to preserve adaptive genetic variation within species (Fraser & Bernatchez, 2001). Thus, when considering threatened populations and how to effectively manage them, one must consider several aspects. The concept of the "Evolutionarily Significant Unit" (ESU) was developed to assign protection to priority groups below formal taxonomic levels (Fraser & Bernatchez, 2001; Ryder, 1986). Throughout the years different variations of what defines an ESU have been put forward until Fraser and Bernatchez (2001) proposed an "Adaptive Evolutionary Conservation" approach. This approach allows for a more flexible definition of what constitutes an ESU based on unique aspects of a particular case and takes into account both genetic and ecological factors (Fraser & Bernatchez, 2001).

When considering natural populations, the genetic structure within them can arise from several factors such as historical processes, physical environmental barriers, and life-history traits (Balloux

& Lugon-Moulin, 2002). In marine environments, it was assumed initially that there would be little evident population structure as there are no obvious barriers restricting gene flow between marine populations (Hauser & Carvalho, 2008; Waples, 1998; Ward et al., 1994). However, since then multiple studies have found evidence of genetic structure in several different marine populations over a range of species (e.g. Hoelzel 1998; Carreras et al*.,* 2007; Natoli et al*.,* 2008; Baker et al*.,* 2013; Ferreira et al*.,* 2015; Morin et al., 2017). Potential factors which can allow for isolation and genetic differentiation in marine environments are ocean current patterns and seafloor topology, among several other oceanographical features (Waples, 1998).

In attempts to study and highlight the potential population structure within different ecosystems the use of molecular markers has become a common tool (Balloux & Lugon-Moulin, 2002; Ellegren, 2004; Hauser & Seeb, 2008; Selkoe & Toonen, 2006; Waples, 1998). Several different molecular markers have been proposed for application in conservation genetics studies such as allozymes, mitochondrial DNA (mtDNA), Single Nucleotide Polymorphisms (SNPs), and microsatellites (Hauser & Seeb, 2008; Wan et al., 2004). At first, the most widely used marker in population genetic studies was allozymes, however, the low variability of alleles reduced the statistical power of these markers (Hauser & Seeb, 2008). On the other hand, mtDNA markers are extremely useful in resolving phylogenetic relationships and taxonomic complications within species but, due to maternal inheritance patterns, are not always informative on aspects of populations that are key in conservation planning (Wan et al., 2004). This leaves nuclear genetic markers, such as SNPs or microsatellites, which reflect biparental genetic variation and structure in populations and are more useful and informative for conservation biology studies (Wan et al., 2004). But by using a combination of mtDNA and nuclear genetic markers vital information from two different genome levels can be obtained about the study population, given that multiple markers can be combined and analyzed, which will allow for more comprehensive conclusions to be drawn.

1.3.2 | Microsatellite genetic markers

Microsatellite genetic markers, also known as short tandem repeats (STRs) are extremely useful in molecular ecology studies (Barbara et al., 2007; Hardy et al., 2003). These are usually repeats of between 1–6 nucleotides, which can vary in the number of repeats, and are found in high frequency within nuclear genomes across most taxa (Selkoe & Toonen, 2006). They are useful for ecologists as they can potentially provide insight into finer-scale ecological questions (Selkoe & Toonen, 2006) which is attributed to their various advantages. First, their characteristic high mutation rate makes them informative on demography and connectivity between populations and so they are useful in population genetics investigations (Selkoe & Toonen, 2006). Furthermore, unlike other molecular markers (such as allozymes), microsatellites offer the advantage of using thousands of potentially polymorphic loci (Schlötterer, 2000), increasing the power and precision needed for comparing populations and individuals (Selkoe & Toonen, 2006). And lastly, from a more practical point of view, microsatellite genotyping can be generated from poor quality and small amounts of DNA which allows for use of samples from dead specimens, such as strandings, or very old specimens from museums' collections (Bourret et al., 2008). This is of particular importance for studies on cetacean species, allowing for the use of sloughed skin and strandings (Valsecchi & Amos, 1996).

Nuclear markers like these are often used in studies investigating population structure, relatedness, evolutionary processes, and genetic mapping (Vieira et al., 2016). One of the drawbacks of the use of microsatellite markers is that there is uncertainty surrounding the appropriate mutation model which is needed in the interpretation of results (Balloux & Lugon-Moulin, 2002; Hauser & Carvalho, 2008; Hauser & Seeb, 2008; Selkoe & Toonen, 2006; R S Waples, 1998). There have been several mutational models proposed (Bhargava & Fuentes, 2010; Ellegren, 2004), but for population genetic studies the Stepwise Mutation Model (SMM) is the most common one because of its simplicity (Selkoe & Toonen, 2006). This uncertainty associated with assigning an accurate mutation model for microsatellite markers comes from the wide variability in several of their characteristics. The variation of nuclear markers in genomes of different taxa can be attributed to a wide array of factors that interact and influence the characteristics of the microsatellite such as length and mutation rate (Buschiazzo & Gemmell, 2006). The variation in length is attributed to the insertion or deletion of repeat motifs in an individual which, most often, is a result of replication slippage error (Vieira et al., 2016). Mutation rate, on the other hand, is influenced by slippage and the structure of the marker which refers to whether it is a di, tri, or tetranucleotide repeat. In this case, studies show that dinucleotide repeats show a 1.5–2.0 times greater mutation rate in comparison to tetranucleotide repeats (Chakraborty et al., 1997).

In a review completed by Bourette et al. (2008), the variation in microsatellites in cetacean species specifically was investigated. This review showed that variation of markers used for cetaceans can, to a large extent, be attributed to the structure of the marker itself and differences in flanking region sequences. Of the 246 markers reported in the review, dinucleotide repeats were found most frequently, followed by tetranucleotide and trinucleotide repeats (Bourret et al., 2008). However, other studies looking at microsatellite markers in cetacean species did observe a slower mutation rate in comparison to other organisms (Schlotterer et al., 1991), despite the high frequency of dinucleotide repeats. In addition to these findings, the nucleotide composition in investigated markers varied with sequences CA and GATA being the most common in cetacean genomes (Bourret et al., 2008). Insertions and deletion events were mostly observed in the flanking sequence region of the markers reported while substitutions were common in the repeat sequence and flanking region, causing variation amongst distantly related cetacean species' microsatellites markers (Bourret et al., 2008).

Overall, microsatellites remain one of the most informative neutral genetic markers in population studies and are particularly valuable for studying cetacean populations. Among the various advantages these markers confer, two specific aspects increase their value in population studies of cetaceans. First, these markers can be used in identifying individuals within a group which, together with photo-identification data, enables several lines of evidence for population monitoring (Valsecchi & Amos, 1996). In addition to this, microsatellites can be used to investigate the relatedness of individuals within a population, which is important in understanding behaviour of cetacean groups (Valsecchi & Amos, 1996). These two factors are also key for conservation purposes and thus provide strong support for use of these markers in population studies in cetaceans.

1.3.3 | Mitochondrial DNA (mtDNA)

Arranged as a circular double-stranded molecule, the mammalian mitochondrial genome differs from the nuclear genome not only in size and structure but in mutation rate, recombination, and mode of inheritance (Ballard & Whitlock, 2004). Upon the discovery that mtDNA was easily obtainable from animals, relatively cheap to sequence, and potentially valuable on various taxonomic levels, it became a popular and informative marker in investigating animal diversity, including in population genetics research (Zhang & Hewitt, 1996). Molecular markers chosen from the mitogenome are extremely useful in resolving phylogenetic and taxonomic complications (Wan et al., 2004). Since mitochondrial genes are conserved across taxa to differing degrees, each gene can be used at different taxonomical levels to investigate several aspects of a population (Arif & Khan, 2009).

Overall, this genome has shown few differences in gene content amongst vertebrates and generally consists of 37 genes, more specifically, two ribosomal genes (12S and 16S rRNA), 22 transfer RNA (tRNA) genes, and 13 protein-coding genes (Ballard & Whitlock, 2004; Hwang & Kim, 1999). The ribosomal genes are highly conserved amongst taxa and so, are used usually to investigate genetic diversity at the phylum level (Arif & Khan, 2009). In contrast, protein-coding genes, such as Cytochrome B (Cyt B) or NADH dehydrogenase 5 (ND5), are characterized by rapid rates of evolution and are therefore used for assessing genetic diversity or phylogenetic relationships at lower-level categories such as genera or species (Arif & Khan, 2009).

In addition to the genes, there is a non-coding region, known as the mitochondrial control region (Figure 5), which plays an important regulatory role in the initiation of mtDNA replication and transcription (Hwang & Kim, 1999). This region is used frequently as a genetic marker as it is a hypervariable region that can even display differences between individuals of the same species (Hwang & Kim, 1999). Previous studies have shown the importance of such intraspecific variation in population viability and larger scale ecological functioning of ecosystems (Des Roches et al., 2018; Jump et al., 2009). The control region has often been used in investigating phylogenetic relationships and taxonomy in many organisms, especially cetacean species (Hwang & Kim, 1999; Rosel et al., 2017; Zhang & Hewitt, 1996). Previous studies have even made use of sequence data of this ~1Kbp marker for conservation purposes in defining management units of mammals (Onuma et al., 2006; Waits et al., 1998). The various applications of the control region data in investigating several aspects of different populations (both terrestrial and marine) show its impact and value as a genetic marker in conservation genetic studies.

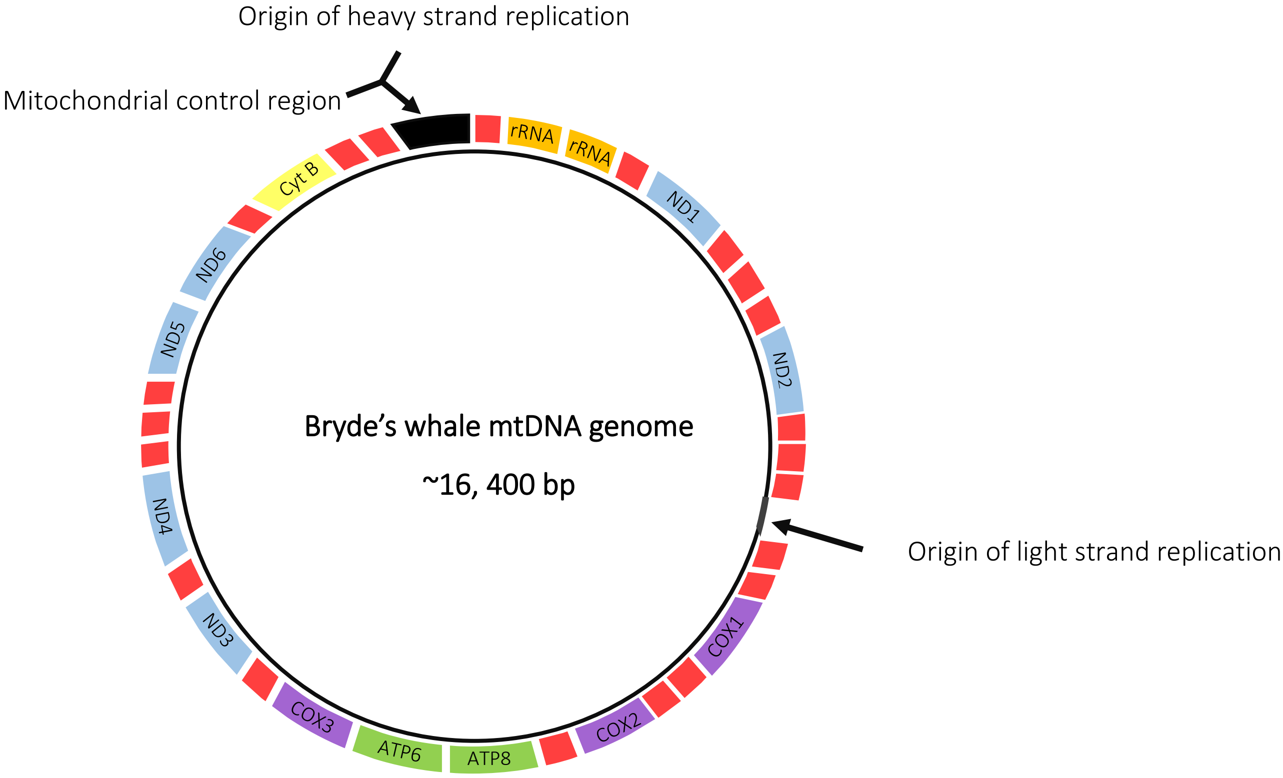

FIGURE 5 Schematic diagram to show the structure and organization of the mitochondrial genome of the Bryde's whale using reference genome *NC_006928.1* from GenBank. Red blocks indicate tRNA.

1.3.4 | Population genetics of Bryde's whales

1.3.4.1 | Global Studies

The ecological differences highlighted between Bryde's whale populations may play a role in causing genetic differentiation between groups (Best, 2001). One of the proposed reasons for the patterns of genetic differentiation seen between Bryde's whale populations is their differences in migratory behaviour, which is complex and less well understood in most species of this family. Since some populations reside in one general area for feeding and breeding purposes it lowers the chance of individuals from different regions associating in, what would normally be, migrations to feeding grounds, which is often where the mixing and breeding of individuals take place (Best, 2001). There have been several reports of distinct populations of Bryde's whales between different oceanic regions (Kanda et al., 2007; Kershaw et al., 2013; Pastene et al., 2015; Rosel & Wilcox, 2014;

Wiseman, 2008; Yoshida & Kato, 1999). The study by Yoshida and Kato (1999), compared offshore populations from the North Pacific with coastal populations from the Solomon Islands, the East China Sea, and Kochi in Japan. Results showed three genetically distinct populations based on mtDNA data where individuals from the East China Sea and Kochi are separate from the North Pacific offshore population on a subspecific level while individuals from the Solomon Island were genetically different from Bryde's whales, suggesting they are, potentially, a separate species (Yoshida & Kato, 1999).

The study by Kanda et al. (2007) was the first peer-reviewed study using microsatellites to analyze population structure in Bryde's whales from inter-oceanic and trans-equatorial regions (Wiseman, 2008). Results from this study revealed four genetically distinct populations of Bryde's whales in the western North Pacific, western South Pacific, eastern South Pacific, and eastern Indian Oceans, respectively. Other studies into the Bryde's whale population that occurs in New Zealand suggested that, although there was evidence of shared mtDNA haplotypes, this population is also distinct from populations in the Northern Pacific and Indian oceans, respectively (Wiseman, 2008). In contrast, Kershaw et al. (2013) used mitochondrial gene segments to confirm the occurrence of two forms of Bryde's whales in coastal and offshore habitats of the Northwest Pacific, Central, and Western Indo-Pacific Oceans. Here, mitochondrial gene evidence confirmed that two different forms of Bryde's whales do occur in coastal and offshore and there is a significant genetic structure between individuals from different oceanic regions (Kershaw et al., 2013). A similar pattern of differentiation was found between Bryde's whales detected between Bryde's whales in the South Atlantic and Pacific and Indian Oceans (Pastene et al., 2015). These studies all support the reliability of using both mtDNA and microsatellite markers in detecting genetic structure between Bryde's whale populations. In addition, the information gathered from these studies highlights the diversity between global Bryde's whale populations, which must be taken into account when building on our understanding of the species (Constantine et al., 2018).

1.3.4.2 | South African Studies

When comparing offshore and inshore populations in the South African region prior studies have highlighted several morphological and ecological differences (Best, 1977, 2001). The ecological differences identified between these two populations increase the chance of genetic differentiation between them. In addition to the lack of migration, the feeding preferences between the populations are a proposed factor in potentially causing genetic differentiation as they are specialized in feeding on different prey sources, which can separate individuals through resource partitioning (Best, 2001). There have been only a few studies completed involving population genetic investigations of Bryde's whales in South Africa (Penry, 2010; Penry et al., 2018). And to this date, no study has been done to investigate the genetic differentiation between offshore and inshore populations of Bryde's whales on the southern African coast using nuclear gene markers.

However, a study conducted by Penry et al. (2018), did find genetic differentiation between offshore and inshore individuals using mitochondrial control region data. Distance computations provided strong evidence for little to no gene flow between offshore and inshore individuals (Φ_{ST} = 0.984 ; $p < 0.001$; Penry et al., 2018), while maximum likelihood analyses showed some of the most interesting results of the study (see Figure 6). Haplotypes one (H1) to four (H4) represent the inshore population and cluster to form a sister taxon with individuals classified under *B. brydei.* These haplotypes also show complete separation from sequences that represent *B. edeni,* thus indicating that the South African inshore Bryde's whale population does not follow the worldwide pattern of coastal Bryde's whales being classified as *B.edeni* but is rather a subspecies of *B. brydei* (Penry et al., 2018).

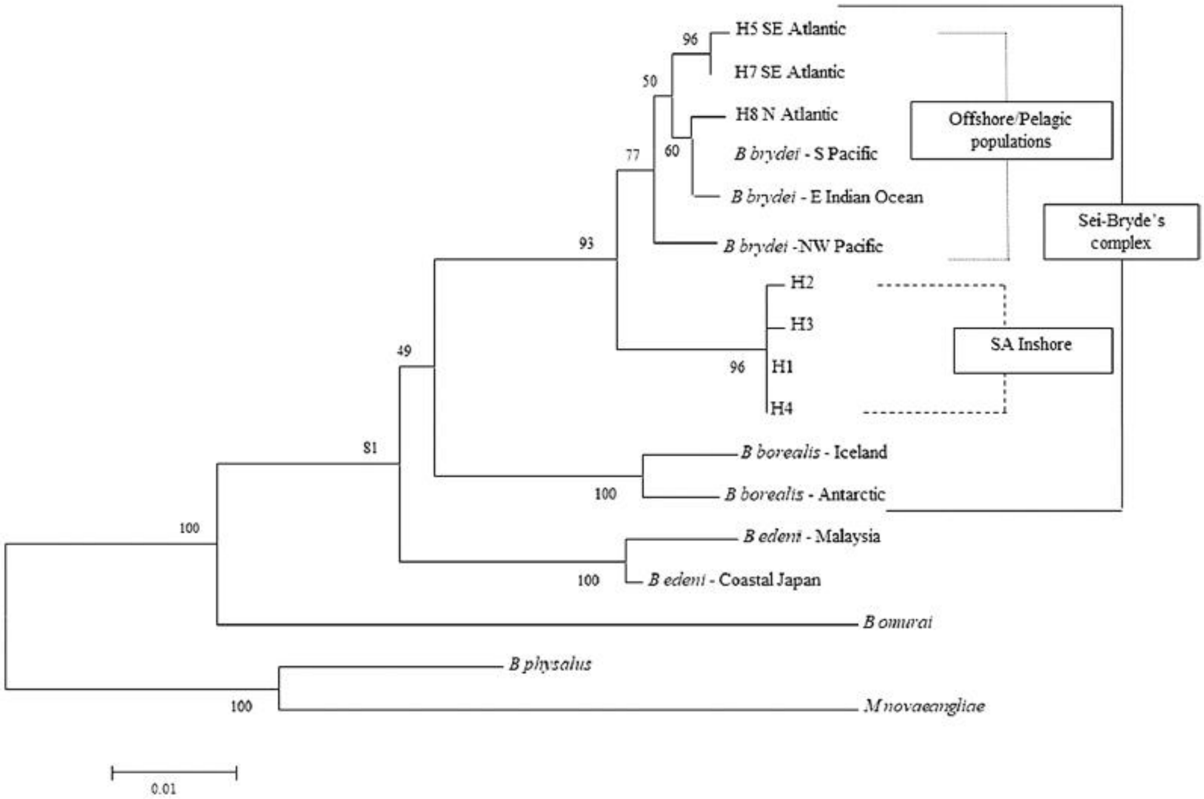

FIGURE 6 Maximum likelihood phylogenetic tree showing relationships between offshore and inshore Bryde's whales in South Africa and Bryde's whales worldwide (Penry et al., 2018)

1.3.41 | Use of population genetics in conservation:

The identification of genetically distinct populations, such as the ones discussed above, is vital for conservation and management purposes (Kanda et al., 2007; Kershaw et al., 2013). Once genetically distinct populations are identified, they may need to be managed as separate entities to prevent the removal of invaluable evolutionary lineages (Kershaw et al., 2013). Such an effective implementation of molecular techniques and population genetic concepts in informing the conservation practices is shown in the study by Rosel and Wilcox (2014). In this case, the "Bryde's-like whales" from the Gulf of Mexico (GOMx) population were investigated in terms of genetic diversity and phylogenetic relationships to Bryde's whales worldwide using mtDNA and microsatellite data. Two key findings from the study were that the GOMx population was evolutionarily distinct from other Bryde's whales worldwide and had a significantly low mitochondrial and nuclear genetic diversity (Rosel & Wilcox, 2014). Both these findings raised concern for the population from a conservation perspective. The small size (< 50 individuals), low genetic diversity, and genetic isolation of the GOMx population in light of the various anthropogenic factors that occur frequently in their distributional range raise concern for the viability of the population (Rosel & Wilcox, 2014). Since the Bryde's-like whales in this area are the only baleen whales found, they make a unique contribution to the ecosystem (Rosel & Wilcox, 2014) and thus, conservation of this species is imperative in maintaining biodiversity within it.

Subsequently, recent studies done by Rosel et al. 2021 provided more evidence of genetic isolation of this GOMx population and resulted in the reclassification of these "Bryde-like like whales". In this study, several genetic, morphological, and ecological (distributional and acoustic) findings emphasized the fact that the GOMx population is distinct from Bryde's whales. Finally, it was concluded that enough evidence was gathered to indicate this population was a new unnamed species (Rosel et al., 2021). This taxonomic clarification resulted in renaming individuals of this population as the Rice whale (*B. ricei).* Identification of this genetically distinct population highlighted a group of animals that requires careful consideration when implementing management strategies concerning fishing and exploitation of energy (such as oil and gas) resources as these are threats with an unknown extent of the impact on this small and critically endangered population (Rosel & Wilcox, 2014; Patricia E Rosel et al., 2016).

In South African waters, the inshore Bryde's whale population is much like the Gulf of Mexico population in terms of its small population size and threats faced relating to competition with fisheries amongst other anthropogenic stresses. Although there are size estimates for the inshore Bryde's whale population in South Africa (Best et al., 1984; Penry, 2010), it is unknown whether individuals form one population or several, smaller subpopulations. Penry (2010) did assess the inshore population structure, possibly defined spatially between the east and west coasts of South Africa. However, Penry (2010) used microsatellite data from 10 loci characterized from four different studies that had locus variability in three related whale species (humpback (*Megaptera novaeangliae*), pilot (*Globicephala spp*), and sperm whales (*Physeter macrocephalus*). Based on nine polymorphic loci the results from this study showed no significant population structure within the inshore population, but due to small sample sizes, and the use of few loci with few alleles, the study was deemed inconclusive from an ecological perspective (*cf.* Hauser & Carvalho, 2008). Therefore, taking into consideration the gaps in knowledge of South Africa's Bryde's whales in conjunction with the anthropogenic threats imposed on them, further investigation into this population is urgently needed.

1.4 PHOTO-IDENTIFICATION

In conjunction with genetic methods, other non-genetic methods can provide additional evidence when assessing population structure such as Photo-identification (photo-ID). This method relies on the visual recognition and resighting of individuals over a period of time (Hoelzel, 1994). Several studies have made use of photo-ID in combination with genetic methods to provide more in-depth information on population demography (Alves et al., 2013; Carroll et al., 2011; Constantine et al., 2012). The recognition of individuals can be achieved through the natural markings found on the animal (Hammond et al., 1990) which could be colour patterns, the shape of features, or scars on the individual (Friday et al., 2008). Thus, the distinctiveness of the marking will range based on the complexity of the colour pattern, shape, or the number and severity of scars (Friday et al., 2008).

For this method to be successful, the markings used in individual recognition need to be well-defined and must stay visible throughout the period of investigation (Hammond et al., 1990). In addition, photo-ID is also reliant on good-quality photographs of individuals that clearly show the distinctive feature (Friday et al., 2008; Hammond et al., 1990).

Photo-ID methods have been employed for individual identification of Bryde's whale worldwide (Chiu, 2009; Hupman et al., 2017; Penry, 2010; Wiseman, 2008). The natural marking used for the identification of these whales is associated with distinctive features on the dorsal fin. The use of scars, cuts, and the shape of the dorsal fin as a marking for individuals is a reliable technique and has been successfully used for the South African inshore population (Penry, 2010). In fact, for Bryde's whales, there is potentially a range of characteristics that can be used for individual identification.

At times, the shape of the fin can be incredibly distinctive due to injury (Figure 6A) making identification relatively easy. Other features such as notches or cuts in the dorsal fin (Figure 6B) can also be used as a distinguishing features. At other times, there is enough variation in the dorsal fin shape for it to be used for identification purposes (Figure 6D). However, there are instances when the dorsal fin is not distinctive at all and cannot be used for photo-identification methods (Figure

6C). Once data are gathered containing pictures of individual dorsal fins these are rated according to their photograph quality (ability to photo-ID) and according to dorsal fin distinctiveness. Thereafter matching of distinctive individuals can be completed and resighting information collated. Together with the location and dates of resightings of unique distinctive individuals, their movement patterns and habitat use can be monitored.

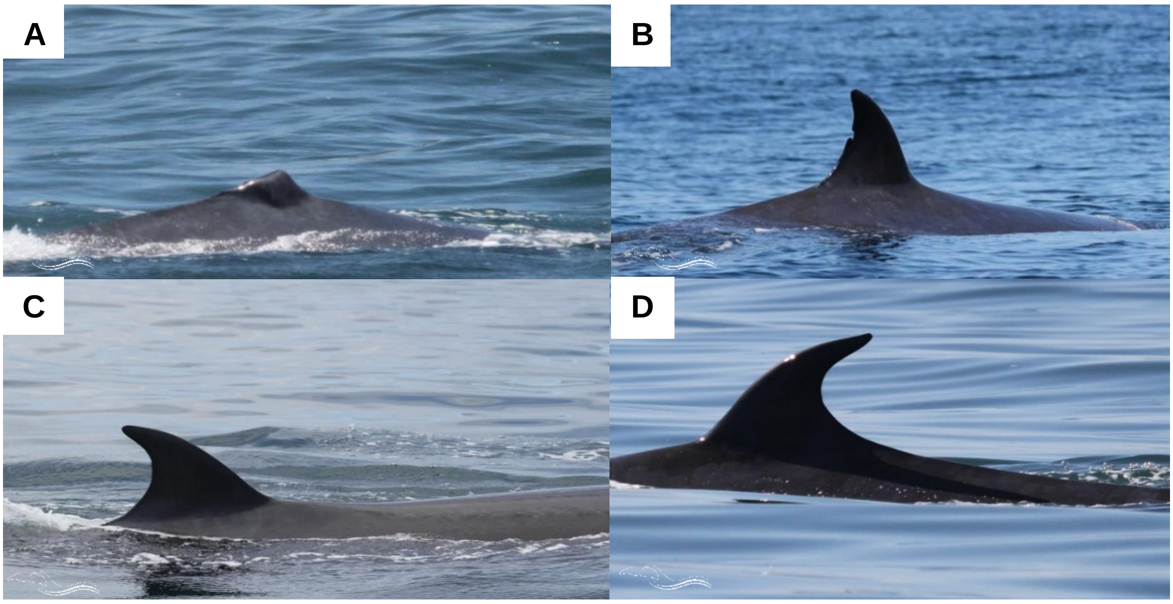

FIGURE 7 A range of characteristics in dorsal fins of the South African inshore Bryde's whales **A)** Distinctive dorsal fin, **B)** Notches on the dorsal fin, **C)** Dorsal fin that is not distinctive **D)** Dorsal fin with a unique shape

1.5 PROJECT RATIONALE

There have been few studies completed on Bryde's whales inhabiting South Africa's waters. Penry et al. (2018) provided the most interesting results in highlighting potential species and subspecies classification of the offshore and inshore populations, respectively. In addition to this, the study also highlighted potentially low gene flow between inshore and offshore populations, suggesting the inshore population is genetically isolated (Penry et al., 2018). In addition, considering its small population size, the inshore Bryde's whale population is of conservation concern (Penry et al., 2016). Furthermore, due to the different oceanographic influences on the west and east coast of South Africa, the inshore population could be structured on this spatial scale (Findlay et al., 1992;

Penry, 2010). Possible subdivisions of the small inshore population can put it at an even higher risk of extinction.

The pilot genetic study, conducted by Penry (2010), did not detect significant levels of differentiation within the population. However, this study used a combination of 10 microsatellite loci, reduced to nine polymorphic loci as one was essentially monomorphic, and 16 and 12 individuals to represent the east and west coast, respectively. This ratio of the number of loci to the number of individuals per population was below the suggested ratio of eight loci for 30 individuals per population for a low F_{ST} value (<0.01) (Cornuet et al., 1999). Therefore, the inferences made from this study were weakly supported (Penry, 2010). In addition, the power of the loci was not tested, which is a suggested step in estimating the levels of F_{ST} that can be detected by the chosen loci (Waples & Gaggiotti, 2006). Only after this test is completed can the failure to reject the null hypothesis, of no significant genetic differentiation, be concluded (Waples & Gaggiotti, 2006). Therefore, an assessment of the population structure of the inshore population of Bryde's whales, using a larger sample size and more informative loci are warranted.

A larger sample size will aid in reducing bias caused by intralocus sampling error (the random error in estimating allele frequencies based on the number of individuals within the sample size), which is a particular problem in marine organisms (Waples, 1998). But the addition of more loci, with more alleles, will also increase the power and ability to detect lower levels of genetic differentiation (Selkoe & Toonen, 2006; Waples, 1998). Furthermore, the addition of more samples from different locations in microsatellite studies can potentially increase the ability to detect fine-scale population structures (Waples, 1998). Hence a combination of samples from previous studies and additional samples collected from different locations representing the west and east coast of South Africa will be used to investigate the potential structure within the inshore Bryde's whale population. Samples collected over different temporal scales are also beneficial and can increase the reliability of results. This is because, if samples are collected over time and patterns of genetic differentiation are consistent then it is unlikely a result of sampling error (Waples, 1998). Thus, the samples that were used ranged over a period of collection from 2005 to 2021. Loci that were informative from Penry (2010) were kept in the panel of loci used for this study, and additional loci were added from recent testing in related species of Balaenopteridae*,* that have shown to have between 8-15 alleles (Tardy et al., 2020).

It is aimed that the results from this study may provide an improved and more detailed understanding of the population structure of Bryde's whales along the South African coastline and aid in the formulation of effective conservation strategies for the population. Effective conservation planning is key for the inshore population of Bryde's whales, given the rise in the number of fatal entanglements in conjunction with its small population size and potential genetic isolation. Furthermore, since the inshore population is largely dependent on fish stocks that are of economic importance, the delineation of subpopulations of Bryde's whales can also inform an ecosystem approach to fisheries management (Penry et al., 2016).

1.6 PROJECT AIMS AND OBJECTIVES:

Research Questions

1) Is there a significant, spatially defined structure in the inshore Bryde's whale (*Balaenoptera edeni brydei*) population in South Africa?
2) What is the current level of neutral genetic diversity of the inshore population?

Aim

To assess the level of genetic diversity and spatial structure in the inshore Bryde's whales (*Balaenoptera edeni brydei*) population in southern Africa.

Objectives:

- To investigate if any spatially defined genetic population structure exists in the inshore Bryde's whale population in South Africa.

- To assess the level of genetic diversity of the inshore Bryde's whale population based on mtDNA and microsatellite markers.

- Assess individual movement patterns to further inform on possible population structure

CHAPTER 2

Genetic structure and diversity of South Africa's inshore Bryde's whale (*Balaenoptera edeni brydei*, Olsen 1913) population

Abstract

South Africa's inshore Bryde's whale (*Balaenoptera edeni brydei*) population is the only resident baleen whale species found in the country's inshore waters. This small, isolated population effectively demonstrates the Bryde's whale's well-known, elusive behaviour which leaves many questions unanswered relating to their biology and ecology. One of these questions links to evidence of population structure across the diverse southern African oceanographic region as well as the level of genetic diversity within the population. In a broader context, the connectivity between the population in South Africa and other regions has remained unclear. Here, microsatellite genotyping and mitochondrial DNA (mtDNA) sequencing were employed to assess these aspects. The analyses of both marker sets show limited evidence of genetic differentiation between the west and south coast of South Africa, thus failing to reject the null hypothesis of a single population. Additionally, the population harbours relatively high neutral nuclear DNA diversity but lower levels of mtDNA diversity, suggestive of possible larger than expected effective population size and sex-biased dispersal. Other mtDNA results suggest that South Africa's inshore population may share connections (ancient or recent) with other regions within the eastern Indian Ocean. These results have important conservation implications as it informs on management strategies of this inshore population. Despite the small population size, a relatively high level of genetic diversity was detected overall which is an important indicator of viability. These results add significant understanding of the demographics of this poorly understood inshore population which is vital in the formulation of effective conservation strategies.

KEYWORDS:

Baleen whale, conservation, microsatellites, mitochondrial DNA, population genetics

2.1 | INTRODUCTION

The Bryde's whale (*Balaenoptera edeni*) is one of the most elusive, least studied, and consequently least understood balaenopterid species worldwide. Their evasive and shy nature creates major gaps in knowledge mainly concerning their ecology, phylogeny, and population connectivity. Although they are currently listed as one of eight species of the family Balaenopteridae (Committee on Taxonomy, 2020) to date, a unanimous agreement on the number of species and subspecies of Bryde's whale has not been established. As a common challenge that is evident throughout cetacean taxa, this has direct implications for the success of conservation and management practices of many populations (Martien et al., 2017; Rosel et al., 2017; Taylor et al., 2017; Taylor et al., 2017). For Bryde's whales in particular, their complicated and confusing history of discovery plays a significant role in the delineation of species and subspecies throughout their global distribution (Best 2001; Sasaki et al*.,* 2006; Penry et al*.,* 2018). The lack of a type specimen for the proposed species *B. brydei* and the absent genetic confirmation of holotype *B. edeni* halts any phylogenetic clarification (Constantine et al., 2018; Penry et al., 2018). In addition to this, general population demographic information is notably lacking for Bryde's whales due to difficulty in monitoring, studying, and collecting of marine specimens in general (Waples, 1998) but especially for this rare species.

Although marine environments lack any obvious barriers to geneflow, studies have shown significant evidence of population structure in marine mammals (e.g Lowther et al., 2012; Baker et al., 2013; Carroll et al*.,* 2015; Alexander et al., 2016). This is demonstrated effectively in Bryde's whales where the few studies completed all highlight the occurrence of genetically distinct populations in different ocean basins (Kanda et al., 2007; Kershaw et al., 2013; Luksenburg et al., 2015; Pastene et al., 2015; Rosel et al*.,* 2021). However, given Bryde's whales' global distribution, some areas have received more research efforts in comparison to others (Constantine et al., 2018). The identification of genetically distinct populations, in general, is an important goal to achieve in the assignment of conservation protection (Kanda et al., 2007). Furthermore, since there is high variability in the ecology, demography, and genetic diversity of Bryde's whale populations globally, population demographic information is key in informing on the best possible management practices for the species. This is one of the suggestions made by Constantine et al. (2018) in describing directions for future research which will develop our understanding of Bryde's whales.

The southern African region serves as an excellent demonstration as one of the areas that require investigation into Bryde's whale population demographics. Three distinct Bryde's whale populations are thought to occur within this region. Two of these proposed populations have an offshore distribution in the Southeast Atlantic and Southwest Indian oceans respectively (Best, 2001). The third and final population is thought to have a restricted inshore distribution around South Africa (Best, 2001). Inshore and offshore individuals are thought to show morphological, ecological, and behavioural level differences (Best, 1977, 2001). In addition, there is some evidence of the genetic distinction between these populations (Penry et al., 2018) but more intensive sampling effort of offshore regions is required before any formal conclusions can be made. A lack of in-depth understanding of the distribution and movements of offshore individuals prevent the clarification of connectivity between proposed subpopulations within the southern African region. However, the residency of the inshore population on South Africa's continental shelf and frequent coastal presence allows for easier sampling in comparison to offshore regions.

Despite the easier sampling access in comparison to offshore regions, South Africa's inshore Bryde's whale population still exemplifies all the challenges of studying the Bryde's whale. This small, resident population is thought to make small-scale movements along the coast of South Africa in pursuit of their main prey source, sardine *(Sardinops sagax)* and anchovy *(Engraulis capensis*) (Best, 2007; Penry et al., 2016; Penry et al., 2011). This sets them apart from other baleen whale species found in South African waters, which undertake annual feeding migrations to the Sub-Antarctic (Best, 2007). Previous studies have concluded that the movements of this inshore population up the east coast of South Africa coincide with the annual "sardine run" into the waters off Kwa-Zulu Natal, making them South Africa's largest resident marine predator which is reliant on this biological event (Penry et al., 2016; Penry et al., 2011). This baleen whale population is believed to be small, consisting of < 1,000 mature individuals overall (Penry et al., 2016). An up-to-date coastal wide population estimate remains one of the critical knowledge gaps for South Africa's inshore Bryde's whales with the only population census completed in the 1980s estimating the presence of approximately 582 individuals (Best et al., 1984), which is clearly out of date. But, following this, a partial update was completed in 2010 by Penry and colleagues using a localized mark-recapture study which estimated 150 individuals occur in the Plettenberg Bay area alone. These two estimates together support the hypothesis of the small population size of the inshore Bryde's whales.

Although the previous work done (Best, 1977, 2001; Penry et al., 2016; Penry, 2010; Penry et al., 2011; Penry et al., 2018) on this inshore population has provided valuable insights, there are still evident gaps in knowledge which have direct impacts on the conservation of the species. Given their small population size, South Africa's inshore Bryde's whales hold a national listing as "Vulnerable" according to D1 (*A taxon with an acute restriction on the number of mature individuals (<1,000) making them particularly vulnerable to stochastic events and other threats;* IUCN 2021) criteria (Penry et al., 2016). This, in combination with the nature of their distribution, increases the impacts that threats such as fatal entanglements and trophic level disruptions, in the form of prey distribution changes due to climate change, have on the viability of this population. In addition, since the inshore populations' main food source consists of some of South Africa' s most economically valuable pelagic fish species, the ecosystem approach to fisheries management in the country must include this population in planning strategies (Penry et al., 2016). However, to be able to implement effective management and conservation strategies there is a need for specific demographic level information of the inshore population.

Some of the main questions that remain unanswered relate to the genetic structure and diversity of the inshore population as well as the connectivity between South Africa and other oceanic regions. A handful of genetic studies have been completed to clarify some of these aspects of the population (Penry, 2010; Penry et al., 2018). Penry (2010) completed a pilot study investigating spatially defined population structure and genetic diversity within the inshore population. However, a low sample size (28 individuals) and number of loci (9 polymorphic loci) impacted the strength of support for the results. Penry et al. (2018) expanded on mitochondrial DNA (mtDNA) phylogenetic investigations within a South African context but did not include a comprehensive global comparison. The present study aimed to build on the valuable findings of Penry (2010) and Penry et al. (2018). Evidence of spatially defined population structure between the west and south coasts of South Africa and levels of genetic diversity of the population was investigated using microsatellite genotyping information. In addition, genetic diversity was also assessed based on mtDNA which included a preliminary assessment of the global connectivity of South Africa's inshore Bryde's whales based on a biased set of available haplotype sequences.

2.2 | MATERIALS AND METHODS

2.2.1 | Samples and biopsy procedure

A combination of biopsies taken from live Bryde's whales and tissue taken from strandings added up to a total sample size of 112 used in this study. These samples were collected from several different locations along the South African, Namibian, and Madagascan coastline for 2005-2022 (Figure 1, Table 1). Sampling completed (n=97) before 2021 was according to the protocol indicated in Penry (2010) and Penry et al. (2018). Biopsy sampling through 2021-2022 was completed on several free-ranging animals (n=15) in Walker Bay and Plettenberg Bay by the MRI Whale Unit, University of Pretoria. Biopsy samples were taken using a Barnett Panzer V (150 lb draw) crossbow. Biopsy darts fired from the crossbow are fitted with a hollow curing tip which removes a sample of skin and sometimes blubber (<20 mm l x 10 mm w). This tip is made of surgical quality stainless steel and fitted with a stopper (approximately 40 mm diameter) to prevent the tip from penetrating more than 20 mm into the skin. In practice, penetration is usually less than this as the angle of impact is rarely 90 degrees. Small barbs inside the tip retain the sample until it is retrieved. When an animal is located, it is approached sideways at a slow speed (1-4 knots) up to a minimum distance of 50 m, to ensure the best chance of hitting the target while disturbing its natural behaviour as little as possible. Once the animal is struck and the bolt rebounds and floats on the water, the boat is stopped, and the animal is allowed to move off after which the bolt and sample are collected. Subsequently, the tip (with the biopsy sample inside) is carefully removed from the bolt and both tip and sample are put into a tube to be stored immediately in an icebox until back in the laboratory where the sample is removed from the tip and stored at -20 °C until further analyses. At all times when handling the tip and/or sample, the researcher is wearing surgical gloves to avoid cross-contamination. If the target is missed, another two attempts are made to collect the biopsy sample. If no samples are obtained after three attempts or 20 mins of approaches (whichever is first) attempts will cease and the animal will be left.

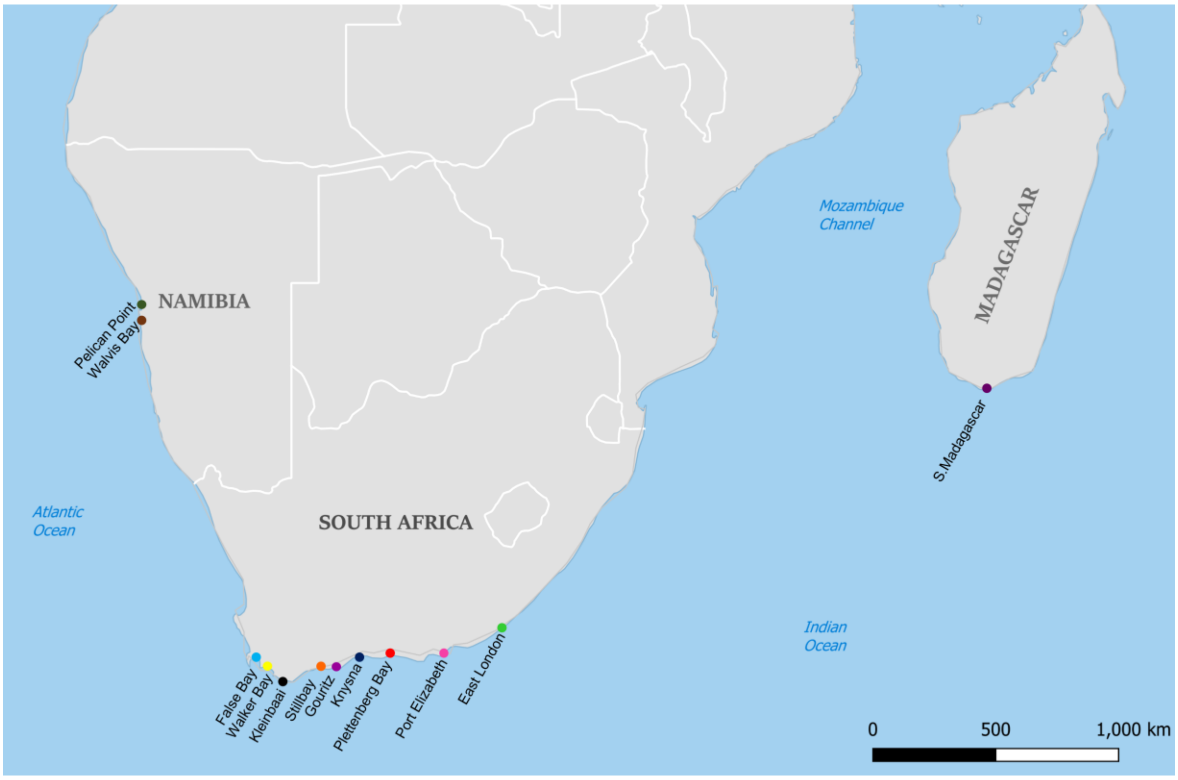

FIGURE 1 Map of the southern African region indicating the locations of Bryde's whale samples

TABLE 1 Location and number of samples used for genetic analyses

Area of southern African Coast	**Location**	**Number of samples**	**Contributor**
South African West Coast	False Bay	45	Penry (2010), Penry et al. (2018)
	Walker Bay	12	MRIWU
	Kleinbaai	1	Penry (2010), Penry et al. (2018)
	Stillbay	1	Penry (2010), Penry et al. (2018)

South African South Coast	Gouritz	1	Penry (2010), Penry et al. (2018)
	Knysna	1	Penry (2010), Penry et al. (2018)
	Plettenberg Bay	40	Penry (2010), Penry et al. (2018)
	Port Elizabeth	4	Penry (2010), Penry et al. (2018)
	East London	1	Penry (2010), Penry et al. (2018)
Madagascar	Southern Madagascan coast	1	Penry (2010), Penry et al. (2018)
Namibia	Western Namibian coast	2	Penry (2010), Penry et al. (2018)
Unknown	Unknown	4	Penry (2010), Penry et al. (2018)

2.2.2 | DNA extractions

DNA extractions of all available samples were completed using the DNeasy Blood and Tissue Kit (Qiagen, Germantown, United States) according to the manufacturer’s protocol. The skin was separated from blubber and muscle tissue before extraction. These extractions were completed on all skin samples from 2005-2022 to ensure consistency throughout. Thereafter skin samples were cut into smaller pieces using a sterilized scalpel and digested in ATL buffer and Proteinase K (10 mg/ml) (Roche, Mannheim, Germany). Although the manufacturer’s protocol suggested that samples should be incubated at 55 °C for three hours, these samples were vortexed and incubated overnight to ensure effective tissue digestion. Following the overnight digestion, samples were then vortexed and incubated with RNase A (0.3 mg/ml) (Roche Mannheim, Germany) for 30 mins at 37

°C. Thereafter, AL buffer was added to samples, and they were vortexed and incubated for a further 10 minutes at 70 °C. To complete the digestion process, ethanol (99.99% EtOH) (Merck, Modderfontein, South Africa) was added to all samples followed by a 30 s vortex step. To begin the elution process, four centrifugation rounds were completed for all extractions. First, each extraction solution was transferred to a new DNeasy mini-column and centrifuged for 1 min at 8000 rpm. Then two wash steps were completed with AW1 and AW1 wash buffers whereby samples were centrifuged for 1 minute at 8 000 rpm and 3 mins at 13 000 rpm respectively. The final and additional centrifugation step was completed for all samples for 2 mins at 13 000 rpm. This step was to dry the membrane of the column thus no buffers were added at this step. DNA was eluted from all samples in 50 µl of Sabax® water (Adcock Ingram, Midrand, South Africa), after an incubation period of 30 mins. To complete the elution, samples were then centrifuged for 1 min at 8 000 rpm. To confirm and assess the presence and quality of DNA, samples were visualized on a 1% agarose gel, and images were viewed on a Molecular Imager Gel Doc™ XR+ Imaging System (Bio Rad, CA, USA). Thereafter the concentrations of the extractions were measured using the NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

2.2.3 | Amplification of microsatellite loci

A combination of microsatellite loci used previously in Bryde's whales (Penry, 2010) and newly characterized loci (Tardy et al., 2020), tested in the fin whale (*Balaenoptera physalus*), were used in this study. Initially, 15 loci in two panels (Table 2) were tested for consistent amplification; these panels were designed in Multiplex Manager v.1.2 (Holleley & Geerts, 2009). After testing all loci, EV37 was discarded as it was not showing consistent amplification. Thus, a total of 14 loci were used for further analyses of the available samples. To amplify the chosen loci the Multiplex PCR Master Mix (Qiagen, Hilden, Germany) was used. Each sample was made up of 10µl reactions which consisted of 3 µl of PCR Master Mix, 0.3 pmol of respective forward and reverse primer and approximately 10 ng of genomic DNA. The following PCR conditions were used for amplification of the loci: one cycle at 95 °C for 15 mins followed by 25 cycles at 94 °C for 1 min 30 s, 54 °C for 30 s and 72 °C for 1 minute 30 s with the final elongation step run at 72 °C for 20 mins. This cycle was used to amplify most of the loci except GT122, GGAA520, and GATA98 which were separately amplified for 30 cycles rather than 25 as these markers showed lower amplification in comparison to the rest.

The next optimization phase consisted of establishing size ranges for Bryde's whales and testing for polymorphism across the amplified markers. A small batch of samples was amplified, and each marker was analyzed to find the size range of new markers, count the number of alleles and assess the presence of heterozygotes and homozygotes. Markers with two or more alleles were kept in for further analyses. No monomorphic markers were detected amongst the tested loci. Thereafter, all microsatellite genotyping results were scored using GeneMapper6 V.6 (ThermoFischer). Overall, 106 individuals were genotyped successfully at 14 polymorphic loci. If an individual had missing data at more than two loci, they were not included in the final dataset. Three loci (Bp1043662, GATA53, GATA098) were sequenced (using unlabeled primers in the standard DNA sequencing approach as described for mtDNA below) in homozygotes to confirm the scoring of alleles.

TABLE 2 Forward and reverse primer sequences for amplification of microsatellite loci and observed amplicon size range in *Balaenoptera edeni*

Locus	**Sequence**	**Size range (bp) in *Balaenoptera brydei***	**Panel Number**
Bp740180[1]	F: TAT CAC TCT TCA GCA GCG CA R: AAA TGC TGC GGC TCT TAG GT	170-200	1
Bp531923[1]	F: GGC TGT CAG TGG TTG ATC TGT R: CCA TGA CTT TAC GGT GGG AGA	190-230	1
Bp1418059[1]	F: TCC CAT CTT GGC ATC TGC TT R: CCC AAT TAT TGA TGC CAG ATGAT	270-290	1
Bp1430262[1]	F: TTC CTC AGT CAG AAA CGC GG R: AGT GGC CAT GTA CAA GTG CA	250-280	1
GT122[2]	F: CCT TTT AAA ACC CAG AAT GTAG	135-160	1

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[1] Tardy et al. (2020)

[2] Bérubé et al. (2005)

	R: TGT TTG CAG TGA CGA ATG AAAGG		
GGAA520[3]	F: TAG CAG AYC TGA GTT ATT TCC R: TAG CAT TTT AGT CTT GGG TGG	190-230	1
GATA028[3]	F: CGCTGA TAG ATT AGT CTA GG R: AAA GAC TGA GAT CTA TAG TTA	180-260	1
GATA098[3]	F: TGT ACC CTG GAT GGA TAG R: TCA CCT TAT TTT GTC TGT CTG	75-100	1
GATA53[3]	F: ATT GGC AGT GGC AGG AGA R: GAC ACA GAG ATC TAG AAG GAG	290-330	1
Bp1178841[1]	F: GGC AGG AAG ACA TAT AAA TCA GCA R: CCC ACT GCC TAG AAA GCC TT	230-270	2
Bp1043662[1]	F: ACC AAG AGC CTT AGC ATG TCC R: TCC AAG GAA GGA GTT AAG GCA	260-290	2
EV1[4]	F: CCC TGC TCC CCA TTC TC R: ATA AAC TCT AAT ACA CTT	120-160	2
GATA417[3]	F: CTG AGA TAG CAG TTA CAT GGG R: TCT GCT CAG GAA ATT TTC AAG	200-230	2
GT023[2]	F: CAT TTC CTA CCC ACC TGT R: GTT AGG CTC TGC ACT CTG	90-120	2
EV37[4]	F: AGC TTG ATT TGG AAG TCA TGA R: TAG TAG AGC CGT GAT AAA GTGC	-	2

2.2.4 | Polymerase Chain Reaction (PCR)

A standard PCR procedure was used to amplify the mtDNA control region in all Bryde's whale samples from 2021 and 2022. Amplification was done in 10 µl reactions with the preparation of a

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[3] Palsbøll et al. (1997)

[4] Valsecchi & Amos (1996)

master mix according to the number of samples being amplified. Primers (Whitehead Scientific (Pty) Ltd Brackenfell, South Africa) that were used for amplification of this region were designed for this project specifically but can potentially be used in other baleen whale species (Table 3). A single reaction consisted of the following: 1 mM $MgCl_2$ (Merck KGaA, Darmstadt, Germany), 1x PCR Buffer (JMR Holdings, Kent, UK), 3.2 mM dNTP's (0.8 mM each) (Promega Corporation, Madison, WI, USA), 1x Bovine Serum Albumin (BSA) (Roche Mannheim, Germany), 3 units of Supertherm Taq polymerase (JMR Holdings, Kent, UK), 10 nM of each primer and ddH_2O to make up to a final volume of 10 µl. Approximately 50 ng of genomic DNA was used in each PCR reaction. All reactions were conducted on the ABI 2720 Thermo Cycler (Applied Biosystems, Foster City, CA, USA) and cycle conditions for amplification of the mtDNA control region started with one cycle at 94° C for 1 min, followed by 35 cycles of 94 °C for 30 s, 72 °C for 45 s, 58 °C for 30 s and a final elongation step of 72° C for 2 mins. All PCR products were visualized on 1% agarose gel which was completed to confirm successful amplification, whereby control region products showed an approximately 900bp amplicon size.

TABLE 3 Primers and sequences used for amplification of the control region

Primer name (Ref NC_006928.1)	**Sequence 5'—3'**	**Region amplified**
L15499	ACA CTC CAC CAT CAG CAC CCA AAG C	5' end of the control region
H35	CCA TCT AGA CAT TTT CAG	3' end of the control region
H15861	CTG GTT TCA CGC GGC CAT G	Conserved block of the control region

2.2.5 | Agarose gel electrophoresis

To allow for separation and visualization of PCR amplified products, 1% agarose gels were made. For 1% gels, 0.35g of Agarose (D1 Low EEO, Conda, Madrid, Spain) was dissolved in 35 mL of 1X TAE (10mM Tris free base, Disodium EDTA, Glacial Acetic Acid, ddH20). Electrophoresis of gels was completed at 100 V for 25-30 mins of PCR products of mtDNA and microsatellite loci to allow for effective visualization.

2.2.6 | PCR clean-up using Sephadex

All PCR amplified products were prepared for cycle sequencing reactions by using the Sephadex clean-up protocol. Prepared Sephadex (GE Healthcare Bio-Sciences, Uppsala, Sweden) slurry was added to a collection tube and centrifuged for 2 mins 30 s at 2 600 rpm to form a "Slurry slope" onto which the PCR products would be dispensed. After this centrifugation step, the column was placed into a clean 1.5 ml Eppendorf tube and the entire PCR product was dispensed into the middle of the slope in small, constant drops taking care not to disturb the slope. This was followed by another centrifugation step for 2 mins and 30 s at 2 600 rpm. The flow-through from this step was used as the cleaned PCR product for cycle sequencing reactions. To confirm efficiency of this clean-up procedure, the PCR products were visualized on a 1% agarose gel.

2.2.7 | Cycle sequencing

A standard cycle sequencing protocol using BigDye™ Terminator 3.1 cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA) was followed for all samples that were sequenced in both directions. For this, 10 µl reactions were prepared for each sample. Each reaction consisted of using one-eighth BigDye™ Terminator 3.1 ready reaction mix, 0.5X sequencing buffer, 3.2 nM of forward or reverse primer (Table 3), approximately 50 ng of DNA and ddH_2O to make up to a final volume of 10 µl. All cycle sequencing reactions were completed on an ABI 2720 Thermo Cycler (Applied Biosystems, Foster City, CA, USA). Cycle conditions for cycle sequencing reactions consisted of 25 cycles of 96 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 mins as per the manufacturer's instructions.

2.2.8 | Cycle sequencing product clean up

In preparation for automated sequencing, all cycle sequenced products were precipitated using a Sephadex clean-up procedure according to the prescribed protocol. Following the completion of cycle sequencing, 10 µl of ddH20 is added to each sample, bringing the total volume used for clean-up to 20 µl. Thereafter the same procedure for the clean-up of cycle sequenced products was followed as for the clean-up of PCR products. Cycle-sequenced products were stored at 4 °C until automated sequencing was completed

2.2.8 | Automated sequencing

After the purification of cycle sequenced products for the mtDNA control region, all samples were submitted for automated sequencing on an ABI 3500xl DNA Analyzer. completed at the Natural and Agricultural Sciences (NAS) DNA Sequencing Facility at the University of Pretoria.

2.2.9 | Data Analysis

2.2.9.1 | Microsatellite genotyping analysis

Once all samples were scored individually to ensure the accuracy of allele calls, all genotypes were analyzed in a specific workflow. Before this was started, multi-locus matching was completed in GenAlEx (Peakall & Smouse, 2006) to find and remove duplicates from the dataset as these would skew downstream analyses. Next, relatives within the dataset were identified using Friends and Family v.22 (de Jager et al. 2017) using the Wang (2002) method. Friends and Family v.22 was used as it gives a comprehensive list of individuals which are related to one another allowing for the efficient removal of one relative of the pair from the dataset for further analyses. Definitions of orders of relatedness (*r*) were according to values suggested by Queller and Goodnight (1989).

Following identification, one of each set of relatives was also removed. Once duplicates and relatives have been removed from the data the presence of null alleles, allele dropout, and scoring error was assessed using Micro-checker v.2.2.3 (Van Oosterhout et al., 2004). Thereafter markers were tested for linkage disequilibrium (LD) and Hardy-Weinberg equilibrium (HWE) using Arlequin v.3.5.2.2 (Schneider et al., 2010). Correction for multiple testing was completed using the Bonferroni correction (αEW=α/k; Rice, 1989). The allele frequency for each marker was then calculated and a frequency distribution plot was drawn in GenAlEx. Thereafter the power of the loci was tested for differentiating subpopulations as well as identification of individuals and relatives. The power of loci in the detection of subpopulations was assessed using PowSim (Ryman & Palm, 2006) while the probability of identity (P_{ID}) and sib-identity (P_{IS}) was calculated in Cervus v.3.0.7 (Kalinowski et al., 2007). Summary statistics such as allelic richness, observed, expected, and unbiased heterozygosity, and inbreeding coefficients were calculated per locus and across loci in FSTAT v.2.9.4 (Goudet, 2001). After the tests were concluded on respective aspects, the patterns of structure and relationships were analyzed. To assess and plot the patterns of variation within the dataset a Principal Coordinates Analysis (PCoA) was run in GenAlEx. Since this was only able to plot two principal coordinates (PCs), a Factorial Correspondence Analyses (FCA) was also run in Genetix v.4.05 (Belkhir et al., 2004) to be able to plot three PCs. Thereafter estimation of number of subpopulations (K) was estimated in STRUCTURE 2.3.4 (Pritchard et al., 2000) on two versions of the dataset, one including samples from Namibia and Madagascar while the other excluded individuals. This analysis was done to assess if these individual genotypes skewed the overall result. All STRUCTURE analyses were run applying the Admixture Model with allele frequencies set as correlated. Analysis was completed over a burn-in 200000 simulations followed by 700000 Markov chain Monte Carlo (MCMC) reps for a *K* of one to six with 20 iterations per *K* . Mean posterior probability Q of each individual's assignment and best estimation of *K* were computed using CLUMPAK (Kopelman et al., 2015) whereas probability of *K* was estimated in Structure Harvester (Earl & Vonholdt, 2012). The threshold for assignment to one population was set a Q of 0.8 (Rohde et al., 2015). In addition to this, pairwise F_{ST} within South African samples (between the west and south coast) and between South Africa and Namibia and Madagascar respectively, were calculated in GenAlEx while R_{ST} values (which allow for finer scale detection, considering allele size variation) were calculated in FSTAT.

2.2.9.2 | Mitochondrial DNA sequences

Two datasets containing different combinations of Bryde's whale mtDNA control region sequences were made for analyses to be done on both local and global scales. The first dataset was composed of sequences generated in the present study using the designed primers (Table 2) combined with mtDNA sequences generated previously by Penry et al. (2018) for South African Bryde's whales specifically. The dataset from Penry et al. (2018) also contained two sequence entries from South Africa's offshore population. The second dataset contained sequences of Bryde's whales globally accessed from GenBank combined with sequences from South Africa. Sequences from both datasets were aligned using the MAFFT v.7 (Katoh et al., 2019) and trimmed on MEGA v.11 (Kumar et al., 2018). Because of the biased area sampling within the global dataset, specifically from Japan and South Africa, a reduced subset of data was made to serve as a more representative sample pool (Table 4). To do this, a random draw of ten samples from Japan and South Africa entries was completed using a simplified code in R v. 4.1.2. These sequences were then combined with the remainder of global data entries. Thereafter summary statistics for each dataset were calculated using DnaSP v.6 (Rozas et al., 2017); these included the nucleotide and haplotype diversity (Nei, 1987) as well as the number of polymorphic sites and haplotypes. TCS allele networks (Clement et al., 2002) were then generated in PopART (Clement et al., 2002) for each dataset. For the global dataset, haplotype information was also used to construct phylogenetic trees in Splitstree v.4.17.2

(Huson & Bryan, 2006) to provide a perspective on the connectivity of South Africa's populations in a global context.

TABLE 4 List of samples used in the global Bryde's whale reduced dataset

Oceanic region	Location	GenBank Accession number or Sample name
North Pacific Ocean	Japan	EF068024.1 LC361211.1 EF068023.1 LC361214.1 AF146387.1 EF068027.1 EF068027.1 EF068042.1 EF068014.1 EF068046.1 LC361215.1 DQ340979.1
Indian Ocean (North and East)	Java	EF068060.1 EF068061.1 EF068062.1 EF068063.1
	Bangladesh	KC261305.1
	Maldives	JX090150.1
North Atlantic Ocean	Southern Caribbean	KF916567.1 KF916566.1 KF916568.1
	Portugal	EU030282.1
South Atlantic Ocean	Brazil	KT191132.1 KT191134.1 KT191133.1 KT191131.1
South Pacific Ocean	Peru	EF068051.1 EF068057.1 EF068059.1 EF068056.1 EF068055.1 EF068054.1 EF068058.1 EF068052.1
	Fiji	EF068049.1 EF068050.1
South Indian Ocean ***South Atlantic Ocean***	South Africa	SA B edeni 11 BW38 SA B edeni 28 BW9 SA B edeni 15 BW24 SA B edeni 10 Madagascar Mdouble SA B edeni 29 SA B edeni 12 (Offshore) BB002 Walvis Bay Jan12 (Likely Offshore)

2.3 | RESULTS

2.3.1 | Microsatellites

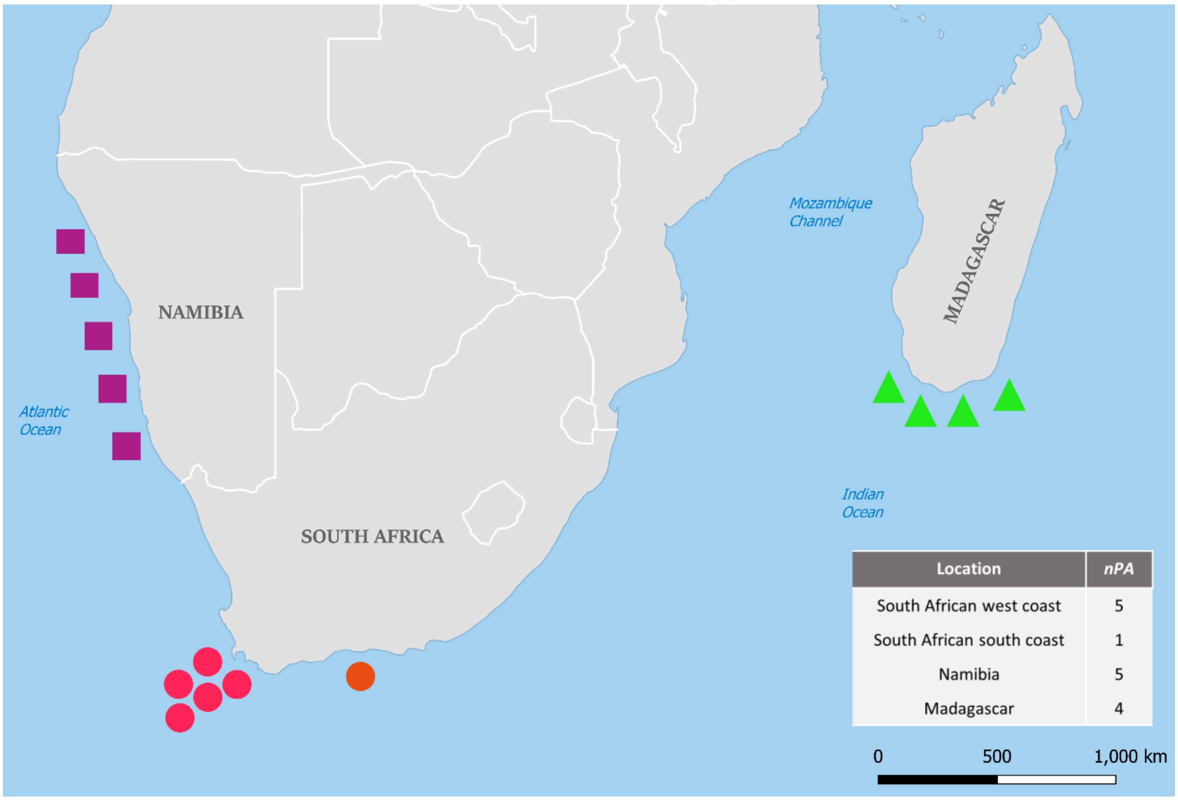
2.3.1.1 | Microsatellite genotyping

In total, 106 genotypes were generated across the available samples. Of the 15 loci tested on *B.edeni* in this study, 14 polymorphic loci were successfully amplified across the majority of the 106 individuals. Five of these loci were previously analyzed in Bryde's whales (Penry, 2010) and were thus expected to amplify, whereas nine loci were newly chosen; three were previously tested on other Bryde's whale populations worldwide (Kanda et al., 2007; Rosel & Wilcox, 2014; Wiseman, 2008) and the remaining six loci were tested for the first time in the present study. There was no evidence of null alleles, large allele dropout, or scoring error across the loci. No significant LD or deviation from HWE ($P > 0.05$) was detected following correction for multiple testing. Of the nine newly chosen loci only one did not amplify in any samples (EV37). Between 12 and 14 loci amplified in all individuals, resulting in an average of 13 loci genotyped per individual. The number of alleles and repeat sequences amongst the 14 loci varied (Table 5). The number of alleles detected at each locus ranged from two to eight with an average of 3.90. One of the newly tested loci (Bp 1043662) was originally reported by Tardy et al. (2020) as a tetranucleotide (AGAT), however, the sequencing of this locus showed it is dinucleotide (GT) in South Africa's inshore Bryde's whales. In addition, a lower number of alleles were detected across the new loci in the inshore individuals when compared to the number of alleles detected in the fin whale (*B.physalus;* Tardy et al., 2020).

Summary statistics for the 14 polymorphic loci are summarized in Table 5. The average allelic richness across loci was calculated as approximately 3.35, with the lowest detected as 1.99 (GT122) and the highest calculated as 6.61 (GATA028) for a minimum number of 35 individuals. The unbiased heterozygosity ranged from 0.25 (GT122) to 0.76 (GATA098) with an average of 0.53 across all loci. Of the 63 alleles detected across all loci and individuals in the southern African coastal region, there were several private alleles present. Five private alleles were detected in an individual sampled in Madagascan waters, four private alleles in one individual sampled in Namibian waters, and six private alleles were detected in six individuals sampled in South African waters (not detected in Madagascar and Namibia), five of which were sampled on the west coast and one on the south coast (Figure 2).

TABLE 5 Repeat sequence and summary statistics of per microsatellite locus and across loci: Number of alleles (N_A), Observed (H_o) and expected (H_e) and unbiased expected (uH_e) heterozygosity and Allelic richness (A_R) based on a minimum sample size of 35 individuals per locus

Locus	**Repeat sequence**	**Summary statistic**				
		N_A	H_o	H_e	**uHe**	A_R
GT122	*GT*	4	0.257	0.252	0.387	1.994
Bp1430262	*AC*	2	0.389	0.253	0.379	1.998
Bp531923	*AC*	2	0.247	0.144	0.245	2.813
Bp1418059	*AC*	3	0.354	0.250	0.353	2.000
Bp740180	*AC*	6	0.499	0.324	0.527	4.604
Bp1178841	*AC*	2	0.701	0.472	0.701	2.000
EV1	*AC/TC*	5	0.810	0.516	0.761	3.981
GT023	*GT*	4	0.537	0.450	0.596	3.673
Bp1043662	*GT*	3	0.292	0.319	0.365	2.000
GATA028	*GATA*	8	0.767	0.588	0.835	6.609
GATA098	*GATA*	6	0.586	0.464	0.595	5.917
GATA53	*GATA*	4	0.595	0.525	0.763	3.895
GGAA520	*GGAA*	3	0.565	0.429	0.559	3.000
GATA417	*GATA*	3	0.274	0.272	0.317	2.427
Overall	-	*63*	*0.491*	*0.376*	*0.527*	*3.350*



Location	*nPA*
South African west coast	5
South African south coast	1
Namibia	5
Madagascar	4

FIGURE 2 Map of the southern African coastal region depicting distribution and number of private alleles (*nPA*) in Madagascan (n=1), Namibian (n=1), and South African (n=6) individuals

2.3.1.2 | Individual identification and relatedness

Multi-locus matching was used to identify resampled individuals within the dataset, by generating a unique genotype for each data entry based on the 14 polymorphic loci. The power to detect individuals was high, with an average P_{ID} of 8.00^{e-7}. Overall, 85 unique genotypes were generated from 106 samples. This means that 21 individuals were resampled, which is supported by the 21 matching genotypes found. The sampling location of these matches showed that individuals were resampled in the same area of the coast within the same year, except for one. Individual 55 was sampled in 2012 in False Bay and resampled again in the same area in 2021 (Table 1, Suppl. Material).

The power to detect siblings was also high with an average P_{IS} value of 1.73^{e-3}. Relatedness values within the dataset ranged from ~-0.4 ≤ r ≥~0.5 (Figure 3) with an average relatedness of $r = 0.093$ calculated amongst the individuals within the dataset across sampling locations. Overall 85 unique genotypes and a total of 37 relatives were detected, seven of which were inferred first-order relationships ($0.375 \leq r \geq 0.625$), which could be parent-offspring pairs or sibling level relationships. The remaining 30, were second-order relatives ($0.125 \leq r \geq 0.3749$). Mean inbreeding coefficients over all loci and individuals show no significant levels of inbreeding within the population ($F_{IS} = -0.013$, $p > 0.05$). This combined with results shown in the frequency distribution plot (Figure 3) shows that, despite the high number of relatives detected, the majority of individuals are unrelated.

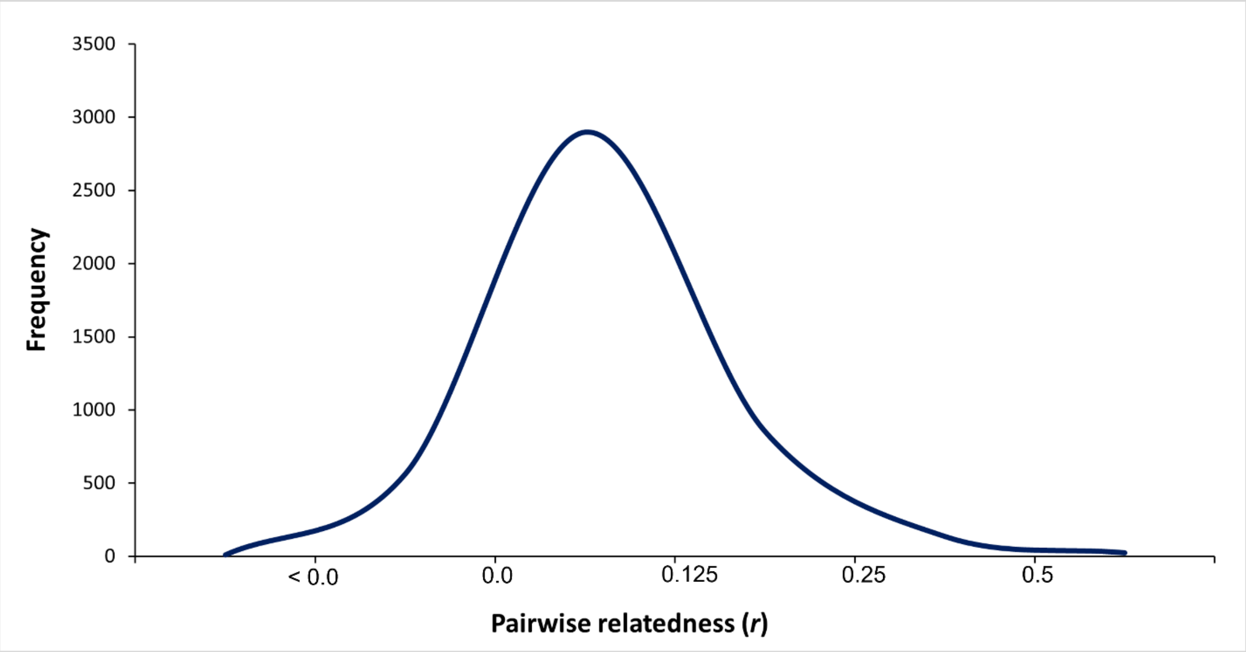


FIGURE 3. Frequency distribution plot of pairwise relatedness values between Bryde's whales in the southern African region

The different sampling locations along the coastline were separated broadly between the west and south coasts of southern Africa (Figure 4). The majority of the detected relatives were sampled in the same area of the coastline (n=24), but some were located in different areas (n=13). Of the 13 relatives that were sampled at different locations, only two were first-order relatives while the remaining 11 were second-order relatives (Figure 4). The farthest geographical distance between second-order relatives was between the south coast of South Africa and Pelican Point (Namibia) (r = 0.27).

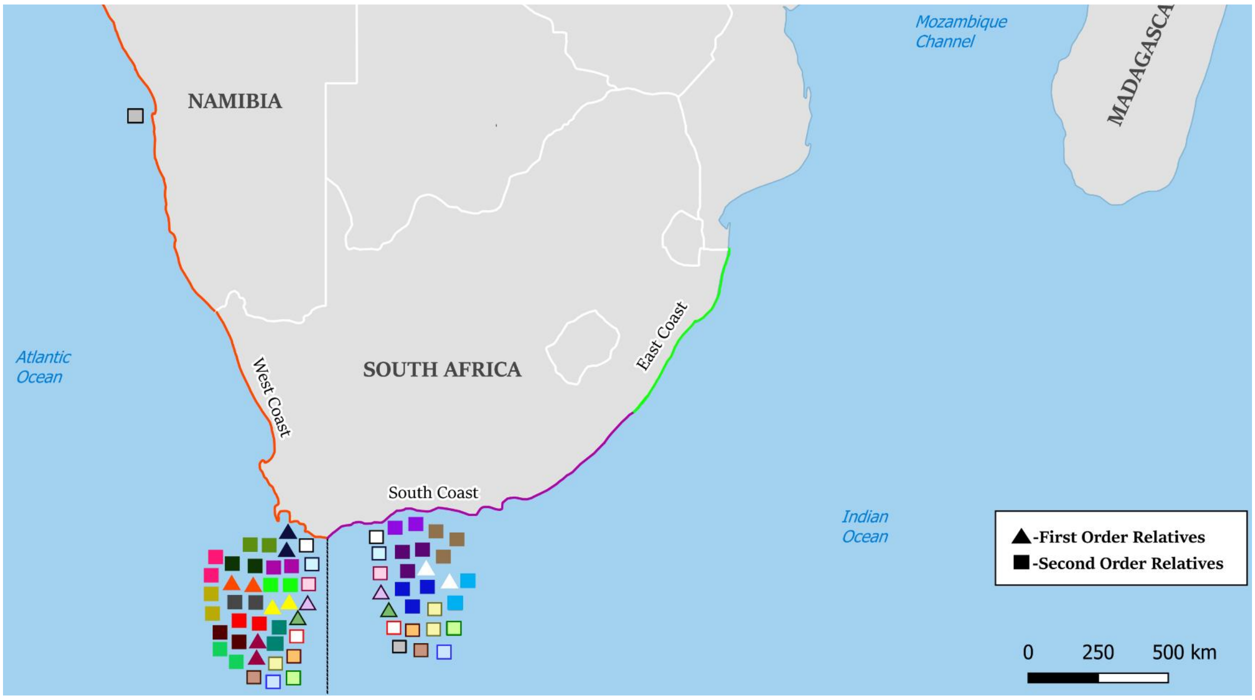


FIGURE 4 Distribution of relatives detected within the dataset. Relatives that were sampled on different areas of the coast are indicated with a darker outline. Relatives that were sampled on the same area of the coast are indicated by a solid colour

2.3.1.3 | Genetic differentiation

The detection of genetic clusters between different sampling locations over the South African coastal region was assessed using multiple different tests. Pairwise F_{ST} values, as well as R_{ST}, were calculated for individuals sampled in South African waters (west coast vs south coast), for 55 unrelated individuals in total (Table 6). The small sample size of Madagascar and Namibia prevented an accurate estimate of these statistics. Within the South African samples significant but extremely low genetic differentiation was detected between samples from the west and south coast shown by the small F_{ST} value (F_{ST} = 0.008, $p < 0.05$. However, R_{ST} values (R_{ST} = 0.006*)* between samples from South Africa were lower and non-significant when compared to the F_{ST} values (Table 6).

TABLE 6 Comparison of pairwise F_{ST} and R_{ST} values from samples of different locations. **: Significant at the 5% nominal level, NA: Significance could not be tested due to low sample size, NS: Not significant.*

Groups compared	F_{ST} value	R_{ST} value
South African west coast vs. *South African south coast*	0.008*	0.006^{NS}

Similar patterns of differentiation, as suggested by the F_{ST} values, are illustrated in a PCoA (Figure 5). South African samples are scattered across the plot forming no defined clusters, though some samples are notably different from the rest (BW21, BW43). The sample of unknown location clusters with South African individuals sampled on the west coast (SRFI2008). The Namibian samples display two different results, whereby the individual sampled at Pelican Point seems more genetically similar to individuals sampled in South African waters. However, the individual sampled in Walvis Bay appeared far more different in comparison and is separated from the rest of the individuals sampled in South Africa and Madagascar. The Madagascan individual is more different from South African and Namibian individuals as it does not cluster closely with either.

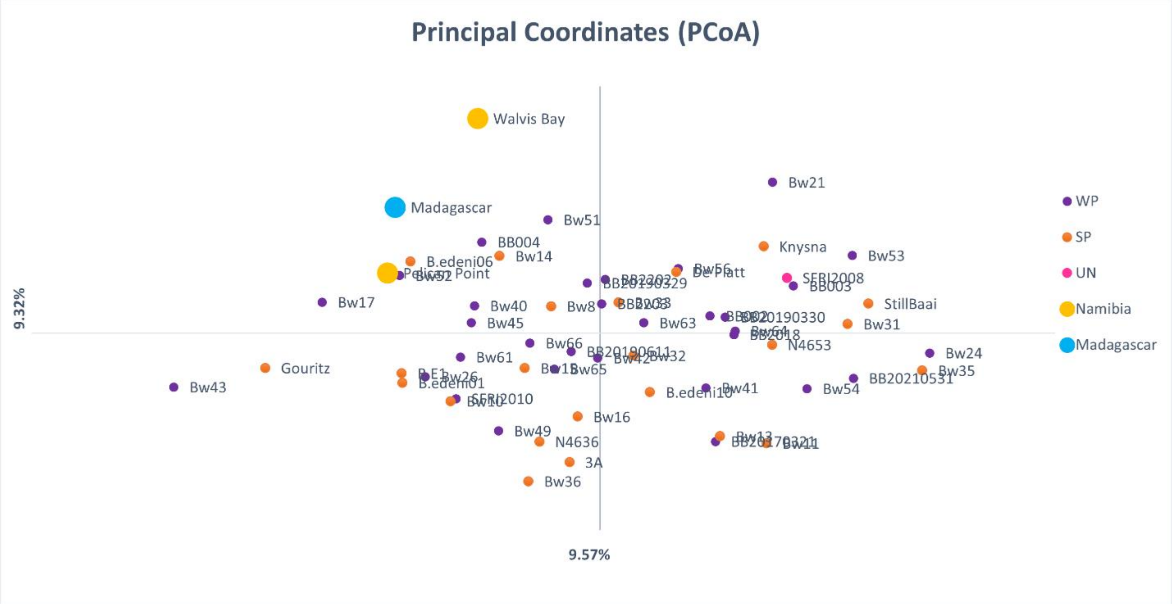


FIGURE 5 Principal Coordinates Analysis (PCoA) of 59 Bryde's whale genotypes sampled in the southern African coastal region. ***WP***= Individuals sampled on the South African West Coast, ***SP***= Individuals sampled on the South African South Coast, ***UN***= Unknown location

Figure 6 shows the FCA plot, with the three factors explaining 37.27% of the genetic variance within the dataset. Despite the higher resolution, the same pattern is displayed in Figure 5. South African genotypes cluster as one group, with a few notable outliers while one Namibian and the Madagascan individuals are separated and different (Figure 6). The second Namibian individual is once again appearing to be more similar to South African genotypes by clustering closely with them.

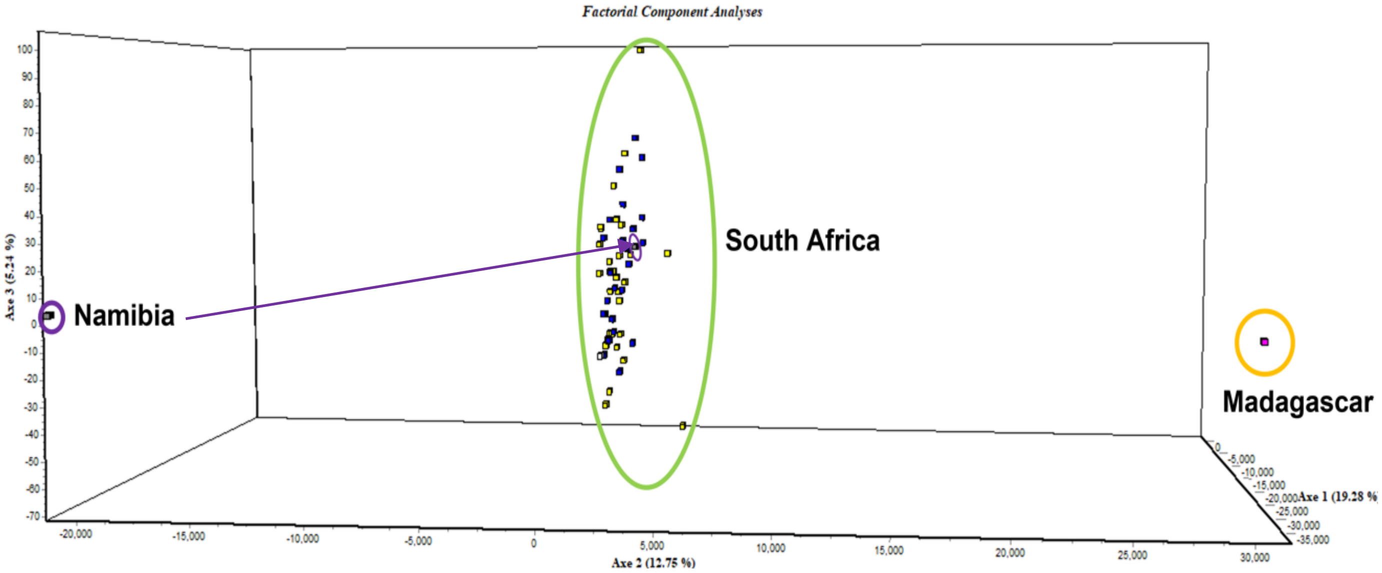


FIGURE 6 Factorial Correspondence Analysis (FCA) based on 59 Bryde's whale genotypes. The arrow indicates the location of the second Namibian sample

Structure analyses of all samples across South Africa at *K*=2 subpopulations is shown in Figure 7. The y-axis of the plots indicates every individual's probability (Q) of assignment to K subpopulations. All of the South African individuals showed similar values of Q (Q = ~0.5) assignment to each subpopulation. Delta K suggested *K*=2 subpopulations but Ln(K) graphs showed *K*=1 was most likely (Figure 1, Suppl. Material). The result of Ln(k) is supported by all South African individuals showing similar values of Q to either population at *K*=2.

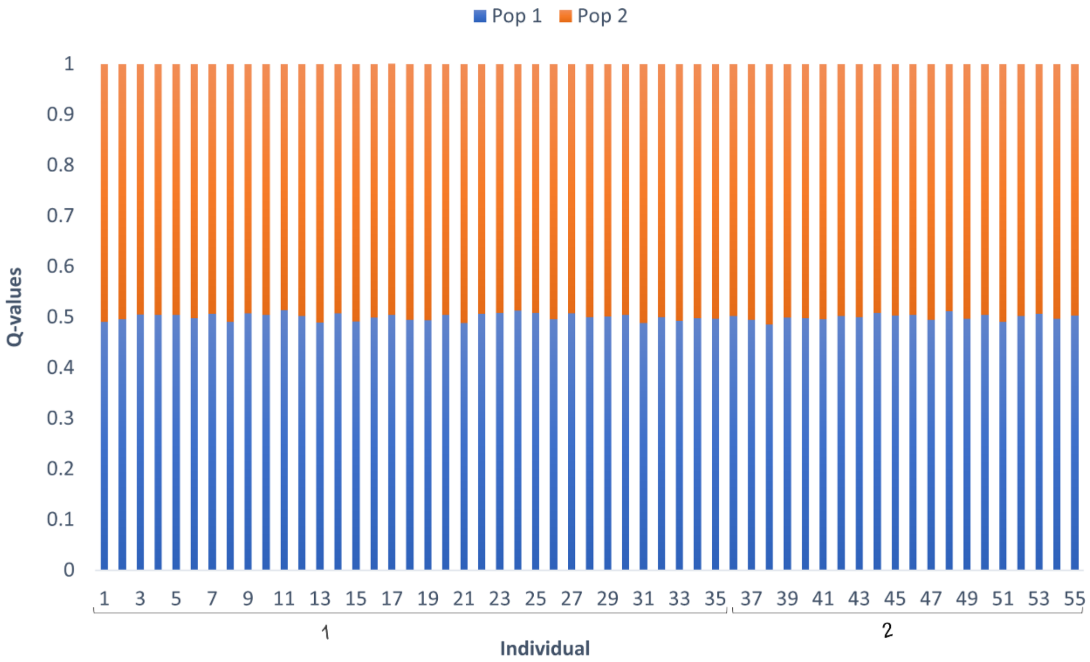


FIGURE 7 Structure plot sorted by Q-value based on 55 Bryde's whale genotypes sampled within South Africa for *K*=2 sorted on Q-value

2.3.2 | Mitochondrial DNA (mtDNA)

2.3.2.1 | Southern African diversity

In total, 20 additional control region sequences were generated successfully using newly designed primers, which produced a ~820 bp length fragment. When combined with mtDNA control region sequences generated previously, 115 sequences of ~636 bp in length made up a final dataset for Bryde's whales of southern Africa. Amongst the 20 newly generated sequences, only three haplotypes were detected across the~820 bp fragment. However, in the overall southern African data, five haplotypes were detected based on the shorter fragment (Figure 8); the haplotype and nucleotide diversities were estimated at 0.149 and 0.00074 respectively (Table 7). Haplotype 1 represents the two sequences from individuals of the offshore population within the South African dataset, clearly separated by nine mutational steps (Figure 8). Haplotype 2 is represented in 103 inshore individuals, one individual sampled in Madagascan waters and one individual sampled in

Namibian waters whereas haplotype 3 was represented by five individuals of the South African inshore population. Both Haplotypes 4 and 5 were represented by one individual of the South African inshore population.

TABLE 7 Summary statistics for different datasets of mtDNA control region sequences

Dataset	**Number of sequences**	**Fragment length**	**Number of polymorphic sites**	**Nucleotide diversity**	**Number of haplotypes**	**Haplotype diversity**
Newly generated sequences	20	820	3	0.00040	3	0.195
Southern African dataset	115	636	13	0.00074	5	0.149
Global dataset	201	308	54	0.02160	64	0.719
Reduced global dataset	52	255	28	0.02153	35	0.958

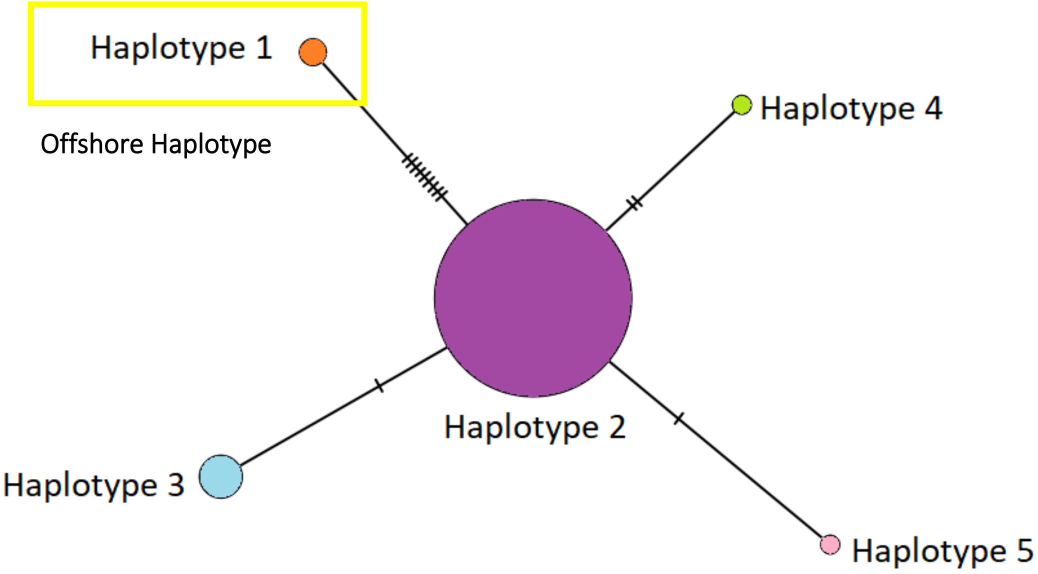


FIGURE 8 TCS allele network based on southern African Bryde's whale mtDNA control region data. Cross hatches indicate the number of mutational steps between haplotypes

2.3.2.2 | Global population comparisons

The global Bryde's whale dataset consisted of 201 control region sequences of~308bp before being reduced. The final reduced dataset for global Bryde's whale consisted of 52 representative sequences which were used for further analyses. This reduced dataset was used to ensure non-biased results from a high number of sequences from South Africa and Japan Results from the larger global dataset showed 64 unique haplotypes over 201 sequence entries, which were derived based on 60 polymorphic sites. Overall haplotype and nucleotide diversity values were estimated as 0.719 and 0.02160 respectively. Within the reduced dataset, 35 unique haplotypes were derived from 28 polymorphic sites. Haplotype and nucleotide diversity was calculated as 0.958 and 0.02128 respectively.

When looking at haplotype patterns of divergence within the global context both allele and phylogenetic networks show similar results throughout the Bryde's whale populations. Most of the unique haplotypes detected originate from the North Pacific Ocean, more specifically Japan. Of 35 unique haplotypes, South African inshore Bryde's whale sequences are represented by Haplotypes 18 and 19 while South Africa's offshore samples are Haplotypes 16 and 17 (Figures 9 & 10). The highest frequency haplotype for South African samples (Hap_18) is shared with an individual sampled in Java (Figure 9). In both figure 9 and 10, haplotypes pertinent to the South African inshore population appear to cluster with individuals sampled in Java (Hap_33), the Maldives (Hap_34), and Bangladesh (Hap_35), which are within the Eastern and Northern Indian Ocean regions respectively. Figure 10 also gives a broader picture of all the possible connections between haplotypes of different regions. In addition, results clearly show divergence between haplotypes of individuals of the South African inshore and offshore populations, as these do not cluster close together in either network. Haplotypes of individuals from the South African offshore population cluster closely with individuals sampled in the southern Caribbean (Hap_24, Hap_25), Portugal (Hap_24), and Brazil (Hap_26) from the Northern and Southern Atlantic Ocean regions respectively (Figure 10). Furthermore, the network in Figure 9 shows a clear separation between the representative sequences used for the reduced dataset, whereby haplotypes from the Indo-Pacific Ocean range seem more closely related and separate from haplotypes sampled from the Atlantic and Northern Pacific Ocean range. The spatial distribution of haplotypes over the global ocean regions is shown in Figure 11. Haplotypes that consist of sequences from different regions are coloured in grey, outlined in the colour of their respective regions (Figure 11).

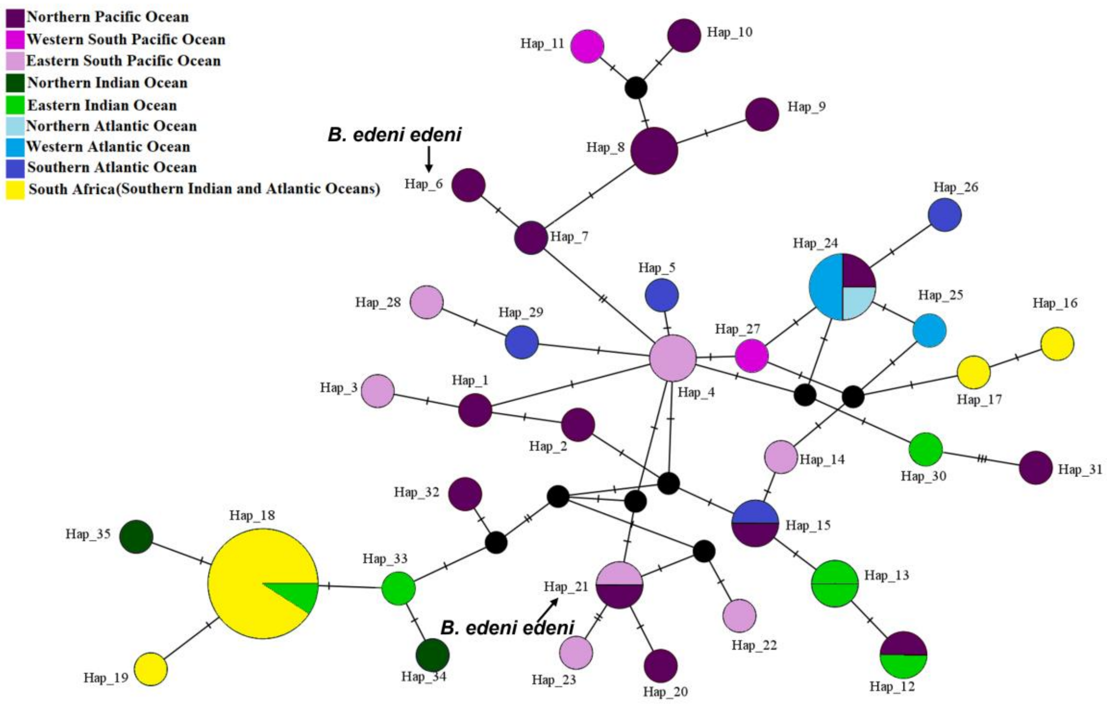


FIGURE 9 TCS allele network based on global Bryde's whale mtDNA control region data. Each ocean region is represented by a different shade of colour shown in the legend. Haplotypes listed as *B. edeni edeni* are indicated in the figure, the unlabeled haplotypes are listed as *B. edeni brydei*

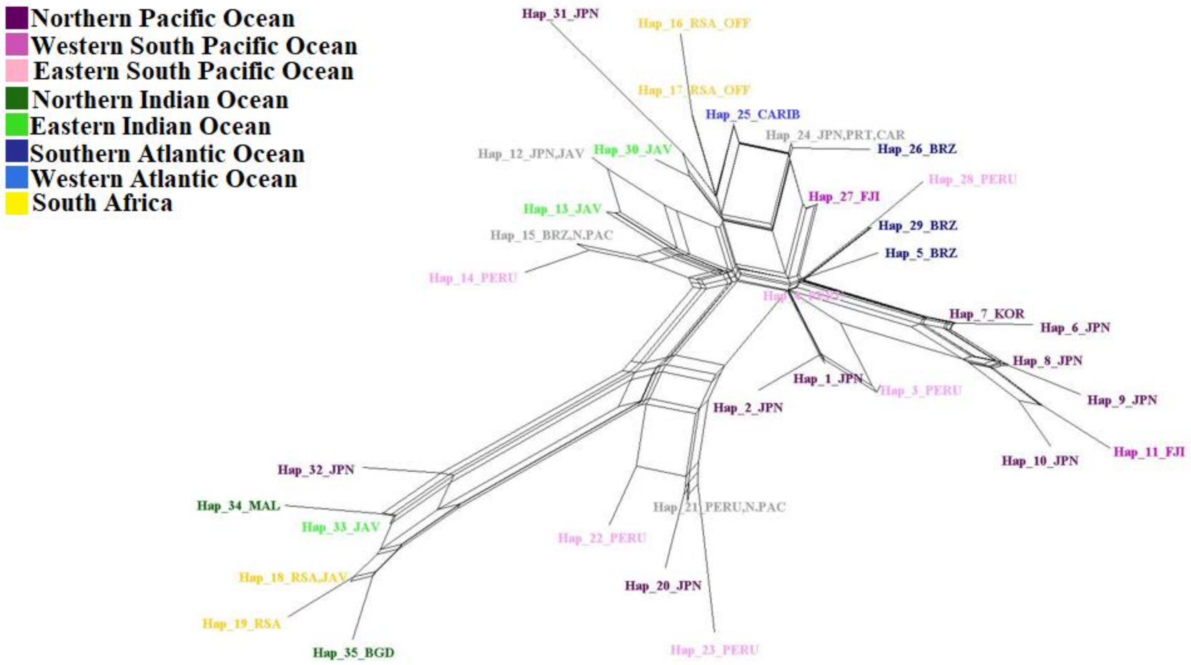


FIGURE 10 Phylogenetic network based on global Bryde's whale mtDNA control region haplotype data. The country of origin of samples is represented by a three-letter abbreviation

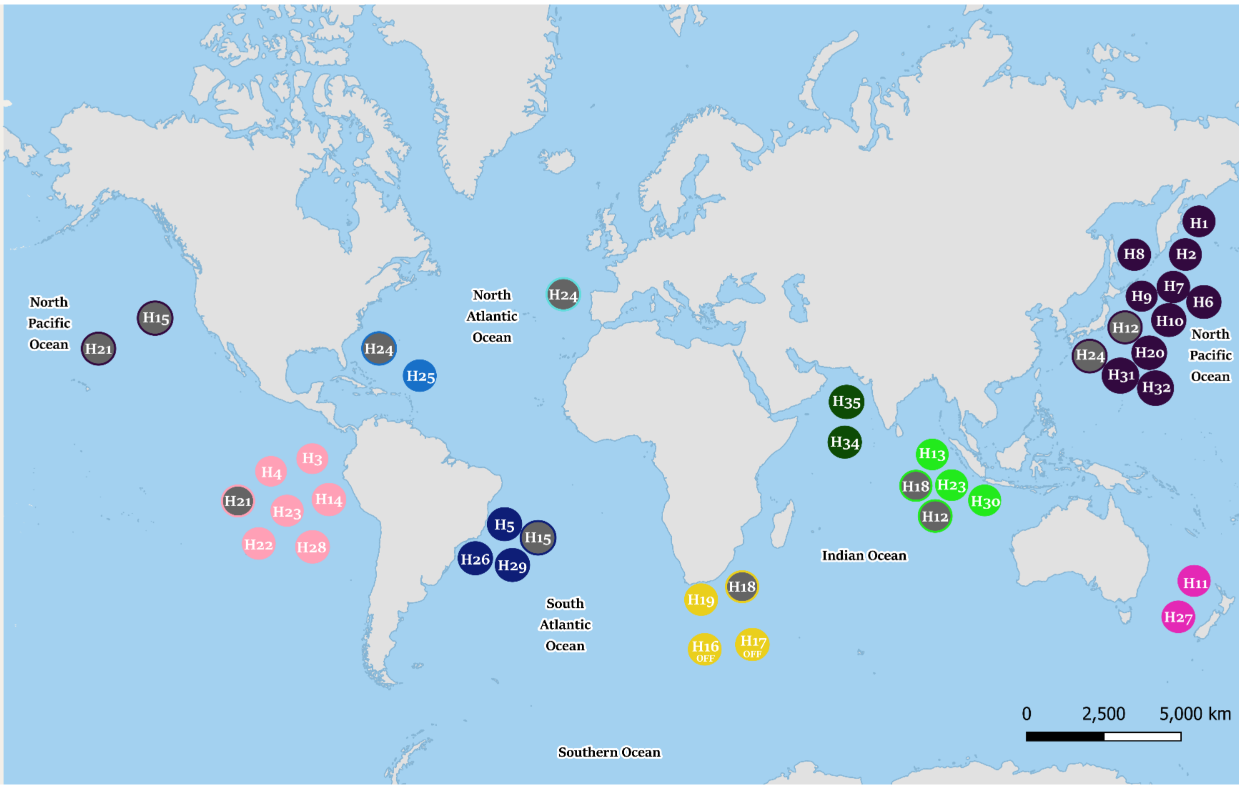


FIGURE 11 Spatial distribution of global Bryde's whale haplotypes on a world map showing oceanic regions. Shared haplotypes are coloured in grey.

2.4 | DISCUSSION

The overall results of this study build on previous research and confirm that the Bryde’s whales occurring in the inshore waters of South Africa form a single population with limited evidence of genetic differentiation between the west and south coast of the country. This population, despite being small, shows high levels of nuclear genetic diversity but low mtDNA diversity. Within a broader global context, results suggest that there is potential geneflow across the Indian Ocean, displayed by shared haplotypes from different locations, but this result may also be caused by incomplete linage sorting. Nonetheless, this likely is an ancient connection between regions. Furthermore, preliminary results suggest that South Africa’s inshore and offshore populations are connected to other populations in two different oceanic regions. Overall, these results show that this inshore population may still be viable despite being small and having a restricted coastal distribution.

2.4.1 | Genetic diversity

2.4.1.1 | South Africa’s inshore Bryde’s whales

Results of this study suggest that moderate levels of nuclear genetic diversity are being maintained within the South African population, with levels higher than previously detected (Penry, 2010), likely due to increased sample size and a larger number of polymorphic loci analyzed. Nonetheless, the levels of nuclear genetic diversity are much lower than other Bryde’s whale populations worldwide, for example, those found in the Pacific and East Indian Oceans (Kanda et al., 2007; Wiseman, 2008). It is believed that this is related to the ecological differences between these populations, as populations from the Pacific and East Indian Oceans are migratory and therefore, have a larger distributional range and larger effective population sizes, resulting in higher levels of nuclear genetic diversity.

A baleen whale population that shares a similar ecology to the South African inshore Bryde's whales is the newly classified Rice's whale (*B .ricei*) (Rosel et al., 2021). Although they are now considered a new species, the Rice's whale was initially thought to be a Bryde's whale population residing in the Gulf of Mexico (GOMx) (Rosel & Wilcox, 2014). Given their close phylogenetic relationship to Bryde's whales and shared ecological traits with the South African inshore population, a comparison of genetic diversity between the two is warranted. The Rice's whale is currently listed as "Critically Endangered", given its small population size and extremely restricted distribution (Rosel et al., 2021). Rosel and Wilcox (2014) studied the genetic diversity of this resident baleen whale in the GOMx and found extremely low genetic diversity in both mtDNA and microsatellite markers. Results from this study clearly show that, despite the ecological similarities, South Africa's inshore Bryde's whales have slightly higher levels of nuclear genetic diversity in comparison to the critically endangered Rice's whales.

Contrasting to the above, South Africa's inshore Bryde's whale population shows low mitochondrial diversity as found in previous work (Penry et al., 2018). In fact, the number of unique haplotypes, as well as haplotype and nucleotide diversities detected within South Africa is lower than what was found in Bryde's whale populations from New Zealand (Wiseman, 2008), South America (Pastene et al., 2015) and the Pacific and Indian Oceans (Kanda et al., 2007), and more similar to the levels found in the Rice's whale population (Rosel & Wilcox, 2014). It is believed that the difference in distribution and migratory behaviour between these populations may account for the contrast in diversity. Furthermore, as in the Rice's whale population (Rosel & Wilcox, 2014) there was also evidence of one high-frequency haplotype likely due to the small population size of the South African inshore population (< 1,000 individuals; Penry et al., 2016)

The factors that influence the patterns of genetic diversity in cetaceans are complex, as different elements drive the maintenance of diversity in mitochondrial and nuclear genomes (Vachon et al., 2018). As such, neutral nuclear diversity is most often influenced by changes in effective population size(N_e) and/or geneflow whereas mitochondrial diversity, also affected by N_e, is mainly influenced by social structure and/or latitudinal range (Vachon et al., 2018). In general, Vachon et al. (2018) suggest that cetacean populations marked with low mitochondrial diversity arise from females being the less dispersive sex or individuals being restricted to a smaller area. Given what we know about the Bryde's whale population inhabiting the inshore waters of South Africa, the latter seems the most likely reason for the low mitochondrial diversity. Although little is known about the reproductive behaviour of South Africa's Bryde's whales, it is also possible that females tend to stay in the same areas where they were born (i.e showing philopatry) also influencing the levels of mitochondrial diversity. On the other hand, high nuclear diversity is characteristic of larger cetacean populations, and consequently larger effective population sizes, distributed over a range of environmental habitats (Vachon et al., 2018). An accurate population size estimate is outstanding for the South African inshore Bryde's whales; therefore, it is possible that the census and effective population may be larger than was originally suggested. N_e estimates are one of the most important indicators of the population's ability to respond to environmental change (Teixeira & Huber, 2021). Together, these results show us that the inshore Bryde's whale population may still be viable despite their restricted distribution. It also highlights the need to use the indication of high neutral genetic diversity to estimate N_e for South Africa's inshore Bryde's whales to gain a better understanding of the population's viability.

2.4.1.2 | Global populations

On a global scale, the patterns of Bryde's whale mtDNA diversity provide insight into the ecological and evolutionary differences between different populations. A previous assessment of global data on Bryde's whales' diversity reported 73 haplotypes over a 305bp alignment (Rosel et al., 2021) which differs from the 65 haplotypes calculated for the 308bp alignment in this study. However, the addition of several South African sequences to the dataset may account for this difference. In general, the high number of unique haplotypes from the North Pacific region is a direct contrast to the low number of unique haplotypes of the coastal populations in Brazil and South Africa. These results reflect how differences in the distributions of populations influence their mitochondrial diversity. Populations from the Pacific Ocean are more migratory, occupying a wider latitudinal range and allowing for more maternal gene flow. As stated by Vachon et al. (2018) cetacean populations such as the Bryde's whales in the North Pacific are usually characterized by this high level of mitochondrial diversity. Whereas populations with a smaller, coastal distribution (e.g in Brazil or South Africa), show a much lower diversity most likely as a result of their restricted movements. Furthermore, these coastal populations could have been founded by individuals from larger groups and now share little gene flow with broader regions, thus lowering their mitochondrial diversity due to founder effects (Vachon et al., 2018). However, the skewed patterns in diversity between areas may also be a result of biased sampling. A more representative sampling of certain areas is required before any formal conclusions on the differences in genetic diversity can be made. Nonetheless, these results provide preliminary insights into the differences between populations of Bryde's whales found worldwide and the careful consideration that needs to be given to differences in ecology before concluding the possible processes shaping genetic diversity.

2.4.2 | Population differentiation

2.4.2.1 | Southern African region

Results show that no clear, differentiated genetic clusters are present within the inshore Bryde's whale population of South Africa and suggest that these whales form one population. This result is in alignment with previous findings (Penry, 2010). However, genetic structure within natural populations lies on a continuum from highly structured to complete panmixia (Waples & Gaggiotti, 2006). Thus, there was still evidence of low genetic differentiation between individuals from the west and south coasts of South Africa which means this may not be a fully randomly mixing population. Considering their distribution in the very different oceanic regimes that surround the South African coastal region influencing the population structure of other marine organisms (Findlay, 1989) this result provides some insight into the movement patterns of these whales across oceanic regimes. Interestingly, relatives were most often found in the same area of the coastline. It is difficult to suggest ecological reasons which could drive these low levels of genetic differentiation, as several aspects of South African Bryde's whale ecology and biology remain unclear. Consideration must be given to the processes that might facilitate and prevent the mixing of individuals, such as breeding throughout the year as suggested by Best (2007), or long-term site fidelity to areas where individuals are born (philopatry). However, it needs to be acknowledged that the obtained results may be affected by the limited number of sample locations in both oceanic regimes. Future research should therefore focus on obtaining samples from more localities along the South African west, south, and even east coast to form stronger conclusions on population structure. Overall, a combination of genetic- and photo-identification data on the population will provide a better picture of the distribution and movements of individuals between areas of the coastline.

Preliminary results on genetic differentiation within the greater southern African coastal region support the initial proposal of three distinct Bryde's whale populations, including an inshore and offshore population in southern Africa and a population on the Madagascan coast (Best, 2001). Microsatellite genotype results provide further proof that individuals from these populations are indeed genetically distinct from each other. In fact, measures of genetic differentiation were similar to other those found between other distinct Bryde's whale populations found in different oceanic regions (Kanda et al., 2007).

Differentiation between individuals within the greater southern African coastal region based on mtDNA variation is slightly more complex. The occurrence of separate haplotypes in offshore and inshore individuals coincides with results found previously (Penry et al., 2018) and suggests strong differentiation. Previous phylogenetic analyses also displayed a clear separation between the offshore and inshore haplotypes, and this clear divergence has further been demonstrated on both morphological and ecological levels (Penry et al., 2018), with individuals differing in body size and scarring patterns as well as distribution, main prey resources, and other ecological factors (Best, 2001). Interestingly, an individual sampled in Namibia grouped, unexpectedly, with individuals of the inshore population indicating that the inshore population distribution may extend further north along the southern African west coast than originally thought. Also unexpectedly, the Madagascan individual shared a haplotype with the southern African inshore population. Considering nuclear data suggest significant differentiation between the two regions, the shared haplotype is believed to be a retention of ancient polymorphism. This may suggest that separate individuals from the same larger, older population may have colonized the South African and Madagascan coasts. Unfortunately, the low sample size from Namibia and Madagascar limits the conclusions that can be drawn and shows the importance of obtaining more samples from these areas for further analysis.

2.4.2.2 | Global context

The comparison of global Bryde's whale mtDNA sequences allowed for a preliminary assessment of the connectivity of South Africa's Bryde's whales to other oceanic regions. The shared haplotype between an individual sampled in South Africa's inshore waters and another individual was not detected previously and highlights the gaps in understanding for Bryde's whales, as the South

African inshore population is thought to have a restricted distribution along the coastline of the country (Best, 2001; Penry et al., 2016). This result suggests potential gene flow or, most likely, retention of ancestral polymorphism between inshore individuals and other populations found in the Eastern Indian Ocean. Further analyses regarding a time of divergence are needed to fully understand this result.

In addition, the phylogenetic networks indicate a link between the southern African offshore population and the population in the North-Western Atlantic Ocean, as was shown previously by Penry et al. (2018). This means that both allele and phylogenetic networks suggest a separate origin and connectivity (historical and/or current) for inshore and offshore individuals of southern Africa. However, further analyses are required to date these proposed divergence events and provide possible scenarios to explain the recurring patterns. In addition, to provide more clarity and understanding of the connectivity of the southern African Bryde's whales within a broader global context, more representative sampling is required of other Bryde's whale populations globally.

2.5 | CONCLUSION

The results of this study provide insight into both ecological and evolutionary aspects of the Bryde's whale population which reside in the inshore waters of South Africa. They show a relatively high level of nuclear genetic diversity and no significant inbreeding, which begins to indicate the viability of South Africa's only resident baleen whale. Nonetheless, the low number of haplotypes as well as low nucleotide and haplotypic diversities align with other small Bryde's whale-like populations which are currently of conservation concern (Rosel & Wilcox, 2014; Rosel et al., 2021). The differing nuclear and mitochondrial diversities raise important ecological questions regarding sex-based dispersal and the adaptive abilities of the population. This study highlights the significant biological and ecological gaps in knowledge that are evident for the species, which must be combined with genetic information to improve our understanding of the Bryde's whale. Nonetheless, the genetic information gained from this study provides a basis on which other ecological questions and research can be built. Based on the results of this study there is a greater emphasis on the importance of estimating N_e and investigating adaptive genetic variation for this population which will provide information on its viability and, in turn, inform on conservation strategies for the species.

When looking at patterns of genetic differentiation, the results confirm the inshore Bryde's whales from South Africa form a single population, with no highly differentiated spatial population structure along the coast. Within the larger southern African region, results confirm the three previously proposed populations within the region (Best, 2001), but stresses the need for increased sample size from both Namibia and Madagascar. On a broader, evolutionary, and global scale, results indicate inshore and offshore individuals from southern Africa are connected to populations in different oceanic regions. This is important when aiming to understand the history and origins of these populations, and in resolving the greater Bryde's whale phylogenetic connections globally which were deemed a research priority in previous conservation assessments (Penry et al., 2016). Overall, the results of this study provide important information in building our knowledge of South Africa's resident baleen whale and highlight the important areas for future genetic and ecological research of this population, which will inform conservation and management strategies, as well as for the understanding of the species in general.

2.6 | SUPPLEMENTARY MATERIALS

TABLE S1 Individual number, sample IDs, and sampling location of genotyped individuals

Individual Number	Sample ID	Sampling Location
Individual 1	BB001	Walker Bay
Individual 2	BB002	Walker Bay
Individual 3	BB003	Walker Bay
Individual 4	BB004	Walker Bay
Individual 5	BB005	Walker Bay
Individual 6	BB006	Walker Bay
Individual 7	Bw17	False Bay
Individual 8	Bw18	False Bay

Individual 9	Bw19	False Bay
Individual 10	Bw20	False Bay
Individual 11	Bw21	False Bay
Individual 12	Bw22	False Bay
Individual 13	Bw23	False Bay
Individual 14	Bw24	False Bay
Individual 15	Bw25	False Bay
Individual 16	Bw26	False Bay
Individual 17	Bw39	False Bay
Individual 18	Bw40	False Bay
Individual 19	Bw41	False Bay
Individual 20	Bw42	False Bay
Individual 21	Bw43	False Bay
Individual 22	Bw44	False Bay
Individual 23	Bw45	False Bay
Individual 24	Bw46	False Bay
Individual 25	Bw47	False Bay
Individual 26	Bw48	False Bay
Individual 27	Bw49	False Bay
Individual 28	Bw50	False Bay
Individual 29	Bw51	False Bay
Individual 30	Bw52	False Bay
Individual 31	Bw53	False Bay
Individual 32	Bw54	False Bay
Individual 33	Bw55	False Bay
Individual 34	Bw56	False Bay
Individual 35	Bw57	False Bay
Individual 36	Bw58	False Bay

Individual 37	Bw60	False Bay
Individual 38	Bw61	False Bay
Individual 39	Bw62	False Bay
Individual 40	Bw63	False Bay
Individual 41	Bw64	False Bay
Individual 42	Bw65	False Bay
Individual 43	Bw66	False Bay
Individual 44	BB2201	Walker Bay
Individual 45	BB2202	Walker Bay
Individual 46	BB2203	Walker Bay
Individual 47	BB2204	Walker Bay
Individual 48	BB2205	Walker Bay
Individual 49	BB20170321	False Bay
Individual 50	BB2018	False Bay
Individual 51	BB20190328	False Bay
Individual 52	BB20190329	False Bay
Individual 53	BB20190330	False Bay
Individual 54	BB20190611	False Bay
Individual 55	BB20210531	False Bay
Individual 56	SFRI2010	False Bay
Individual 57	Bw16	East London
Individual 58	N4636	Port Elizabeth
Individual 59	N4653	Port Elizabeth
Individual 60	Pe1	Port Elizabeth
Individual 61	Bw1	Plettenberg Bay
Individual 62	Bw2	Plettenberg Bay
Individual 63	Bw3	Plettenberg Bay
Individual 64	Bw5	Plettenberg Bay

Individual 65	Bw6	Plettenberg Bay
Individual 66	Bw7	Plettenberg Bay
Individual 67	Bw8	Plettenberg Bay
Individual 68	Bw9	Plettenberg Bay
Individual 69	Bw10	Plettenberg Bay
Individual 70	Bw11	Plettenberg Bay
Individual 71	Bw12	Plettenberg Bay
Individual 72	Bw13	Plettenberg Bay
Individual 73	Bw14	Plettenberg Bay
Individual 74	Bw15	Plettenberg Bay
Individual 75	Bw27	Plettenberg Bay
Individual 76	Bw28	Plettenberg Bay
Individual 77	Bw29	Plettenberg Bay
Individual 78	Bw30	Plettenberg Bay
Individual 79	Bw31	Plettenberg Bay
Individual 80	Bw32	Plettenberg Bay
Individual 81	Bw33	Plettenberg Bay
Individual 82	Bw34	Plettenberg Bay
Individual 83	Bw35	Plettenberg Bay
Individual 84	Bw36	Plettenberg Bay
Individual 85	Bw37	Plettenberg Bay
Individual 86	Bw38	Plettenberg Bay
Individual 87	1A	Plettenberg Bay
Individual 88	2A	Plettenberg Bay
Individual 89	3A	Plettenberg Bay
Individual 90	B.E1	Plettenberg Bay
Individual 91	B.edeni01	Plettenberg Bay
Individual 92	B.edeni03	Plettenberg Bay

Individual 93	B.edeni04	Plettenberg Bay
Individual 94	B.edeni05	Plettenberg Bay
Individual 95	B.edeni06	Plettenberg Bay
Individual 96	B.edeni07	Plettenberg Bay
Individual 97	B.edeni08	Plettenberg Bay
Individual 98	B.edeni10	Plettenberg Bay
Individual 99	De Platt	De Platt
Individual 100	Gouritz	Gouritz river mouth
Individual 101	StillBay	Stillbay
Individual 102	Knysna	Buffalo Bay
Individual 103	SFRI2008	Unknown location
Individual 104	Pelican Point	Namibia
Individual 105	Walvis Bay	Namibia
Individual 106	Madagascar	Southern Madagascar

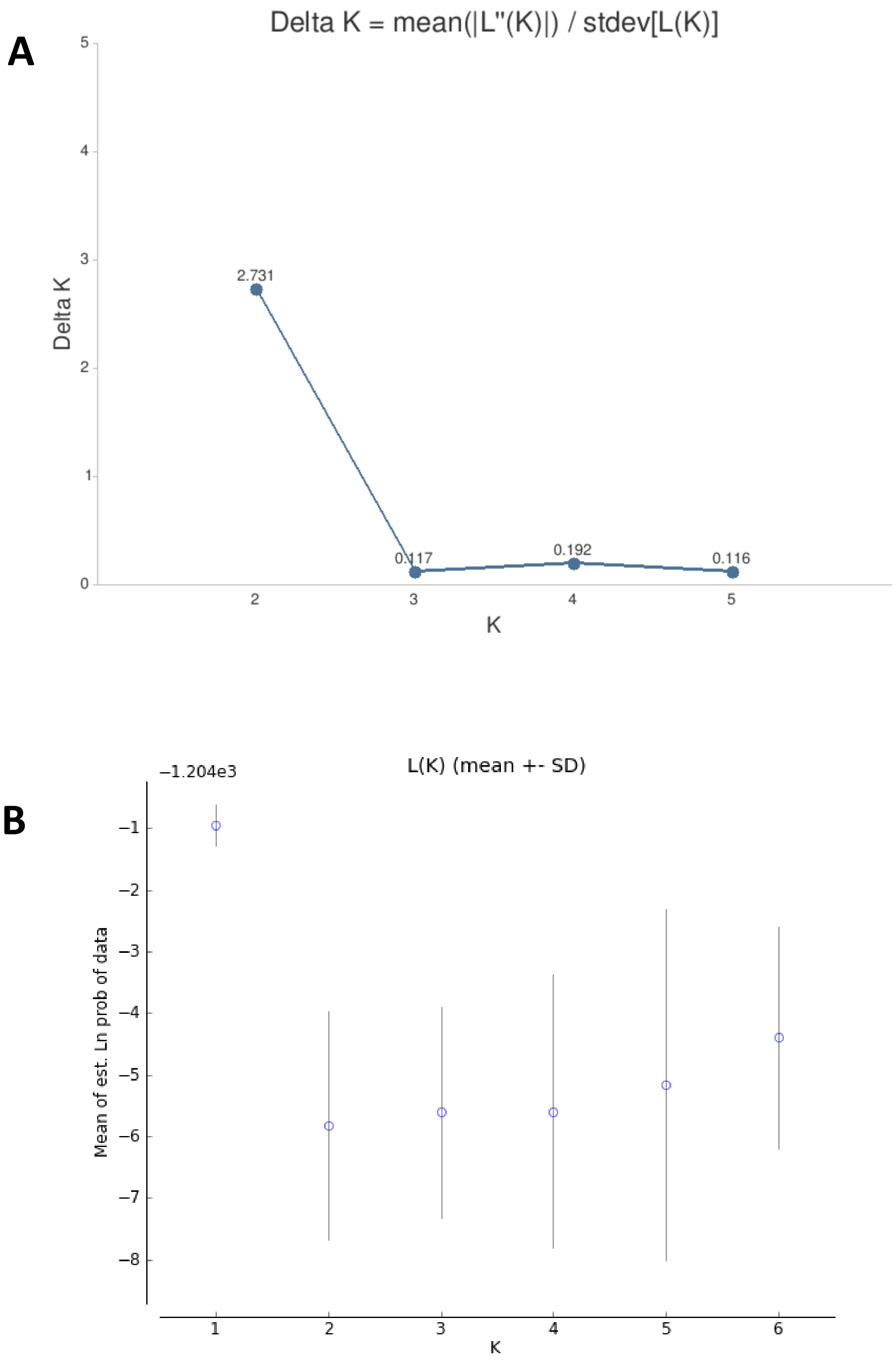


FIGURE S1 Probability of K subpopulations (A) and ln(K) (B) graphs of STRUCTURE run containing South African individuals

CHAPTER 3

Assessing movement patterns of South Africa’s inshore Bryde’s whale (*Balaenoptera edeni brydei*) using photo-identification methods.

Abstract

Despite its year-round residency in South African waters, the South African inshore Bryde's whale population (*Balaenoptera edeni brydei*) is notoriously elusive and thus several ecological knowledge gaps exist for this baleen whale species in the country. Although it is generally accepted that these whales make small scale movements along the coastline of South Africa in pursuit of their main prey sources, their fine scale movement patterns remain unclear. In the present study, photo-identification images of inshore Bryde's whales were collated over 18 years (2004–2022) across several areas on the west and south coasts of South Africa. These images were analyzed to assess the movement patterns of individuals across the different areas as well as formalize a national catalogue for this population. In total, 246 photographs were matched, resulting in 183 uniquely identified individuals comprising the current national catalogue . In total, 14 matches were found across regions, with 2 individuals moving up to 400 km and 600 km, a distance not previously recorded. The results of this study seem to indicate that individuals may show a degree of site-fidelity to specific regions, although far distances can also be covered. This is an important baseline of ecological information that can be further developed and used in ongoing research and monitoring of this vulnerable inshore population.

KEYWORDS:

Ecology, habitat-use, population monitoring, site-fidelity, South African coastal region

3.1 | INTRODUCTION

Photo-identification (photo-ID) of marine mammals, such as cetaceans, makes use of natural markings on individuals which are maintained over periods of time (Hammond et al*.,* 1990). Photo-ID quickly became the method of choice for studying cetaceans, owing to its non-invasive, and relatively inexpensive means to assess individuals within a natural population (Urian et al., 2015; Elliser et al*.,* 2022). This method has been used extensively on several cetacean species to study various population parameters (e.g Calambokidis et al., 2009; Bröker et al*.,* 2020; Athayde et al., 2020; Elliser et al., 2022). As technology developed so did the means and accuracy of photo-ID techniques. Some studies have even begun to propose adaptations of traditional photo-identification methods, such as using more than one identification feature, to be used in the assessment of generally "poorly marked" cetacean species (Elliser et al., 2022). Overall, photo-ID provides a valuable opportunity to assess individuals in a naturally occurring population and investigate parameters such as population abundance, site fidelity, demographic population structure, and movement patterns.

In the South African coastal region, several photo-ID studies have been completed on both commonly occurring large and small cetacean species such as southern right whales (*Eubalaena australis*; e.g. (Best, 2000)), humpback whales (*Megaptera novaengliae* e.g Barendse and Best, 2011), the Indian Ocean humpback dolphin (*Sousa plumbea* e.g. Vermeulen et al., 2017); Heaviside dolphins (*Cephalorhynchus heavisidii;* Elwen et al., 2009) and inshore Bryde's whales (*Balaenoptera edeni brydei*) e,g. Penry, 2010). Each of these studies used one unique morphological feature of the species, such as callosity patterns, fluke patterns, or scars and notches on the dorsal fin, to identify individuals. However, amongst these photo-ID studies, those on the inshore population of Bryde's whale, South Africa's only resident baleen whale, remain most limited. Evident knowledge gaps in this small population arise from a challenge in observing the species owing to their elusive behaviour. It is generally accepted that these whales show a restricted, coastal distribution within South Africa's waters which extends to the continental shelf (Best, 2001). Previous studies using encounter rates demonstrated the seasonal fluctuations in the occurrence of this species along the southern area of the South African coastline (Penry et al*.,* 2011). In addition, other studies indicate that their movements and distribution are directed by the dispersal patterns of their main prey sources (Best,2007; Penry,2010; Penry et al*.,* 2016) including movements along the east coast of South Africa coinciding with the well -known "sardine-run" (Penry et al., 2016), which involves the northward migration of small-pelagic fish.

Despite these broad overviews of the population, a clear understanding of movement patterns and ranges of individuals remains unresolved. Nonetheless, considering these whales reside in the oceanographically diverse South African coastal region, oceanographic factors may impact the movement of individuals between areas. The strong, warm Agulhas current to the east coast of the country differs in temperature, speed, and overall productivity in comparison to the cool Benguela current on the southern African west coast. Although a previous assessment of photo-ID data suggested that individuals' ranges may be limited, data were not abundant enough to make firm conclusions (Penry, 2010). In the present study, I aimed to collate photo-ID data of the inshore Bryde's whale population of South Africa from previous scientific surveys and citizen science platforms to assess movement at the individual scale and formalize the curation of a national catalogue for South Africa building on the work of Penry (2010). Given its small population size and national listing as "Vulnerable" (Penry et al., 2016), improving our understanding of the species' movement patterns and habitat use is vital to develop effective conservation strategies for South Africa's largest, resident baleen whale species, that faces an increasing number of anthropogenic threats.

3.2 | MATERIALS AND METHODS

3.2.1 | Data collection

Photographs of individual inshore Bryde's whales from several areas of the South African coastline were collated for the period of 2004–2022 and merged with the data from 2004–2010 already processed by Penry (2010). For the purpose of this study, a hypothetical divide was made along the coastline as done previously (Penry, 2010), and taking into account the oceanographic differences; the west coast was defined as the South African coast west of Cape Agulhas, whereas the south coast was defined as the coastline between Cape Agulhas to Algoa Bay to the east (Table 1, Figure 1). Photographic data were collected on dedicated surveys by research groups (MRIWU, SeaSearch, DICT)[1] or were contributed by citizen scientists and tour operators (L. Edwards, D. DeBeer, D. Hurwitz). The procedure of obtaining photographs coincided with the biopsy sampling of individuals on dedicated surveys. Once an animal was located, it was approached sideways slowly (1-4 knots) up to 50 m. As the individual surfaced, several photographs were taken, focusing mainly on the dorsal fin. All photographs were accompanied by gross meta-data, including the year and area of the sighting. As not all images had meta-data regarding precise date, photographs were combined per year, and matching was only done across years and areas

TABLE 1 Area and photo-identification data collection period

Area of South African coastline	**Location**	**The time period of the photographic collection**
West coast	False Bay	2004-2021
	Walker Bay	2011-2022
	Gansbaai	2021-2022

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[1] Mammal Research Institute Whale Unit (MRIWU), University of Pretoria

Dyer Island Conservation Trust (DICT)

South coast	Plettenberg Bay	2004-2018
	Algoa Bay	2008-2022

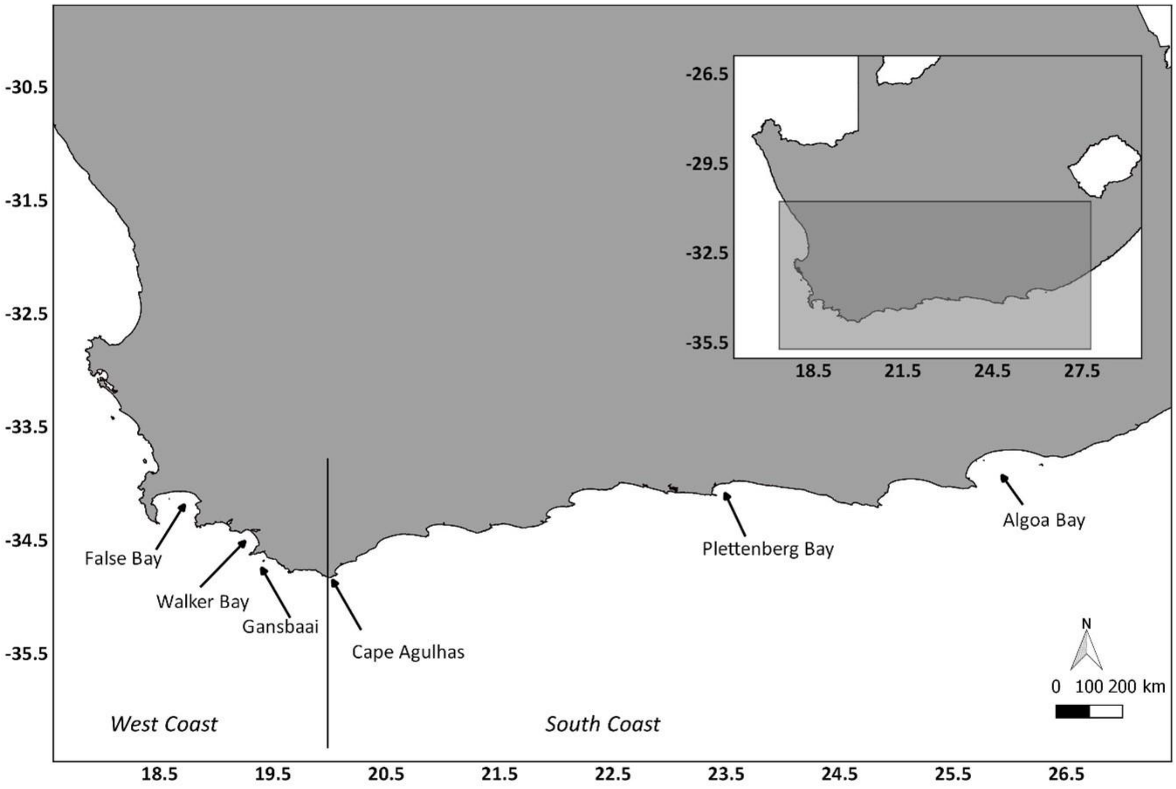

FIGURE 1 Map showing the areas where photo-identification data were collected along the South African coastline. The vertical black line indicates the hypothetical divide between the west and south coasts

3.2.2 | Sorting and assessment of data quality

Photographs were sorted and assessed based on the photograph quality and dorsal fin distinctiveness (see Table 2). First of all, grading photographs were scored on a scale of one to five in terms of quality (Q). Photographs with a Q1–Q3 were deemed unsuitable for photo-identification purposes owing to poor lighting, focus, or angle of the camera to the whale. Photographs were given a Q4 or Q5 score when a clear outline of the entire dorsal fin was visible, and the photograph was at approximately 90 degrees to the whale. Each Q4 or Q5 photograph was then graded for dorsal fin distinctiveness (D). Dorsal fins given a score of D1 were classified as extremely distinctive making the individual easily identifiable (see example in Figure 2). A score of D2 or D3 was assigned to individuals that had one or several notches in the dorsal fin. Individuals were given a D4 score when the dorsal fin shape was sufficiently unique/unusual for identification, but there were no visible notches. Individuals that had no distinctive characteristics on the dorsal fin, (i.e., no scars, notches, or unusual shape) were given a D5 score and excluded from further analyses (see example in Figure 3). Criteria of quality and distinctiveness scoring were adapted from Penry (2010) with slight modifications specifically to the criteria for quality.

TABLE 2 Quality (Q) and distinctiveness (D) criteria of Bryde's whale photo-identification data

	Score	**Criteria**
Photograph quality	Q1	No dorsal fin in the picture Completely out of focus Too far from the animal to get a clear image
	Q2	Water obstructing the dorsal fin and its features
	Q3	Poor lighting/angle/focus on the dorsal fin
	Q4	The entire dorsal fin outline is visible, with moderate lighting/focus
	Q5	Excellent focus and lighting on the entire dorsal fin which fills the frame of the camera. The animal is close and angled approximately 90° to the camera
Dorsal fin distinctiveness	D1	Highly distinctive fin (large notches, part of fin missing, etc.)
	D2	More than one small – medium sized notch in the trailing edge of the dorsal fin.
	D3	One small- medium notch in the trailing edge of the dorsal fin.
	D4	A distinctive shape of dorsal fin (No notches visible)
	D5	Non-distinctive dorsal fin

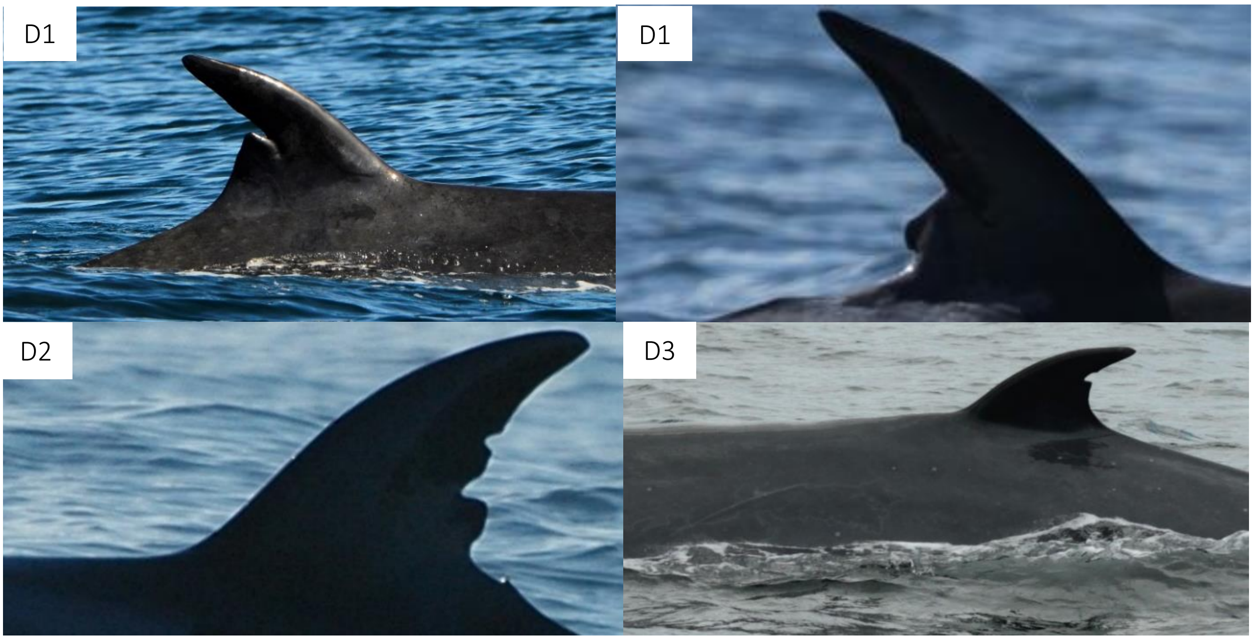

FIGURE 2 Examples of four distinctive (D1-D3) individual Bryde's whales' dorsal fins

FIGURE 3 Examples of four non-distinctive (D5) individual Bryde's whales' dorsal fins

Once dorsal fin distinctiveness was scored, all animals with D1-D4 in Q4 –Q5 photographs were given a photo-identification number which was assigned in chronological order, depending on the date the photo was taken. When possible, photographs of both sides of the dorsal fin were used for each individual. Since Bryde’s whales are largely solitary, individuals were often photographed alone, thus the confidence in knowing both sides of the same individual were obtained was high. Photographs were subsequently cropped to ensure the dorsal fin and its features were easily visible to assist in the matching processes.

3.2.3 | Matching and catalogue curation

All photographs with Q4–Q5 and D1–D4 were organized according to the year of collection and the name of the contributor. Subsequent processing and matching of individual whales were completed using the R package “catRlog” (Keen et al., 2021). This package allows for the organizing and easy visual inspection of photographs for matching purposes. The package creates a platform on which photos in a “catalog” section can be easily compared to all photos stored in the database. On this platform, photographs are positioned next to one another to allow for easy visual comparison. Thereafter, based on the matcher’s visual assessment, the photo can be assigned to one of two categories, “100% match” or “low-confidence match”. Alternatively, if no match is found then the image is assigned to “No match, New whale”. This package then organizes all matching session information in a .csv file for easy referral and further processing. Photo-ID images were matched by eye and several matching sessions were carried out by the author (DP) to increase the accuracy of the results. All obtained matches were reviewed by two additional, experienced researchers (EV, GP) as a final check. If a match was reviewed and there was no disagreement it was accepted as a “100% match”. However, if there was one specific feature that did not seem to match perfectly according to a reviewer it was listed as a “probable match”. If there was more substantial doubt about the initial match, then it was listed as a “low-confidence match”. Once the matching process was completed, the metadata of the individuals was assessed to evaluate the movement patterns and distribution of individual animals. After eliminating duplicate images from the matched individuals, a national catalogue was created by allocating the best photograph of the dorsal fin (from the right- and left-hand side, when possible) for each unique individual, and assigning a unique catalogue number

3.3 | RESULTS

A total of 5,107 photographs were collated and processed from the five different coastal regions including 3,924 from the west coast region and 1,191 from the south coast (Table 3).

TABLE 3 Number of photographs from each coastal region

Area	**Location**	**Number of images collated**
West coast	False Bay	390
	Walker Bay	3400
	Gansbaai	134
South coast	Plettenberg Bay	98
	Algoa Bay	1093

After the quality check, 1,280 images received a score of Q4 or Q5 and were included in the analyses. Subsequently, these images were assessed to ensure the inclusion of only one image (from the right- and left-hand side, when possible) per sighting (i.e., if several good pictures were taken during one surfacing of a Bryde's whale, only the best one was selected for matching purposes). This selection procedure led to 582 photographs of sufficient quality of single sightings to be processed further for individual identification and matching (Figure 4).

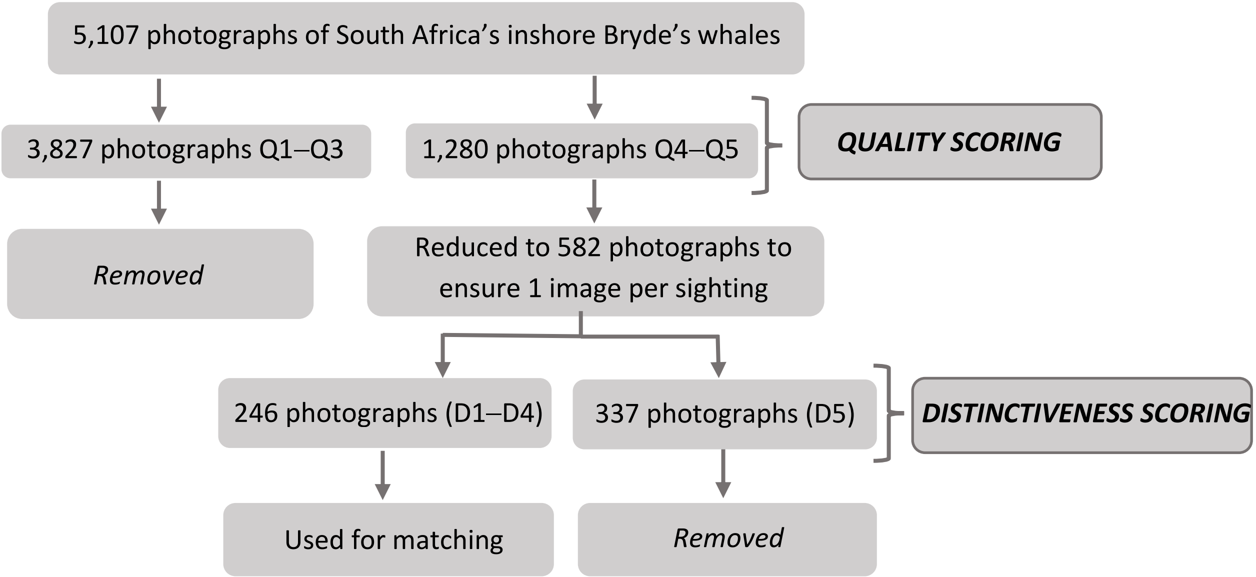

FIGURE 4 Schematic diagram of the data processing to give the final numbers of photographs containing distinctive individuals available for matching

3.3.1 | Distinctive individuals

Of the total of 582 suitable photographs used for analyses, 246 (42%) contained a Bryde's whale dorsal fin which had a distinctiveness score of D1 to D4. The remaining 336 photographs (58%) had non-distinctive dorsal fins (D5) and were removed from further analyses (Figure 4). As meta-data on the precise date was often lacking, the selected 246 images remained organized in folders per area and then per year.

3.3.2 | Matches

Owing to the lack of precise date information (only year was available) from images collated provided by citizen scientists, the initial matching combined within-year sightings in the same area. This resulted in 197 non-unique individuals to be used for matching across years and regions. After matching across years and regions 29 matches of 14 unique individuals could be confirmed. Of these matches, 28 were "100% match" whereas one was considered a "Probable match" of a highly distinctive individual (Figure 5) which was included in further analyses. After matching was completed, the final national catalogue contained 183 unique individuals, of which 7% are D1, 26% are D2, 65% are D3 and 2% are D4. The number of identifiable individuals (D1-D4) varied between regions with the highest number from Algoa Bay (Figure 6).

FIGURE 5 Highly distinctive individual (SAi001) listed as a "Probable match"

3.3.3 | Movement patterns

Of the 14 matched individuals, eight were resighted in the same area across different years whereas six were photographed in different areas (Table 3; Figure 6). Only two individuals were photographed on the both west and south coast. Within the west and south coasts, distances were covered up to a maximum of 150 km and 260 km, respectively. Across the west and south coast, the maximum distance was approximately 600 km (from Algoa Bay to Walker Bay; Figure 6). The timespan over which individuals were resighted ranged from 1 to 14 years (Table 4).

TABLE 4 Matching information of individuals showing the number of times, years, and areas of re-sighting. *AB*= Algoa Bay, *PB*=Plettenberg Bay, *FB*=False Bay, *WB*=Walker Bay and *GB*=Gansbaai

Sighting area	**Location of resighting**	**Individual ID**	**Number of years resighted**	**Years seen**
West and South Coasts	AB, WB	SAi001	3	2007,2009,2021
	PB, FB	SAi053	2	2012,2019
South Coast Only	PB, AB	SAi002	2	2012,2021
	AB	SAi008	2	2018,2021
	AB	SAi009	2	2017,2018
	AB	SAi015	2	2007,2014
	PB, AB	SAi031	3	2011,2021,2022
	AB	SAi039	2	2011,2012
	AB	SAi099	4	2014,2016,2017,2018
	AB	SAi114	2	2016,2017
West Coast only	FB, WB, GB	SAi064	3	2012,2021,2022
	FB	SAi091	2	2013,2015
	FB,GB	SAi095	2	2012,2022
	FB	SAi096	2	2013,2019

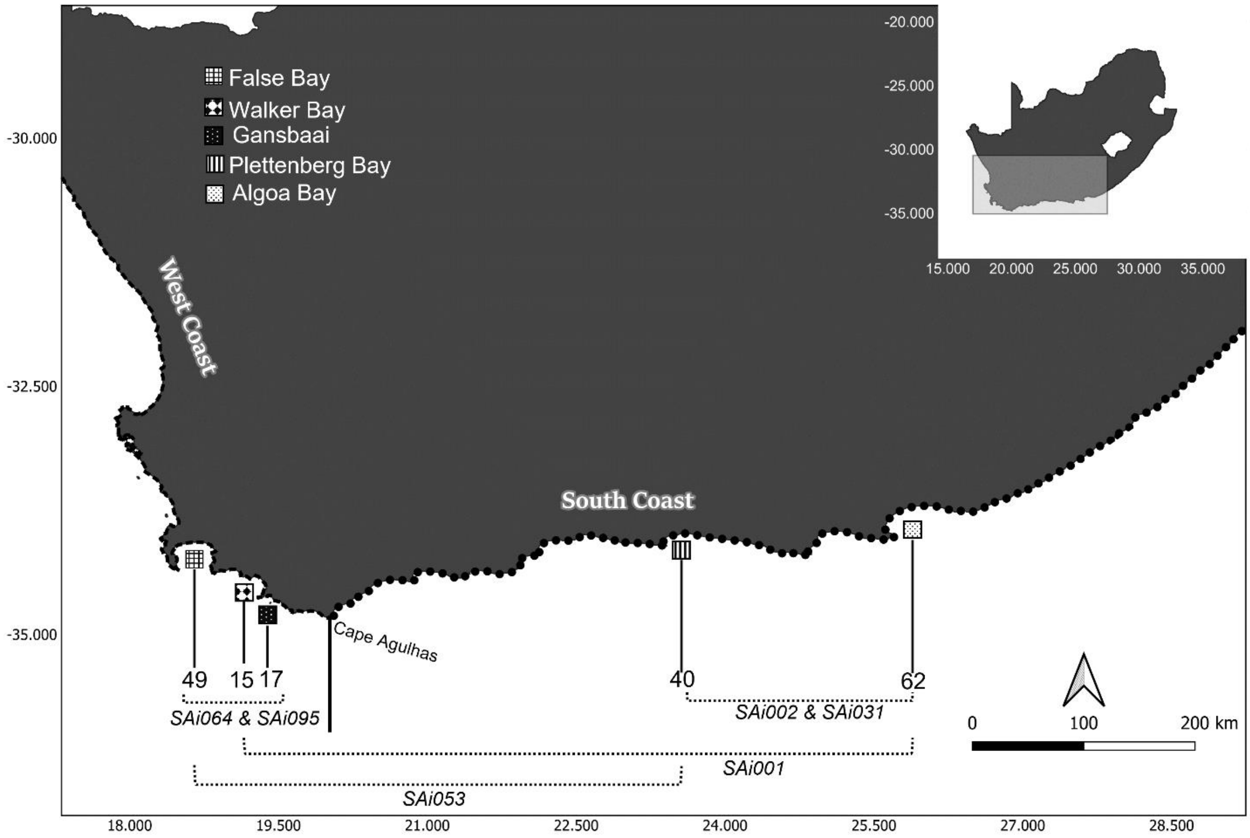

FIGURE 6 Map showing the number and movements of distinctive individuals (D1–D4) between areas on the west and south coasts of South Africa. The first line of numbers reflects the number of distinctive, unique individuals from each area. The dotted line and ID number below indicate the individuals that moved between areas

3.4 | DISCUSSION

This study made use of photo-ID data to assess movement patterns of individual inshore Bryde's whales along the South African coast over a period of 18 years (2004–2022) and to formalize the curation of a national photo-ID catalogue. Given the species' notoriously elusive behaviour the number of usable photographs for the curation of a national catalogue was reduced significantly. Nonetheless, 183 individuals were uniquely identified through distinctive dorsal fins and assigned a national ID which can be used for future monitoring of the population and to better understand their movement. As the available information suggests this population is small (< 1,000 mature individuals, Penry et al., 2016) this study provides a valuable foundation for further investigations into their population dynamics and use of the South African coastline.

In general, the high proportion of non-distinctive dorsal fins is not unexpected for this species and the findings are similar to those in other photo-ID studies on Bryde's whales in Brazil (Athayde et al., 2020) and New Zealand (Tezanos-Pinto et al*.,* 2017) and demonstrate the challenge in using photo-ID methods on Bryde's whales. Consequently, the overall low number of matches in the entire database may reflect this difficulty. Furthermore, their elusive nature makes it difficult to obtain good quality pictures, which complicates the matter further. Nonetheless, some individuals do show clear and unique marks in the dorsal fins which allow for accurate tracking of movement patterns when the animal is resighted. Such individual movement patterns are essential for developing an understanding of the species' habitat use patterns which, in turn, facilitates more accurate conservation planning.

Results of the photo-ID matching show more resighting in the same general area compared to resighting between areas, which could indicate some degree of site fidelity. Nonetheless, a long-distance movement was also recorded, up to 600 km over a span of 14 years. This movement between the west and south coast was previously not detected (Penry, 2010) and now confirms, for the first time, the ability of Bryde's whales to cross the boundary between the different current systems surrounding South Africa. Although this individual (SAi001) was considered a "Probable match", it is one of only three individuals in the catalogue that have no dorsal fin and they are all highly distinguishable from each other. In general, it is believed that Bryde's whale movement patterns are determined by the movements of their main prey species, sardine (*Sardinops sagax*) and anchovy (*Engraulis capensis*) (Best 2007; Penry et al., 2016). Considering that these whales have to forage daily to meet their energetic needs (Best et al., 1984), the highly dispersive nature of their prey is also expected to be reflected in the distribution of Bryde's whales. Sardine and anchovy are characterized by large migratory patterns through autumn and winter which are important biological events that support many marine predators such as Cape Gannets (*Morus capensis)*, African Penguins (*Spheniscus demersus),* and the Bryde's whale (Penry et al*.,* 2016; Coetzee et al., 2018). Although recent studies have identified separate stocks of sardine, individuals from both stocks are known to move across oceanic boundaries in their annual migration (Teske et al., 2021). Previous studies have demonstrated that the seasonal shifts in the movements of Bryde's whales coincide with these annual migrations of fish stocks (Best et al., 1984; Best 2001; Penry et al., 2011, 2016). This relationship between prey species migration and Bryde's whale movement could explain why individuals may cross current systems during feeding aggregations.

The results of this study also revealed that some individuals show a level of long-term site fidelity. This is demonstrated effectively by one individual (SAi015) which was resighted in Algoa Bay in 2007 and again in 2014. Penry (2010) also showed that two different individuals were sighted on six and seven occasions, respectively, in Plettenberg Bay in a five-year period (both within and between years). This study, and previous observations therefore strongly support that individuals tend to return to the same areas, but the motivation for this is not fully understood. The lifespan of a Bryde's whale has been estimated at approximately 50 years (Bannister et al., 1996), making them a long-lived marine mammal. Although it is possible that Bryde's whales have wider ranging movements than is currently known they are predominantly resident on the continental shelf of South Africa. These two ecological traits (long lifespan and residency) of the species, therefore, make resighting over long periods of time more probable.

3.5 | CONCLUSION

The results of this study show that, albeit challenging, photo-ID can be used as a method to track individual movements of inshore Bryde's whales in South Africa. Although some individuals appear to stay within a region (i.e west or south coast), the distances moved by two individuals exceeded those seen in previous studies, with one individual covering approximately 600 km across different ocean regimes. This finding supports a possible wider distributional range, at least for some individuals, than was previously known. This study also consolidated a national catalogue for the species, which now comprises of 183 uniquely marked individuals, and will be useful for ongoing research and monitoring of the population.

These results provide important baseline information on the ecology of South Africa's only resident baleen whale, which is vital for the conservation and management of the species. Since so many ecological aspects of this species remain unknown, any additional information will fill ecological knowledge gaps. An increased and continuous photo-ID effort is therefore highly encouraged to improve our knowledge base of this vulnerable population along our coastline. Alternatives to this method may also be employed to assess the movements of individuals, such as genetic mark-recapture or telemetry to provide more accurate data collection. In addition, future research should further investigate the role of the complex environmental and oceanographic conditions of the South African coastline that may influence the movements and site fidelity of Bryde's whales. This will provide additional support for conservation management.

CHAPTER 4

General discussion and conclusions

4.1 | GENERAL DISCUSSION

The aims of this dissertation were to determine if there is any spatially defined population structure while assessing the level of genetic diversity within South Africa's inshore Bryde's whales. These aims were achieved using both nuclear genetic markers and photo-ID methods. By combining results from these two techniques multiple lines of evidence can be drawn together to assess individuals within the South African inshore population. In addition, mtDNA data were also used to investigate structure and diversity within a broader global context to begin to understand the origins and history of South Africa's Bryde's whales. The overall results of this dissertation indicate that the inshore Bryde's whales form a single population with evidence of low genetic structure between the west and east coast of South Africa.

These results suggest that, although a single population, it is not completely randomly mixing which would indicate complete panmixia (Hauser & Carvalho, 2008). When overlaying photo-ID data of movement patterns of individuals with genetic results, stronger support for this low level of differentiation is demonstrated. Some individuals have shown residency within the same general area of the coastline over several years which was also found in previous photo-ID studies of the population (Penry, 2010). This long-term residency in certain areas lowers the chance of mixing between all individuals within the population, causing the low levels of differentiation that are evident in the population. There were a few instances of uniquely marked individuals crossing boundaries between oceanic systems surrounding South Africa which is a result not recorded in previous studies, which supports the genetic evidence of a single population. However, the ecological reasons for these movement patterns are unclear. In terms of genetic diversity, the inshore population shows patterns of high nuclear diversity but low mtDNA diversity. High nuclear diversity can be an indication of gene flow or a larger N_e of the population than previously thought (Teixeira & Huber, 2021) whereas low mtDNA diversity can be indicative of differences in dispersal ranges between sexes (Vachon et al., 2018). Within the greater southern African region, the inshore population appears to be genetically distinct from individuals from Namibian and Madagascan populations, although a more concerted sampling effort is required to confirm this. On a global scale, evidence shows that South Africa's inshore population makes different connections to oceanic regions in comparison to South Africa's offshore population.

Results suggest that there are three distinct populations between South Africa, Namibia, and Madagascar as outlined previously (Best, 2001). These are preliminary results, however, and are an important indication of the need for greater sampling within the region to begin to fully understand the connectivity between individuals of these three populations. On a global scale, the results provided initial insight into the connectivity between oceanic regions as well as the origins of South Africa's Bryde's whale populations. Two separate connections of offshore and inshore individuals to different oceanic regions could mean distinct origins between these two populations. In addition, the shared haplotype between South African inshore individuals and the East Indian Ocean provides evidence of an ancient connection between these regions. Several studies have been completed that show the genetic distinction of Bryde's whales between oceanic regions (Kanda et al., 2007; Kershaw et al., 2013; Pastene et al., 2015; Wiseman, 2008; Yoshida & Kato, 1999) however when combining data from areas in this study, results support the need for a more representative sample baseline to be able to unravel these connections.

Overall, the genetic, and photo-ID results provide important demographic and ecological insight into the inshore population that contributes significantly to the understanding of South Africa's largest resident baleen whale species, which is imperative in conservation efforts. In addition, a strong baseline of genetic and photo-ID information is now available for the inshore population which can be developed over time, increasing efficiency in monitoring the population. These results also give an initial insight into the connection between South Africa's inshore Bryde's whales within broader contexts (i.e the southern African region and globally). This is key information in deepening the historical origins of this population and in directing future research in assessing the current level of geneflow between South Africa's populations and others found globally.

4.2 | IMPLICATIONS

As one of the most elusive marine mammals, South Africa's inshore Bryde's whales are a challenge to study and many ecological aspects of the population remain unclear. Nonetheless, the results of this dissertation have clarified one of the major questions which are key in conservation planning. These whales form a single population; thus, gene flow is occurring between regions of the coastline which also results in a high nuclear diversity level.

In addition, these results highlight the need to estimate N_e for this population which will inform conservation and management planning for this species in South Africa's coastal waters. On a global scale, these results have implications in beginning to understand the connection between global populations and South Africa, which is an important knowledge gap that urgently needs to be filled. Overall, these results have increased the knowledge and information available on South Africa's only resident baleen whale which will serve as a foundation for further research on population monitoring and conservation.

4.3. | FUTURE WORK

The results of this study have established a baseline genetic database for South Africa's inshore Bryde's whale population. Future work will involve effort in the development of this database, aiming to get samples from a wider region of the South African coast, including from further up both the west and east coasts. This will lend to a more developed database of the genetic identity of the individuals in the population which can be combined with photo-ID data. Such a database would then provide a comprehensive and effective strategy to assess the movements of individuals. Furthermore, the census and effective population size estimates of the inshore Bryde's whales can also be calculated from a more established database, which will inform calculations of prey biomass in-take of Bryde's whales, critical information needed for an ecosystem approach to fisheries management in South Africa. In addition, estimates of N_e are the most informative on investigating the viability of a population from a genetic perspective (Teixeira & Huber, 2021), and will therefore provide critical information on the conservation status of the inshore Bryde's whale population.

In addition to expanding the database for South Africa specifically, a stronger sampling effort of Bryde’s whales within the greater southern African region and globally is urgently needed. Populations in Namibia and Madagascar require further investigation as they appear to be genetically distinct from South Africa’s inshore population. But in addition to this, a more representative sampling of neglected areas on a global scale is also required. This will enable further resolution of where exactly the South African inshore population fits in phylogenetically. Since sampling Bryde’s whales are difficult, future work will include obtaining a few more representative samples while expanding on the genome coverage in analyzing the connection between populations. The wider genome coverage will increase the power of detection of differentiation and phylogenetic analyses and thus account for the lower number of samples potentially used.

CHAPTER 5

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