Population genetics of species on the genera Tursiops and Delphinus within the Gulf of California and along the western coast of Baja California.

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Population genetics of species on the genera *Tursiops* and *Delphinus* within the Gulf of California and along the western coast of Baja California.

By

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Abstract

This present study investigated the evolution of population genetic structure of two closely related cetacean species, bottlenose (*Tursiops truncatus*) and common dolphins (*Delphinus spp.*) within the Gulf of California (GC) and along the West Coast of Baja California. In this study, we found evidence of strong genetic differentiation in both bottlenose and common dolphin populations in the absence of physical barriers. The comparison of the patterns of population genetic differentiation found here for bottlenose and common dolphins supports the hypothesis of local habitat dependence and resource specialization at both the population and putative species level. Fine-geographic scale structure was detected in coastal bottlenose dolphins, which seemed to be strongly associated to the biogeographic subdivision of the Gulf of California and western coast of Baja California. This result suggests that gene flow among bottlenose dolphin coastal populations might be restricted by local dependence on diverse ecological conditions. In contrast, the long-beaked common dolphin genetic structure did not reflect the habitat heterogeneity of the region to the same extent. The difference in foraging specialization between coastal and offshore populations of both bottlenose and common dolphins is reflected in the pattern of genetic structure observed at a broader geographic scale.

Overall, the results support the hypothesis that local habitat dependence promotes population differentiation in the absence of physical boundaries to dispersal in these highly mobile species. This study provides an unusual insight into the conditions that lead to incipient speciation in these groups. Divergence among common dolphin populations appears to be associated with changes in the paleoceanographic conditions of the region to the extent that reciprocal monophyly between the sympatric *D. delphis* and *D. capensis* forms has evolved within the Holocene timeframe.

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Chapter 1

General Introduction

1.1 Evolution of population structure

The evolution of population structure arises when conspecific populations maintain reproductive isolation that may lead to the development of distinct traits. There are a number of barriers to gene flow that can lead to reproductive isolation, such as physical (e.g. rivers, mountain, land bridges), ecological (e.g. resource specialization), behavioral (mating strategies, social structure). Population structure can occur in allopatry, when populations are physically separated; in parapatry, when populations occur in adjacent or overlapped areas and in sympatry, when populations co-habit the same area. These three scenarios can promote different levels or patterns of population structure, from finely structured populations by means of assortative mating and restricted gene flow, to single panmitic populations showing random mating and high levels of gene flow. It is widely accepted, that the marine environment does not confer apparent barriers to dispersal for several marine species, for instance the blue fin tuna, *Thunnus thynnus*, (Block et al. 2001) and the European eel, *Anguilla anguilla*, (Wirth and Bernatchez 2001). However, genetic molecular analyses have revealed that in many pelagic species, with high dispersal capabilities, this generalization is not strict. For instance, in the Pacific Ocean off the coast of Mexico, populations of dolphinfish, *Coryphaena hyppurus*, have been shown to be genetically structured (Rocha-Olivares et al. 2006). The variability in local resources and habitat conditions may promote local niche specialization and result in population differentiation, despite high dispersal capabilities in many marine species (Hoelzel 1998). In cetacean species, resource specialization is evident in cases of differential niche use, which might lead to assortative mating and/or physical separation within local environments. This may then result in divergence or sympatric speciation due to genetic drift within isolated sub-population (Hoelzel 1998). Social structure also has an impact on shaping population structure in many social mammalian species determined by the patterns of grouping or aggregation and dispersal needs and capabilities of both sexes (Sugg et al. 1996, Dobson et al. 2004, Guschanski et al. 2008).

1.1.1 Resource specialization

Resource specialization, such as habitat or local food availability can lead to intraspecific differentiation in cetacean species (Hoelzel 1998). The best known example resource specialization resulting in population genetic differentiation is the killer whale, *Orcinus orca*. In the North Pacific comparisons between fish (resident) and marine mammal (transient) foraging specialists revealed strong genetic differentiation at both mitochondrial and microsatellite level (Hoelzel et al. 1998). In the North Atlantic, three genetically divergent ecotypes can also be distinguished: the fish specialists "resident" and "offshore", and the marine mammal "transient" foraging specialist (Foote et al. 2009).

The spinner dolphin, *Stenella longirostris*, are distributed worldwide and at least four subspecies have been described based on their morphological characteristics, distribution and habitat preferences (Perrin and Gilpatrick 1994). For example, the spinner dolphins around French Polynesia, seem to be isolated from other pelagic populations. These dolphins have insular habitat preference; however, they show high levels of genetic diversity that may be explained by their particular social structure and metapopulation dynamics (Oremus et al. 2007).

Morphological differentiation within the genus *Sotalia* led to the description of five different species of the genus in the 19th century. Among these species, riverine and coastal ecotypes were distinguished (Rice 1998). Further revisions of the taxonomic status of the genus *Sotalia* recognized only one species, *S. fluvitalis*, which include the coastal (*S. fluvitalis guianensis)* and riverine (*S. fluvitalis fluvitalis*) ecotypes as subspecies (Rice 1998). However, multi-locus genetic divergence and phylogenetic patterns, in addition to the morphological and biogeographical patterns, strongly support the recognition of these two *Sotalia* subspecies as full species (Caballero et al. 2007).

1.1.2 Social structure

The pattern of individual aggregation by age and sex, temporality and how they disperse may shape and define different levels of population structure, which is also influenced by the extent of philopatry of one or both genders. For example, in Uganda the mountain gorillas (*Gorilla beringei beringei*) showed differences in the extent of genetic

structure among females and males, it has been suggested that female preference for natal habitat has influenced dispersal decisions, thus population genetic structure is mainly shaped by female dispersal, despite equal dispersal capabilities of both sexes (Guschanski et al. 2008). Fine-scale genetic structure was detected in the Ethiopian wolf (*Canis simensis*), mediated by restricted male gene flow among cohesive groups or packs of related kin (Randall et al. 2010)

Social behavior in marine mammals is basically mediated through mating and foraging locations and strategies (Connor 2002). Mating systems comprises for the way individuals obtain a mate, the number of individuals with which they mate, the time they stay together and the allocation of the parental care. In general, cetaceans are considered polygynous species, (prolonged association of one male with more than one female); where females invest heavily in the offspring and males invest less and afford higher dispersal and access more females, resulting in higher levels of gene flow (Hughes 1998). However, promiscuity has been detected in killer whale, *O. orca;* analyses of individual genotypes of killer whales suggested that mating occur mainly outside the natal pods, but is still highly selective (Pilot et al. 2010). Male-biased dispersal has been observed in several cetacean species; for instance: in sperm whale, *Physeter macrocephalus,* (Lyrholm et al. 1999), dall's porpoise, *Phocenoides dalli* (Escorza-Treviño and Dizon 2000), striped dolphin (*Stenella coeruleoalba)* (Gaspari et al. 2007) and Australian bottlenose dolphin *Tusiops* (Krutzen et al. 2003, Moller and Beheregaray 2004).

Foraging strategies were found to promote sociality, besides the advantage of potentially reducing the predation risk, it benefits foraging performance by cooperative feeding and finding prey in patchy environments such as the ocean. For instance, transient killer whale groups are able to consume other big cetaceans such as baleen and sperm whales (Reeves et al. 2007); and bottlenose dolphins form cooperative groups to pursue and feed on school of fish (Wursig 1986, Barros and Wells 1998). Social cohesion and foraging specialization were suggested as the major factors shaping genetic structure in pilot whales, *Globicephala melas* (Amos et al. 1991) .

In general, population and social structure of the oceanic dolphins are poorly understood. Bottlenose dolphins are known to swim with associates that are not necessarily relatives, in this fission-fusion type of society, groups are not stable through

time as individuals join or leave groups eventually (Connor et al. 2000). Observations in the Ionian Sea revealed similar patterns of association among common dolphins (Bruno et al. 2004). Bottlenose dolphins may also form alliances of few individuals, mainly males (Connor *et al*. 1992), and larger groups of both genders that have shown to highly stable over a long period of time (Lusseau *et al*. 2003). Common and pantropical spotted dolphins *Stenella atenuata* have been shown to segregate by gender and age (Perrin and Reilly 1984, Perrin 2002). Common dolphins in the Atlantic Ocean and English Channel, do not exhibit a matrilineal or kinship based society. Instead they seem to have a fluid social structure, with some segregation by gender (Neumann et al. 2002, Bruno et al. 2004, Viricel et al. 2008). In some cases variations on social structure are suspected to be habitat dependent as observed in spinner dolphins in Hawaii (Karczmarski et al. 2005).

1.2 Gulf of California biogeography overview

The Gulf of California (GC) is well known not only for its oceanographic heterogeneity and high level of biodiversity, but also for the high level of endemism. This indicates that the GC has been isolated for a significant time before bio-diversification led to its characteristic fauna and flora (Briggs 1995, Brusca et al. 2005). Indeed, based on oceanographic features and species distribution, a number of bioregions with singular characteristics have been defined within the gulf (Figure 1).

The GC has been divided into at least four bioregions (described later), according to their oceanographic and ecological features (Santamaría-del Ángel et al. 1994). Examples in several taxa support the hypothesis that the habitat features within the GC have influenced the intra-specific diversification, even in highly mobile animals. Similar patterns of geographic population structure have been detected in several taxa from marine invertebrates such as crab and shrimp (Correa-Sandoval and Rodriguez-Cortes 1998, De la Rosa Veléz et al. 2000), fishes (Walker 1960, Riginos and Nachman 2001), and even in California sea lions (Schramm et al. 2009). The analyses of the genetic structure of California sea lions indicate a significant differentiation of rookeries from the Pacific Ocean and the GC, and among rookeries across the length of the gulf (Schramm et al. 2009). Furthermore, the analyses of metals in California sea lion bones suggest a similar regional pattern, clustering the gulf rookeries into four groups (Szteren 2006).

Morphological and genetic differentiation among Pacific and GC populations has also been found in several fish species (Walker 1960, Bernardi et al. 2003, Pondella et al. 2005).

The GC is a long, narrow, subtropical and semi-enclosed sea, located between the Peninsula of Baja California and the northwest Mexican mainland (22-32º N and 105-107º W); it is 1400 km long, and has a maximum width of 200 km (Roden and Groves 1959). Depth is uneven across the length of the GC, simulating an ascendant gradient towards the mouth of the gulf. The Upper Gulf is the shallowest region (0-50 m), except for the Wagner deep basin which is 200m depth (). The Midriff Islands region is characterized by deep basins that cause abrupt changes in depth in this area. In the central

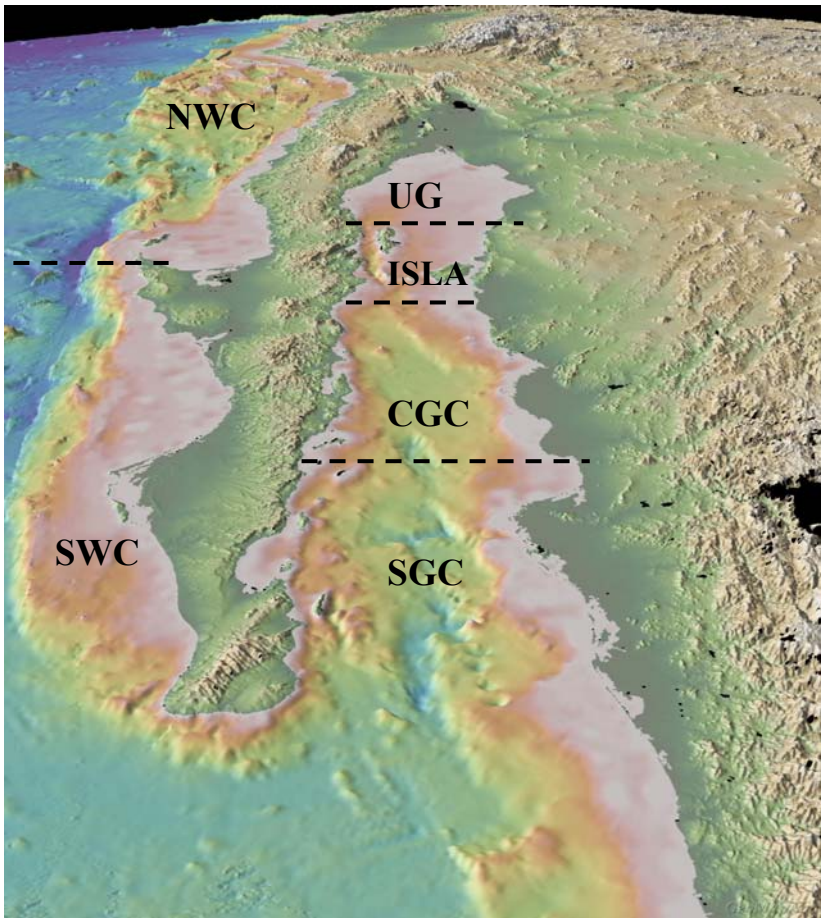

Figure 1 1 Bioregions division within the Gulf of California and western coast of Baja California. NWC: Northwest coast of Baja California, SWC: South west coast of Baja California, UG: Upper gulf, ISLA: Midriff Islands, CGC: Central gulf, SGC: South and mouth of the gulf.

and south region of the GC depth reaches the 3000m depth at the center of the gulf () (Alvarez-Borrego 2002).

In general, water circulation of the GC is characterized by an inflow of deep water from the Pacific Ocean and an outflow of surface water although surface circulation is variable and complex (Castro *et al*. 1994, Mascarenhas *et al*. 2004). There appear to be four main processes that advect nutrients to the photic zone and contribute to generate one of the highest levels of primary production in any ocean worldwide. These are, wind-driven mixing and coastal upwelling (primarily along mainland coast), tidal mixing and turbulence in the Midriff Islands region and thermohaline circulation that moves intermediate waters into mixed layer and coastal trapped waves (Douglas *et al* 2007). Mesoscale gyres and jets are also involved in nutrient transport across the GC (Glaxiola-Castro *et al*. 1999). The coast along the peninsula is mostly rocky shores with some scattered sandy stretches and a narrow shelf with no drainage from rivers. The mainland shores, on the other hand, are characterized by long sand beaches, large costal lagoons, open muddy bays, and a wide continental shelf with large supplies of freshwater (Lluch-Cota *et al.* 2007).

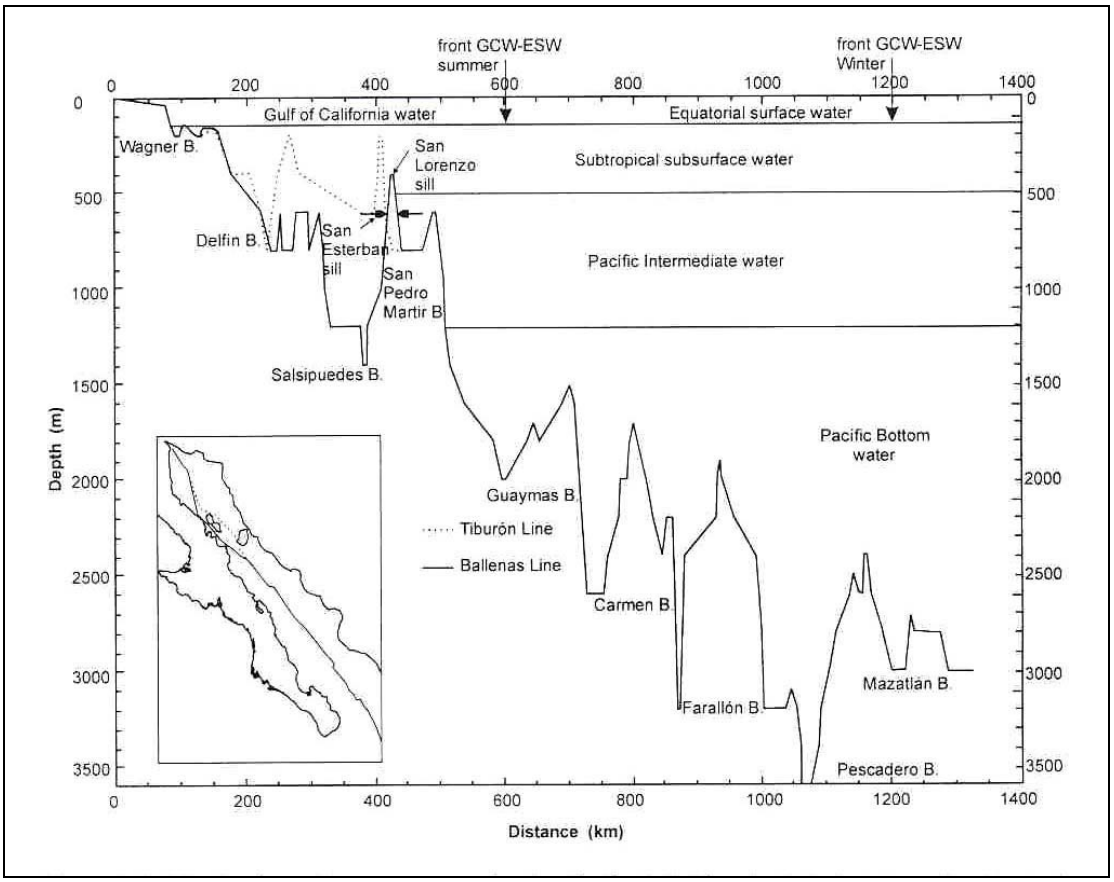

Figure 1. 2 Depth profile a cross the length of the Gulf of California (from Álvarez-Borrego 2002).

The GC climate is influenced by the eastern tropical Pacific Ocean (ETP). The differential warming of the ocean and land and seasonal interplay in atmospheric circulation between the tropics and mid-latitudes result in a monsoon climate. The GC monsoon has a strong variation of winds, sea surface temperature (SST) and rainfall (Bordoni et al. 2004). Winds in the Gulf are variable; in the offshore regions northwesterly winds prevail in winter (November to May) causing mixing and upwelling that enhance nutrient supply and high primary productivity (Alvarez-Borrego and Lara-Lara 1991). Because of winter winds, the GC is an evaporative basin with annual evaporation exceeding precipitation (Beron-Vera and Ripa 2002), being up to 3m/yr in the northern region (Bray 1988). Evaporation in the northern region leads to the formation of Gulf Water, which then sinks and flows south (Bray 1988). Prevailing winds during summer and autumn are mostly southeasterly, but are more diffuse than other seasons, causing the primary productivity to decline, mainly in the peninsular margin

(Alvarez-Borrego and Lara-Lara 1991, Douglas et al. 2007)). During summer SST exceeds 29ºC due to increased insolation and the introduction of tropical Pacific surface waters via the Mexican Counter Current; by mid-summer a thick layer (up to 150 m) of warm water (>28 ºC) extends along the central and southern region of the Gulf. This generates a deep thermocline, which delays the vertical advection of nutrients (Douglas et al. 2007).

Waters with low oxygen and high nutrient concentration are very shallow and it takes relatively little energy to bring these nutrients to the euphotic zone (Alvarez-Borrego and Lara-Lara 1991). Upwelling occurs on the east coast of the GC during winter and spring with northwesterly winds, and on the west coast of the GC during summer with southwesterly winds.

Tidal mixing is particularly strong in the northern region and inner regions of the gulf, particularly around the Midriff Islands. Compared with the Pacific coast of Baja, the GC has warmer surface temperatures from April to September, but comparable during the remaining months (Alvarez-Borrego and Lara-Lara 1991).

Surface nutrient concentrations tend to increase from the mouth to the north part of the gulf; in the south region of the gulf the nutrient distribution and concentration is more like that of the open ocean. The highest surface concentrations of nitrate and silica have been found in the Canal de Ballenas (13 μM and 29 μM, respectively; (Alvarez-Borrego and Lara-Lara 1991). Upwelling areas in the gulf have some of the highest surface concentrations of nutrients in any of the oceans of the world (Alvarez-Borrego et al. 1978)

Across the GC an east-west productivity gradient persists year round in the central and southern regions, where pigment concentrations measured on the eastern side are two to three times higher than on the western margin, except in summer (Douglas et al. 2007). There is a north-south productivity gradient in the central portion and the western side of the gulf with the highest values in the northern region and Midriff Islands (Douglas et al. 2007).

In terms of the distribution of primary productivity, the GC has been divided into four distinct regions: the Upper Gulf, Midriff Islands-Canal de Ballenas, Central Gulf and Mouth or South Gulf (Roden and Emilson 1979). The Upper Gulf (between the mouth of

the Colorado River and the Midriff Islands), is characterized by strong tidal currents and convective overturn during winter while the Central Gulf is a transitional region between the Upper Gulf and the South Gulf (Lluch-Belda et al. 2003).

The Pacific coast of the Peninsula de Baja California, western coast of Baja California (WCBC), is influenced by the California Current System (CCS), which is the eastern boundary gyre of the North Pacific and a large transitional area. The CCS includes the California Current (CC), which flows southward, and the California Undercurrent (CUC), which has a surface flow northward along the coast of Baja California and Southern California. However, during upwelling season (normally April-September) the CC covers the CUC, resulting in a singular southward flow (Soto-Mardones et al. 2004). Salinity and sea surface temperature is higher in the gulf than in the WCBC, which is influenced by the CC. The WCBC is dominated by the formation of complex dynamic structures such as eddies and meanders that have a strong influence on several biological processes; such structures are associated with high productivity areas (Aguirre-Hernandez et al. 2004). The region around Bahía Vizcaíno is characterized by the formation of anticyclonic eddies, which are likely to be driven by the CC flow trend to follow the coast (Soto-Mardones et al. 2004). However, off Punta Eugenia eddies tend to rotate cyclonically as the CUC along the coast have the propensity to reverse its flow direction. Thus, this area is well known as a transition region (Lluch-Belda et al. 2003, Soto-Mardones et al. 2004).

1.3 Conservation issues within the study area

The GC is globally recognized as a priority area for conservation; therefore a precise knowledge of its biodiversity is needed to achieve conservation goals (Olson and Dinerstein 2002). Effective conservation depends on accurate information about stock boundaries, abundance and habitat requirements. Moreover, the distinction of demographically isolated units is the stepping stone for management and conservation actions. In cetacean species the definition of demographic isolated units has been challenged by the extent of intraspecific polymorphism and in some cases the complexity of their taxonomic status.

The bottlenose and common dolphin are widely distributed in Mexican waters; being the most abundant small cetaceans within the GC. However, our knowledge of these species remains scarce. Overall, morphological and preliminary genetic analyses suggest that the geographic genetic structure of the bottlenose and common dolphin is related to their local habitat preferences, as observed elsewhere in the world (Natoli et al. 2004, Natoli et al. 2005, Natoli et al. 2006, Moller et al. 2007, Bilgmann et al. 2008, Rosel et al. 2009, Wiszniewski et al. 2010). The GC represents a unique scenario, given its particular characteristics, to test the hypothesis of local adaptation and to asses the extent of genetic structure in these two species that play a key role as upper-level predators in the GC and western coast of Baja California. Given the unique habitat diversity found within the gulf there is likely to be fine-scale stock structure in these species as observed elsewhere in the world. This study will incorporate molecular genetic and ecological data to address this hypothesis and to estimate the level of genetic structure in two close phylogenetically related cetacean species. Thus, these results, along with other species zoogeographic patterns will lead to a better understanding of the evolutionary forces that are taking place in this exceptional ecosystem.

In addition to international treaties, Mexican law provides special protection to free-ranging dolphin populations. Despite the designation of at least 16 Natural Protected Areas in the region, serious threats to marine fauna remain; dolphins continue to be killed in fisheries by-catch (e.g. common dolphins in the tuna fishery) and by destruction of the habitat, such as the development of resorts and marinas. Moreover, future live capture of dolphins for public display would be contingent on population assessments conducted by scientific institutions. Therefore, management authorities need to better understand the structure, dynamics and vulnerability of dolphin populations.

This study consequently will have an immediate impact on the conservation and management of these delphinid species by providing management data to the Mexican federal authorities. Hence they can effectively create, implement and enforce official regulations for the protection of dolphins and their habitat in the country. The present study also will have important long-term impact through the identification of necessary boundaries for protective areas, and by facilitating our understanding of the processes that lead to population structure in these species, and in the role of critical habitat.

1.4 Aims and hypotheses of the study

This study was designed to evaluate the extent of population structure of the cetacean species on the genera *Tursiops* and *Delphinus* inhabiting within the Gulf of California and western coast of Baja California. The high variety of habitats the found in the GC and adjacent waters represent an exceptional scenario to test the hypothesis that ecological complexity leads to local habitat dependence and population differentiation in these highly mobile species. This study tested the hypothesis that populations of bottlenose and common dolphin that inhabit within the Gulf of California and western coast of Baja California, were genetically structured resembling the habitat partitioning observed in the study area. Of specific interest in this study was to estimate the extent of genetic differentiation between ecotypes, coastal and offshore, of the genus *Tursiops*, and long and short-beaked forms of the genus *Delphinus*. Overall this study aimed to pool total evidence: morphological and ecological, from independent studies, and genetic to contribute to our understanding of the general evolutionary processes that are responsible for the high levels biodiversity held in the GC and similar environments. In addition, the results of this study may have an immediate impact towards the encouragement of conservation actions by promoting the identification of management stocks defined by the estimates genetic variation. to better understand the extent and evolution of population differentiation observed in these closely related species.

1.4.1 Particular objectives and hypotheses

Chapter 2

Objective: To evaluate the levels of genetic differentiation between coastal and offshore population and among sampled populations within the Gulf of California and western coast of Baja California.

Hypotheses:

- The bottlenose dolphin population genetic structure resembles the pattern of habitat subdivision.
- Little or restricted gene flow is expected to occur between Gulf of California and Pacific Ocean populations.

- In smaller geographic scale, high levels of population differentiation are expected among populations inhabiting the distinct bioregions within the gulf and the Pacific Ocean.

Chapter 3

Objective: To evaluate the levels of genetic population structure among long-beaked common dolphin population that inhabit within the Gulf of California and western coast of Baja California.

Hypothesis:

- The long-beaked common dolphin normally occurs in large groups year round within the Gulf of California. Thus little or no gene flow is expected between the gulf and western coast of Baja California long-beaked common dolphin populations.

Chapter 4

Objectives:

- To estimate the extent of genetic differentiation and evolutionary divergence of the two Pacific putative species of common dolphins in the Pacific Ocean.
- To investigate the molecular phylogenetic relationships between the two Pacific short and long-beaked forms.
- To test the hypothesis that the occurrence of long-beaked forms everywhere is a result of local diversification.

Hypothesis:

- The long and short-beaked common dolphin forms are genetically distinct at the species level.
- Local habitat changes promoted the diversification of the long-beaked common dolphin in the Pacific Ocean.

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Chapter 2

Population genetic structure of the bottlenose dolphin, *Tursiops truncatus,* within the Gulf of California and western coast of Baja California, Mexico.

2.1 Abstract

The Gulf of California (GC) is a semi-closed sea characterized by a wide spectrum of habitats as well as high level of endemism, intra-specific differentiation, and biodiversity. These characteristics have led to the definition of this sea as a bioregion that is distinct from the Pacific Ocean (PO) and now considered a priority area for conservation at an international scale. Therefore an accurate knowledge of its biodiversity and the evolutionary mechanisms taking place in the region is needed to achieve conservation aims. The bottlenose dolphin, *Tursiops truncatus,* is one of the most common cetacean species in the Eastern Tropical Pacific and Gulf of California. Nonetheless, according to the IUCN-Red List the knowledge needed to evaluate their vulnerability and implement effective management strategies is inadequate. It has been suggested that the GC bottlenose dolphins may exhibit a complex pattern of genetic structure, given the evidence of local habitat dependence observed in this species elsewhere. The objective of this study was to better understand the evolutionary processes of population differentiation in bottlenose dolphins. It was hypothesized that the ecological complexity of the GC would lead to local habitat dependence and genetic differentiation. The genetic variation of bottlenose dolphin in the GC and along the West Coast of Baja California was investigated at a fine geographic scale using molecular genetic markers. The results suggest a strong genetic differentiation between coastal and offshore ecotypes, and among coastal bottlenose dolphin populations from the northern gulf, mainland and North West coast of Baja California, for both mtDNA and microsatellite markers. The pattern of fine-scale genetic structure, similar to that seen for this species in other regions, reinforces our understanding that habitat specialization is an important driver in the evolution of population structure in the bottlenose dolphin. This study provides valuable knowledge of bottlenose dolphin genetic diversity, which can ultimately encourage effective conservation both through the identification of local populations in need of separate management, as well as the identification of general processes that may explain population structure in similar environments.

2.2 Introduction

In marine environments, where geographic boundaries to gene flow are not always conspicuous, the definition of distinct populations is challenging, especially for highly mobile animals, such as cetaceans. Cetacean species with a wide distribution and large home range may commonly show a certain degree of population differentiation among putative sympatric populations and with no clear correspondence to geographical barriers or distance (Hoelzel 1998, 2002). Intraspecific differences in habitat use, in particular among small cetacean species, have resulted in population differentiation of phenotypic and genetic traits (Hoelzel 2002). There are still no definitive answers on how ecological forces can drive intra-specific differentiation, but examples are common among delphinid species.

A well known example is the killer whale, *Orcinus orca*, in the Eastern North Pacific. Two foraging specialists have been distinguished, the "transient" populations prey preferably on marine mammals, and the "residents" primarily on fish (Bigg et al. 1990). The geographical ranges of these two specialists overlap; however, analyses of the mtDNA control region and microsatellite data revealed the genetic differentiation between these two populations (Hoelzel et al. 1998a).

The eastern tropical Pacific Ocean (ETP) pan-tropical spotted dolphin (*Stenella attenuata*) is subdivided into two subspecies, the coastal spotted dolphin (*S. attenuata graffmani*) and the offshore spotted dolphin (*S. a. attenuata*), based on morphological data (Perrin 1984). Recent genetic analyses, based on the mitochondrial DNA (mtDNA) control region and microsatellite data, were consistent in showing differentiation between coastal and offshore forms and among coastal population along the ETP coast (Escorza-Treviño et al. 2005).

The spinner dolphin, *Stenella longirostris*, also represents a challenging species. This species has a worldwide distribution and displays high levels of intraspecific differentiation, which had led to the description of at least four subspecies based on their morphological characteristics, distribution and habitat preferences (Perrin and Gilpatrick 1994). Intraspecific differentiation is evident in insular spinner dolphins around French

Polynesia, which seem to be isolated from other pelagic populations (Oremus et al. 2007).

Morphological differentiation within the genus *Sotalia* led to the distinction of riverine and coastal ecotypes (Rice 1998). Further revisions of the taxonomic status of *Sotalia* recognized only one species, *S. fluviatilis*, which include the two coastal and riverine ecotypes as subspecies *S. fluviatilis fluviatilis* (riverine subspecies) and *S. fluviatilis guianensis* (coastal subspecies, Rice 1998). However, multi-loci genetic divergence and phylogenetic patterns, in addition to the morphological and biogeographical patterns, strongly support the recognition of these two *Sotalia* subspecies as full species (Caballero et al. 2007).

However, in most of the previous examples it remains uncertain whether intraspecific differences indicate that these populations are actually on separate evolutionary trajectories or whether they represent the ecological plasticity of these widely distributed species, as suggested for the bottlenose dolphin (Curry and Smith 1997).

Historically the genus *Tursiops* has been the most taxonomically controversial genus among delphinid cetaceans. It exhibits high levels of phenotypic and genotypic polymorphisms resulting in at least 20 nominal species having been described, but only two full species are currently distinguished based on morphological and genetic evidence (Wang et al. 1999). They are *Tursiops truncatus* with a worldwide distribution and *T. aduncus* with a limited distribution in the Indo-Pacific, China and South Africa (Rice 1998).

However, recent molecular genetic analyses suggest that the South African and Asian "*aduncus*" forms represent two distinct species (Natoli et al. 2004). In addition, the extent of phenotypic and genetic differentiation observed in populations of the genus *Tursiops* has again suggested the possible existence of distinct species or subspecies. For instance, the bottlenose dolphin from the Black Sea, *T. truncatus ponticus*, is morphologically and genetically differentiated from their Mediterranean and Atlantic Ocean conspecifics (Natoli et al. 2005, Viaud-Martínez et al. 2008). Evidence from cytochrome b and control region mtDNA together with microsatellite DNA data, also

showed genealogically distinct and reciprocally monophyletic populations suggesting a distinct species of bottlenose dolphin in Southern Australia (Möller et al. 2008).

The bottlenose dolphin also shows high levels of intraspecific variability at a regional scale. For instance, off the coast of Australia highly significant differences were found among bottlenose dolphin populations over distances of 400 km or shorter. Coastal populations off New Zealand also show a significant genetic structure over a small geographic distance (Tezanos-Pinto et al. 2009). Within the Gulf of Mexico, mitochondrial and microsatellite data revealed genetically distinct offshore and inshore populations of bottlenose dolphins (Rosel et al. 2009). In the Northern Bahamas, Parsons et al. (2006) found significant differentiation and low gene flow among bottlenose dolphins from three geographically close sites. The bottlenose dolphins inhabiting the Mediterranean Sea, Black Sea and Eastern-north Atlantic Ocean have also been shown to be genetically differentiated (Natoli et al. 2005).

In the Gulf of California (GC), bottlenose dolphins have also shown evidence of phenotypic, ecological (habitat and prey preferences) and genetic differentiation, which supports the recognition of "coastal" and "offshore" ecotypes as demographically significant population units (Segura et al. 2006). Such a distinction is also true in the North Atlantic basin (Hoelzel et al. 1998b). Moreover, analysis of skull measurements of specimens collected across the length of the GC provides phenotypic evidence for the subdivision of the GC bottlenose dolphin in at least four groups, (Figure 2. 1) (Vidal - Hernandez 1993). Similarly, analyses of the mtDNA control region suggest that more than two population stocks may be present within the GC (Segura et al. 2006). The GC provides a unique opportunity to study the mechanisms shaping any such population differentiation, given its oceanographic heterogeneity and high level of biodiversity (Briggs 1995, Brusca et al. 2005). Indeed, a number of bioregions within the GC and the Pacific Ocean (PO) have been defined based on oceanographic features (Santamaría-del Ángel et al. 1994) and species distribution (Walker 1960, Santamaría-del Ángel et al. 1994, Stepien et al. 2001, Bernardi et al. 2003). Similar patterns of genetic structure within the GC have also been detected in several taxa of marine invertebrates (Correa-Sandoval and Carvacho 1992, De la Rosa Veléz et al. 2000), fish (Riginos and Nachman

2001, Sandoval-Castillo et al. 2004, Lin et al. 2009), and the California sea lion (Schramm et al. 2009).

Ecological factors have been proposed to promote the evolution of fine-scale population structure (non random distribution of genotypes within one basin) in the Western North Atlantic and Gulf of Mexico bottlenose dolphin populations (Rosel et al. 2009). This species is also known to exhibit high dispersal capabilities (Wells et al. 1999), however, this does not seem to prevent the development of fine intra-population subdivision. Their complex social structure, which shows different levels of site fidelity, individual association patterns, and the development of diverse foraging strategies, has been documented in a number of populations; e.g. in Sarasota Bay; (Irvine et al. 1981, Owen et al. 2002); Australia; (Chilvers and Corkeron 2001); Bahamas (Rossbach and Herzing 1999); and Scotland; (Lusseau 2005). In addition, the indications of gene flow among bottlenose dolphin populations suggests that they are not closed demographic units (Connor et al. 2001, Krutzen et al. 2004, Sellas et al. 2005, Moller et al. 2006). Demographic studies of bottlenose dolphin in the GC are limited. However, they have shown a certain level of residency, mostly along coastal areas (e.g. in Bahía Kino, Sonora; (Ballance 1990); Bahía de La Paz, Baja California Sur; (Rojo-Arreola et al. 2001 and Salinas-Zacarías and Aureoles-Gamboa 2002); Bahía Santa María, Sinaloa; (Reza-García, 2001); and Bahía Banderas, Nayarit- Jalisco; (Rodríguez Vázquez 2008).

Within the GC there appears to be seasonal variation in bottlenose dolphin movement. Throughout the summer and autumn months, bottlenose dolphins have been observed along the entire length of the GC (Mangels and Gerrodette 1994), while Silber *et al.* (1994) observed that bottlenose dolphins in the upper gulf are present year-round. A recent study suggested that bottlenose dolphins also show a seasonal variation in occurrence along the length of the gulf associated with jumbo squid (*Dosidiscus gigas*) abundance (Díaz-Gamboa 2009). These studies suggest habitat dependent movement of inshore populations of bottlenose dolphins and prey dependent movement of offshore populations of bottlenose dolphins.

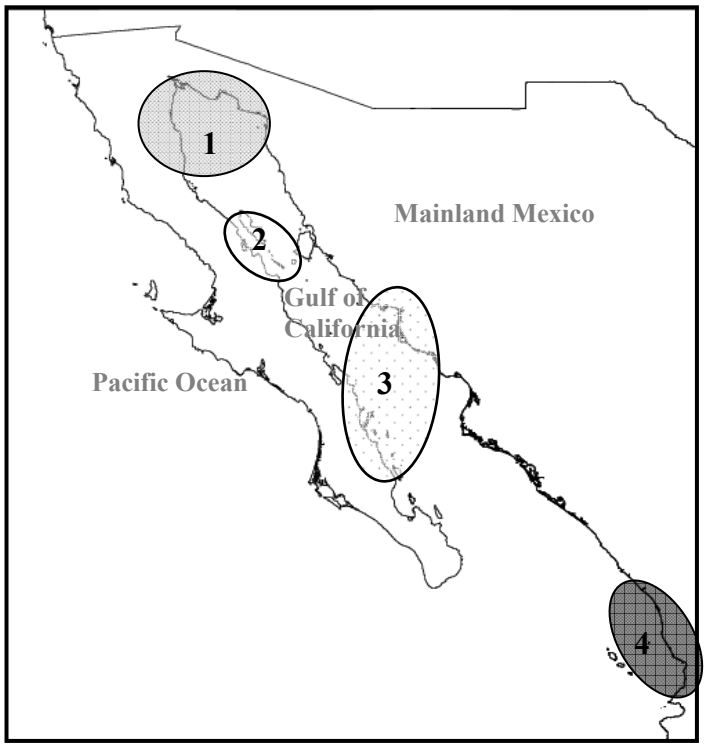

Figure 2. 1 Population subdivision based on skull measurements; 1) Upper Gulf, 2) the Midriff Islands, 3) the Central Gulf (Peninsula side and mainland) and 4) the mouth of the GC along the coast of Nayarit-Jalisco (from Vidal-Hernández 1993)

The bottlenose dolphin shows strong population structure across its worldwide distribution, even where habitat heterogeneity is not as remarkable as within the GC. This study assessed the genetic differentiation at the mtDNA control region and eight microsatellite loci within a fine geographic scale (amongst regions separated by few hundreds of Km); of contiguous bottlenose dolphin populations across a region that shows a high level of habitat heterogeneity. The aim of this study was to test the hypothesis that habitat complexity drives the evolution of the genetic structure of bottlenose dolphins within the GC and the western coast of the Baja California peninsula (WCBC). Thus, the bottlenose dolphin population structure was expected to resemble the pattern of subdivision of the gulf in at least four distinct bioregions: the upper gulf, midriff islands, central and southern gulf; and two regions along the WCBC, the north and south regions delimited by a transition area off Punta Eugenia.

2.3 Methods

2.3.1 Sample collection

Skin biopsy samples were collected from different regions within the Gulf of California and along the western coast of Baja California and South California Bight (n=233). For each locality, surveys were conducted from a small fibreglass boat fitted with an outboard engine. Boats were hired with the local fishermen owners who were experienced with local navigation. The darting system was used to collect skin biopsy samples, which employs a crossbow to deploy a lightweight dart that terminates with a foam stopper and a stainless steel biopsy tip (Palsboll et al. 1991). The biopsy tip is a hollow cylinder that has an incision and a "barb" that retains the tissue. The tip length is designed to penetrate no more than 1.5cm of skin and blubber. Biopsy sampling attempts are directed to the dorsal-lateral region of the back, posterior to the dorsal fin. This sampling method is well-established for small cetacean species and has been extensively used for a number of cetacean species (Barrett-Lennard et al. 1996, Hoelzel et al. 1998b, Krutzen et al. 2002, Natoli et al. 2005, Segura et al. 2006). After the floating dart is recovered, the sample is taken out of the tip. The tip was then sterilized before being used again by rinsing the tip with hydrogen peroxide following by two washes of ethanol and flamed. The skin samples were kept in an ice bucket and later stored in salt/DMSO.

Additional bone and tooth samples (n= 27) were obtained from stranded dolphins collected along the study area and held in three different Osteological Collections (Biology Institute of the National University of Mexico-IBUNAM, School of Science of the National University of Mexico-FC-UNAM, and Centre of research on feeding and development-CIAD-Guaymas). Unfortunately, the poor quality and quantity of DNA only allowed the amplification of nine of these samples. Twenty samples were also obtained from captive dolphins which were originally captured along the coast of Sinaloa (Vallarta Adventures and Dolphin Discovery).

The total number samples used in this study and their distribution for each sampling region are summarized in Table 2. 1 and Figure 2. 1.

2.3.2 DNA extraction and purification

DNA was extracted from biopsies following the phenol-chloroform or salt saturation protocols described by Sambrook *et al*. (2001) and Aljanabi and Martínez (1997), respectively. Bone and tooth samples were processed in an ancient DNA facility in order to prevent cross contamination. DNA from bone and tooth samples was extracted by drilling the solid tissue down to a powder. In preparation for drilling, samples were treated with 10% bleach solution to remove any contaminating DNA that may have collected on the outer surface and rinsed with deionised water. The powder drilled from the outer layer was discarded. The rest of the powder was collected in tubes with 3mL of digestion buffer (0.425 M EDTA pH 8, 0.5% Sodium dodecyl sulphate, 0.05 M tris pH 8.5) and 0.5 mg/mL Proteinase K. The samples were incubated in a rotator overnight at 55 ºC. DNA was then extracted following the spin purification columns purification protocol (QIAGEN, UK).

Table 2. 1 Sample localities and acronyms used in this study and sample sizes

Locality	Acronym	N
North Gulf of California Coastal	NGC	38
South Gulf of California Offshore	SGC	109
Mainland Mexico Coastal	Mainland	32
South western coast of Baja California offshore	SW	32
North western coast of Baja California Coastal	NW	51

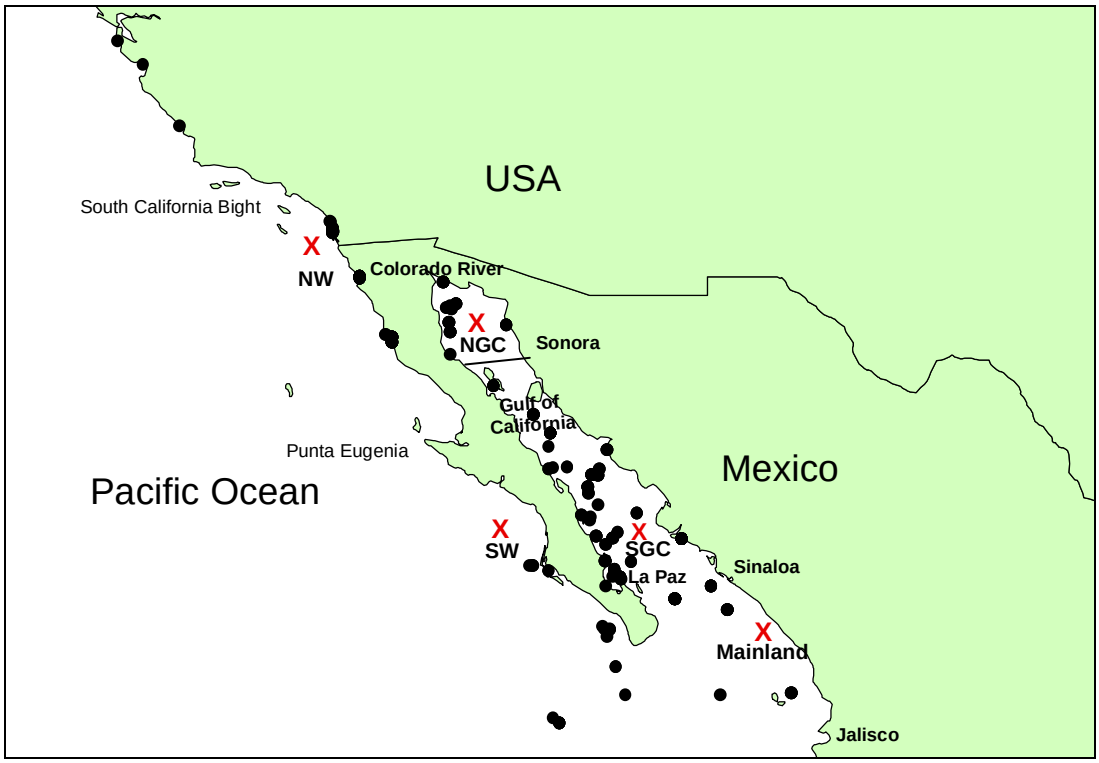

Figure 2.2 Geographic distribution of the sample set. Five sample localities marked with an X, circles might represent more than one dolphin sampled in the same geographic position.

2.3.3 Mitochondrial DNA (mtDNA) analyses

Sequence fragments of the mtDNA control region, 480 base pairs (bp), tRNA proline end, were amplified for 167 samples. Thirty-five new haplotypes were identified among these individual samples and pooled with 32 mtDNA control region haplotypes derived from 96 samples from a previous study conducted in the same geographical region (Segura et al. 2006); Genebank accession numbers DQ105702-DQ105733, referred to as TTGC1-32 herein). The PCRs were performed in 25µL volumes consisting of 10mM Tris-HCl, 50mM KCl, 2.5mM $MgCl_2$, 0.25mM each dNTP, 1.2µM each primer: L15812 (TRO): 5' CCT CCC TAA GAC TCA AGG AAG 3' (Escorza-Treviño et al. 2005) and H16343 (D): 5' CCT GAA GTA AGA ACC AGA TG 3' (Rosel et al. 1994),1.25 U of *Taq* DNA polymerase (NEB, UK), and approximately 50ng of genomic DNA. The thermo cycling profile consisted of a hot start denaturation step of 5 min at 95ºC, followed by 36 amplification cycles of 45 sec at 48ºC, 1 min at 72ºC and 45 sec at 94ºC and a final elongation step of 10 min at 72ºC. PCR products were purified using

purification spin columns (QIAGEN, UK) and then sequenced in an automatic sequencer (ABI 3730 Gene Analyzer, Applied Biosystems).

Sequences were checked with the software CHROMASlite (Technelysiun Pty. Ltd.) to verify base calling and aligned with CLUSTAL X (Jeanmougin et al. 1998). Unique haplotypes were identified using DNAsp version 3 (Rozas and Rozas 1999). The best evolutionary model that fit the mtDNA sequence variation was tested with MODELTEST 3.7 (Posada and Crandall 1998). Haplotype diversity (*h*) and nucleotide diversity (π) to estimate diversity within populations, and fixation indexes (F_{st} and Phi_{st}) to assess differentiation among regional populations were estimated using ARLEQUIN (Schneider et al. 2000). A neighbour-joining phylogenetic reconstruction of mtDNA haplotypes was conducted in PAUP v 4.0 (Swofford 2002) and rooted with homologous sequences from *Delphinus delphis* and *Stenella attenuatta*. As an alternative phylogenetic representation, a median-joining network was also generated with the program NETWORK 4.5.1.0 (Bandelt et al. 1999).

2.3.4 Microsatellite analyses

Eight microsatellite loci: MK5, AAT44, TexVet5 and TexVet7, derived from *T. truncatus* (Rooney et al. 1999, Krutzen 2001, Caldwell 2002, respectively) and KWM1b, KMW2b, KWM12a, derived from *Orcinus orca* (Hoelzel et al. 1998a), and EV37Mn derived from *Megaptera novaeangliae* (Valsecchi et al. 1997), were amplified by PCR The PCRs were performed in 15µL volumes consisting of 10mM Tris-HCl, 50mM KCl, 1.5-2.5mM $MgCl_2$, 0.25mM each dNTP, 1.2µM each primer under the following conditions consisted of a 95ºC hot start denaturation followed by 40 cycles of 1 min at annealing temperature, 45 sec at 72ºC and 45 sec at 95ºC, and a final elongation of 10 min at 72ºC. Specific annealing temperatures for each microsatellite were: MK5: 53ºC, AAT44: 52.6ºC, TexVet5: 50ºC, TexVet7: 50ºC, KWM1b: 49ºC, KMW2b: 43ºC, KWM12a: 56ºC and EV37Mn: 51ºC.

Genotypes across all loci were tested for the presence of allelic dropout and null alleles using the program MICRO-CHECKER (Van Oosterhout et al. 2004). Bi-parental genetic diversity (estimated as observed heterozygosity (*Ho*) and expected heterozygosity (*He*), regional differences in frequencies, deviation from Hardy-Weinberg equilibrium,

and the analysis of variance in allele frequencies among groups of samples (*Fst*) were all computed in ARLEQUIN 2.0 (Schneider et al. 2000). Allelic richness and number of alleles per locus were also estimated using FSTAT 2.9.3 (Goudet 2002). The Bayesian clustering assignment method to estimate population structure was performed as implemented in STRUCTURE (Pritchard et al. 2000), whereby population clusters (K) were detected without a priori assignment to populations. Five independent runs for each putative number of populations (K = 1 - 6) were performed using the correlated allele frequency and admixture models with 1,000,000 repetitions and a burn-in of 500,000. Population structure was inferred by the modal value of ΔK, which correspond to the rate of change in the likelihood of K and proved to be the optimal estimation for genetic population subdivision (Evanno et al. 2005). An alternative Bayesian method to estimate the most probable number of populations (k) and delineate their spatial distribution was conducted by integrating the geographic (lat, long data) and genetic information using the program GENELAND 3.1.5 (Guillot et al. 2005). To determine the most probable number of populations (k) and the posterior probability of population membership for each individual 100,000 MCMC iterations were performed for each K of 1-6. The maximum rate of Poisson process was fixed at 100, spatial uncertainty was set as zero Km and an uncorrelated allele frequency model was selected.

2.3.5 Sex determination and sex-biased dispersal

Sex was determined by amplifying fragments of the gene Zfy/x and SRY. The PCR reactions were performed in 10μL volumes consisting of 10mM Tris-HCl, 50mM KCl, 2.5mM $MgCl_2$, 0.25mM each dNTP, 1 μM each primer: P15EZ: 5' ATA ATC ACA TGG AGA GCC ACA AGC T 3', P23EZ: 5' GCA CTT CTT TGG TAT CTG AGA AAG T 3', Sry-Y53-3c: 5' CCC ATG AAC GCA TTC ATT GTG TGG 3' and Sry- Y53-3d: 5' ATT TTA GCC TTC CGA CGA GGT CGA TA 3', 1.25 U of Klear Taq DNA polymerase (KBiosciences, USA) and approximately 50ng of genomic DNA. The thermo cycling profile consisted of a hot start denaturation step of 15 min at 95ºC, followed by 36 cycles of 45 sec at 60ºC, 1 min at 72ºC and 45 sec at 94ºC and a final elongation step of 10 min at 72ºC. PCR products were verified using agarose gel electrophoresis. The band for the gene Zfy/x appeared between 400 and 500 bp in both sexes, while the

male/SRY band appeared at 200 bp in males only. Sex-biased dispersal was tested by estimations of F_{IS}, F_{ST}, relatedness, mean assignment index and variance of assignment indices using FSTAT 2.9.3 (Goudet 2002). Population subdivision was estimated for derived data sets containing only males or females using ARLEQUIN 2.0 (Schneider et al. 2000). The latter analysis was also carried out for mtDNA data sets.

2.4 Results

2.4.1 Genetic diversity

Among the combined 263 samples, 77 mtDNA control region haplotypes were found, defined by 64 segregating sites (Table 2. 2)**.** No fixed differences were observed across haplotypes from the distinct populations. Haplotype diversity ranged from 0.769 to 0.963, while nucleotide diversities ranged from 0.4 % to 1.7% (Table 2. 3). Twelve haplotypes were shared among all populations; the lack of shared haplotypes is indicative of some extent of genetic structure and of the differentiation between the GC and Pacific Ocean basins (Table 2. 3).

Table 2. 2 Variable sites among the 77 mtDNA control region haplotypes, numbers in the heading row indicate the base pair position of the polymorphic nucleotide. Haplotype absolute frequency in each population.

	111 1111111122 2222222333 3333333333 3333333333 3333344444 4444	NGC	SGC-SW	Main-land	NW
	788899024 4555666601 1233677001 2223333333 4444555667 8889912345 5566				
	9747907018 9468358981 5901435470 3483456789 1239237243 3586869290 1278				
Hap-32	AGCCTCCCGT ATCTAATGCA GTGCCTACGC TTCCAATTTT TCTACTTGTT ATCCTCCTAT CTAT		9		
Hap-68	....T..... .... .C........ CT..T..A..				1
Hap-69	.... .C....C... C...T..A..		1		
Hap-12	....T..... ....T..A.. ...A------ ----		6		
Hap-75	.A........ .C..G...TT A...T..... .A.......C ..C.T..... ...A------ ----	1			
Hap-76	.A........ .C..G...TT ....T..... .A.......C ..C.T..A.. ...A------ ----		1		
Hap-77	.A.....T.. .CTC....TT ....T..... .A.......C ..C.TC.... ...A------ ----		1		
Hap-43	....T..... C.....G... ...A------ ----	1			
Hap-10	....TT..... ....T..A.. ....G...GC T...		4		
Hap-54	.... .C.......C C....C.A.. ......T...		1		
Hap-8	....T..... ....T..A.. ....GC	3	4		
Hap-34	.... CT........ ......T...		1		
Hap-55	....T..... .... .C........ C...TC.A.. ..T.....			1	
Hap-57	....T..... .... .C........ C...T..A.. ..T......C		1		
Hap-35	.... CT...C.... ......T...		1		
Hap-70	.... .C....C... C...T..A.. C		1		
Hap-2	....C..... G.....C.T. ..A....TA. .C......C. C...TC.A.. ......T... ..G.	6	12	5	
Hap-3	....C..... .C....C.T. ....T..... .CT....CC. ....TC.A.. ......T...		3		
Hap-44	....T..... C......... ...------- ----	1	5		6
Hap-73	..T....... ....A.. ....T..... .C.T.....C ....TC.A.. ...------- ----		1		
Hap-37	.... CT...C.... ...------- ----		1		
Hap-29	....T..... C......A.. ...------- ----		1		
Hap-11	....T..... ....T..A.. ...------- ----		6		
Hap-67	....T..... .... .C........ C...T..A.. ...------- ----		1		
Hap-60	....T..... .... .C........ C...TC.A.. ..T......C	1			
Hap-27	.... C......A.. .....T.C..		1		
Hap-16	....T..T.. ....TC.A.. ....GC				17
Hap-51	....C...... .... .C........ C....C.A..			1	
Hap-64	....T..... .... .C........ C...T..A..			3	
Hap-72	.... .C........ C...T..A.. ......T...		1		
Hap-1	....C..... G.....C.T. ....TA. .C......C. C...TC.A.. ......T... ..G.		4		
Hap-22	....T..... ....TC.A.. ....C		5	1	
Hap-52	.... C....C.A.. G.....T...		1		
Hap-50	....C...... .... C....C.A..	1	1		
Hap-30	....C.... ..T..... C......A.. ....C..	1			
Hap-21	....T..... ....T..A.. ....C			1	
Hap-17	....T..... ....TC.A.. ....GC		1		
Hap-31	.... C.........	5	1		
Hap-6	.... .C........ ....T..... ....T..A.. ......T.GC		9		
Hap-13	....T.G... .... .T.....A.. ....GC TC..		13	1	
Hap-7	.... .C........ ....T..... ....T..A.. ....GC	8	11		
Hap-14	....T..T.. .... .T.....A.. ....GC TC..	1			
Hap-23	....T..... ....A.. ....C		4		
Hap-36	.... .T...C.... ......T...		1		
Hap-53	.... .A........ .... C....C.A.. ......T...		2		
Hap-49	....C...... .... C......... C....C.A..		1		
Hap-61	....T..... .... .C........ C...T..A.. ..T.....	1			
Hap-63	....T..... .... .C........ C......A.. ..T.....	1		1	
Hap-15	....T..... ....A.. ....GC	3	3		
Hap-4	.A........ .C...G..T. .... .A......C. .T..TC.... ......T...		2		
Hap-33	.... C.......C.		1		
Hap-38	.... .C........ CT...C.... ...------- ----		1		
Hap-66	....T..... .... .C........ C...T..A.. ......T...			1	
Hap-59	........A. ..T...C... .... .C..G..... C...T..A.. ..T.....	2			
Hap-58	....T...C... .... .C..G..... C...T..A.. ..T......C		2		
Hap-56	....T..... .... .C........ C...TC.A.. ..T..T....			1	
Hap-28	.... C...T..A.. .C...T.C..		1		
Hap-39	.... .C........ .... CT...C....		1		
Hap-40	.... .C........ .... CT...C.... ......T...		1		
Hap-9	......G... ....T..... ....T..A.. ....GC		3	7	
Hap-19	....T..... .C........ ....T..... ....GC			2	
Hap-24	....T..... ....T..A..		3		
Hap-46	.... G......... ....C... C....C.ACC ....C..			2	
Hap-47	....C... C....C.ACC ....C..				16
Hap-71	.... .C........ C...T..A..		1		
Hap-62	....T..... .... .C...G.... C...T..A.. ..T.....	1			
Hap-65	....T..... .... .C........ C..GT..A..			2	
Hap-5	.A........ .C...G..T. .... .AT.....C. .T..TC....	1			
Hap-74	.A.T.T...C .... A...T...AT .A........ .....C.A.. .....TT...		2		1
Hap-18	C......... ....T..... ....TC.A.. ....GC			1	
Hap-20	....T..... ....TC.... ....GC		1	1	
Hap-26	C......... .... C....C.A..				
Hap-41	....T..... CT...C.... ......T...			1	
Hap-42	....T..... C....C.... ......T...				7
Hap-45	.... C....C.AC.		1		3
Hap-25	.... C....C.A..		1		
Hap-48	....T..... C....C.A..				

Table 2. 3 Genetic diversity indices based on mtDNA control region haplotypes. Haplotypic diversity (*H*) and nucleotide diversity (π).

Population	n	*H*	π
NGC	38	0.909	0.017
SGC-SW	141	0.963	0.013
Mainland	32	0.929	0.015
NW	51	0.769	0.004

A total of 245 bottlenose dolphin individuals were genotyped for the eight loci. Insufficient DNA quality prevented the amplification of microsatellite loci from 18 individual samples, 12 of which were teeth samples. No allelic dropout was identified by MICRO-CHECKER. The number of alleles per locus ranged from two in KWM1b to 27 in EV37Mn (Table 2.4). Deviation from HW equilibrium was detected in a few cases (see Table 2.4), after pooling the offshore populations as suggested in STRUCTURE (GC-SWoffshore). HW deviations were found at 4 loci after sequential Bonferroni corrections (p=0.009), however, these deviations were only marginally significant. These loci were retained in subsequent analyses in spite of their significant HW disequilibria, as even before pooling offshore dolphin populations estimates of *Ho* and *He* were similar across all loci, although, only two loci showed significant deviation of HW (Tex Vet 5 and EV37Mn). The observed deviations from HW may also reflect a Wahlund effect within offshore bottlenose dolphins, suggesting the possible existence of substructure that our dataset could not resolve. The north-west coastal ecotype had the highest level of differentiation, followed by the North GC.

Table 2.4 Genetic diversity at microsatellite loci for each population. No alleles: number of different allleles, Ho: Observed heterozygosity, He: expected heterozygosity, H-W: significance for deviation from H-W

Locus		**Populations**			
		NGC	**GC-SW**	**Mainland**	**NW coastal**
		n=27	**n=131**	**n=32**	**n=54**
Tex Vet 5	No alleles	6	13	10	6
	Ho	0.920	0.772	0.562	0.420
	He	0.841	0.879	0.749	0.448
	H-W	0.427	**0.002**	0.009	0.109
KWM12a	No alleles	9	12	8	9
	Ho	0.808	0.782	0.677	0.760
	He	0.805	0.836	0.773	0.715
	H-W	0.159	0.176	0.096	0.925
KWM2b	No alleles	3	5	3	4
	Ho	0.115	0.473	0.226	0.440
	He	0.148	0.474	0.290	0.522
	H-W	1	0.952	0.271	0.066
KWM1b	No alleles	4	10	2	2
	Ho	0.407	0.561	0.074	0.149
	He	0.445	0.526	0.418	0.228
	H-W	1	0.786	**<0.001**	0.098
AAT44	No alleles	4	10	7	8
	Ho	0.555	0.739	0.562	0.704
	He	0.586	0.774	0.589	0.758
	H-W	1	**0.003**	0.362	0.081
MK5	No alleles	12	16	11	7
	Ho	0.833	0.849	0.742	0.686
	He	0.889	0.872	0.855	0.791
	H-W	0.016	0.436	0.502	0.159
Tex Vet 7	No alleles	5	7	5	6
	Ho	0.667	0.504	0.387	0.509
	He	0.655	0.636	0.606	0.581
	H-W	0.825	**0.006**	**0.004**	**0.006**
EV37Mn	No alleles	11	27	16	9
	Ho	0.846	0.854	0.812	0.558
	He	0.857	0.945	0.919	0.675
	H-W	0.108	**0.005**	0.013	**0.002**

2.4.2 Inferring population structure

The clustering analysis performed in STRUCTURE (Pritchard et al. 2000) showed the highest likelihood at K = 5 (number of populations) (Table 2. 5). However, K = 3 also showed a high likelihood, was less variable among iterations than K = 5 and shows a clear pattern of population clustering, supported by other analyses (Figure 2. 3 and Table 2. 5). Moreover, the modal value of ΔK=3 supported this optimal estimation for genetic population clustering. The three populations are: 1) the northern Gulf of California (NGC); 2) the offshore ecotype samples from the Gulf and western coast of Baja California peninsula, consisting of: Midriff islands, central and southern GC and western coast of Baja California putative populations (GC-SW-offshore), and 3) the coastal ecotype from north western coast of Baja California (NW) (Figure 2. 3). The Mainland sample includes a large proportion of individuals that appear to assign better to the SW-offshore sample than to NW coastal (Figure 2. 3). However, differentiation between the Mainland and NW coastal samples is supported by the fixation indexes estimated from both mtDNA and microsatellite markers (Table 2. 8 and Table 2. 9). The GC-WC offshore cluster (cluster 2, Figure 2. 3) showed a possible Wahlund effect (increased homozygosity at TexVet5, AAT44, TexVet7 and EV37Mn loci), and so was reassessed using STRUCUTRE (Pritchard et al. 2000) to test for further population subdivision, but the highest likelihood was associated with K=1.

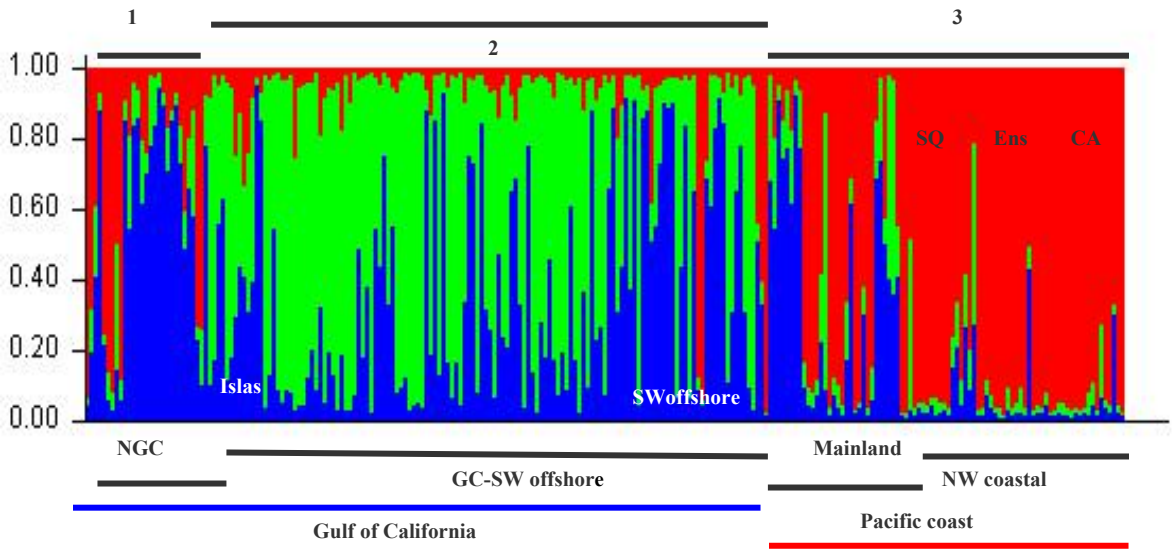

Figure 2. 3 Estimated proportion of the coefficient of admixture for each individual using eight microsatellite loci, K=3, clusters indicated by the lines above the bar graph. The lines below the graph indicate the best reliable population among the samples.

Table 2. 5 Number of putative populations (K) and their posterior probabilities [Ln P(D)] estimated by the Bayesian cluster analysis performed in STRUCTURE.

K	Ln P(D)	Var[LnP(D)]
2	-5938.6	188.9
3	-5916.7	425.8
4	-5984.1	686.1
5	-5901.5	709.1

Overall, the hypothesis of panmixia was rejected. Coastal and offshore ecotypes were high significantly differentiated (mtDNA: F_{st} = 0.029, Φ_{st} = 0.043, p<0.001; microsatellites: F_{st} = 0.046, p<0.001). Fixation indices based on mtDNA sequences including all samples were able to detect population differentiation, but also showed that most of the variance found is within populations (Table 2. 6 and Table 2. 7). All F_{st} and Φ_{st} pairwise population comparisons were also significant after Bonferoni correction, except for the Φ_{st} pairwise comparison between the north GC and the offshore ecotype from GC-SWoffshore (p= 0.081; Table 2. 8). The largest estimates of population differentiation were for pairwise comparisons with the NW coastal population (Table 2.

8). Consistently, a high degree of population differentiation among all putative populations was estimated at the microsatellite loci (F_{st} =0.076, p<0.001). Pair-wise comparisons among all four putative populations were also highly significant (Table 2. 9). The statistical tests for sex-biased dispersal suggested either that both females and males are responsible for the pattern of genetic structure observed, or low power, as no significant differentiation between gender dispersal was found (Table 2. 10).

Table 2. 6 Estimation of genetic hierarchical variation, based on haplotype frequency.

Source of variation	d.f.	Variance component	Percentage of variance
Among populations	3	0.043	8.62
Within populations	251	0.455	91.38
Total	254	0.498	
Fixation index		F_{st} = 0.086. p< 0.001	

Table 2. 7 Estimation of genetic hierarchical molecular, considering genetic molecular diversity

Source of variation	d.f.	Variance component	Percentage of variance
Among populations	3	0.637	17.85
Within populations	251	2.934	82.15
Total	254	3.572	
Fixation index		Φ_{st} = 0.178. p< 0.001	

Table 2. 8 Mitochondrial DNA control region fixation indeces. Pair wise comparisons, below diagonal Fst and above diagonal Φst values, p<0.008 after Bonferroni correction**

	NGC (n=37)	SGC-SW (n=142)	Mainland (n=33)	NW-coastal (n=51)
NGC		0.081	0.087**	0.336**
SGC-WC	0.027**		0.102**	0.257**
Mainland	0.056**	0.033**		0.443**
NW-coastal	0.160**	0.124**	0.154**	

Table 2. 9 Microsatellite Fst pair-wise comparisons, based on 8 loci, p<0.008 after Bonferroni correction**

	NGC (n=27)	SGC-WC (n=136)	Mainland (n=33)	NW-coastal (n=54)
SGC-WC	0.043**			
Mainland	0.033**	0.027**		
NW-coastal	0.087**	0.115**	0.136**	---

Table 2. 10 Statistical test for sex-biased dispersal between males and females over all populations. n= number of individual tested, Ho: observed heterozygosity; He: expected heterozygosity; FIS: inbreeding coefficient; FST: fixation index, Relatedness coefficient, *AIc*: mean corrected assignment index, *vAIc*: variance of the corrected assignment index *AIc*.

	n	F_{is}	F_{st}	Relatedness	*Ho*	*He*	*AIc*	*vAIc*
Females	124	0.1006	0.0641	0.1106	0.6212	0.6907	-0.1102	8.777
Males	104	0.039	0.0885	0.1575	0.6293	0.6548	0.1314	11.356
p-values		0.072	0.419	0.323	0.757	0.075	0.598	0.14

The population genetic analysis conducted in GENELAND supported k = 2 as the most probable number of populations (Figure 2. 4). The resulting clustering and spatial arrangement of population membership patterns correspond primarily to the coastal and offshore ecotypes, and also it showed evidence of some extent of the admixture between ecotypes; for instance individuals with some extent of admixture but genotype highly related to the offshore ecotype were represented as Admixture 1, while individuals with genotypes related to coastal ecotypes were indicated as Admixture 2 (Figure 2. 5).

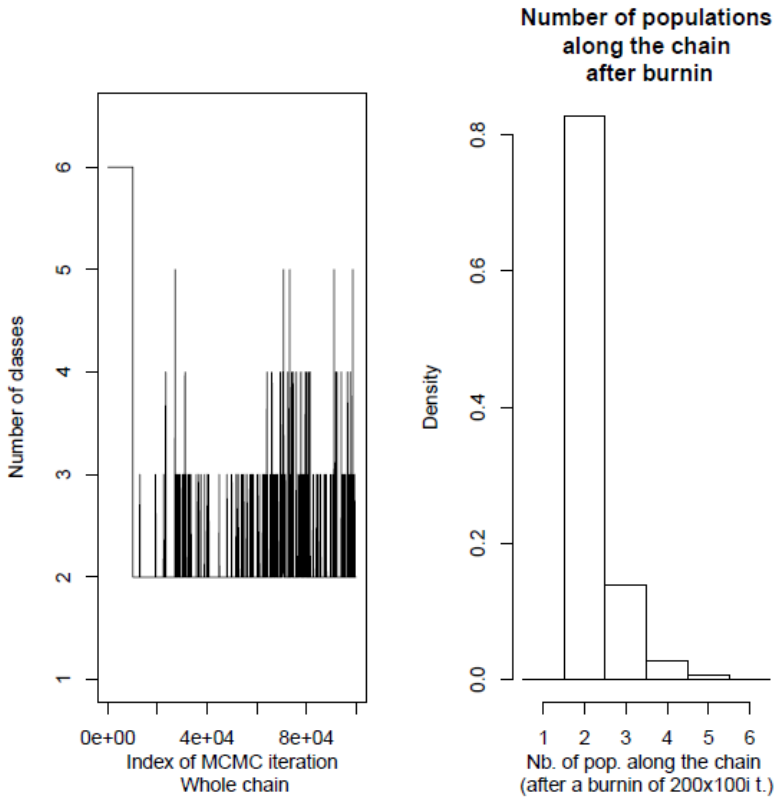

Figure 2. 4 Modal number of population (K=2) simulated after posterior distribution, based on multi-locus genotypes as implemented in GENELAND.

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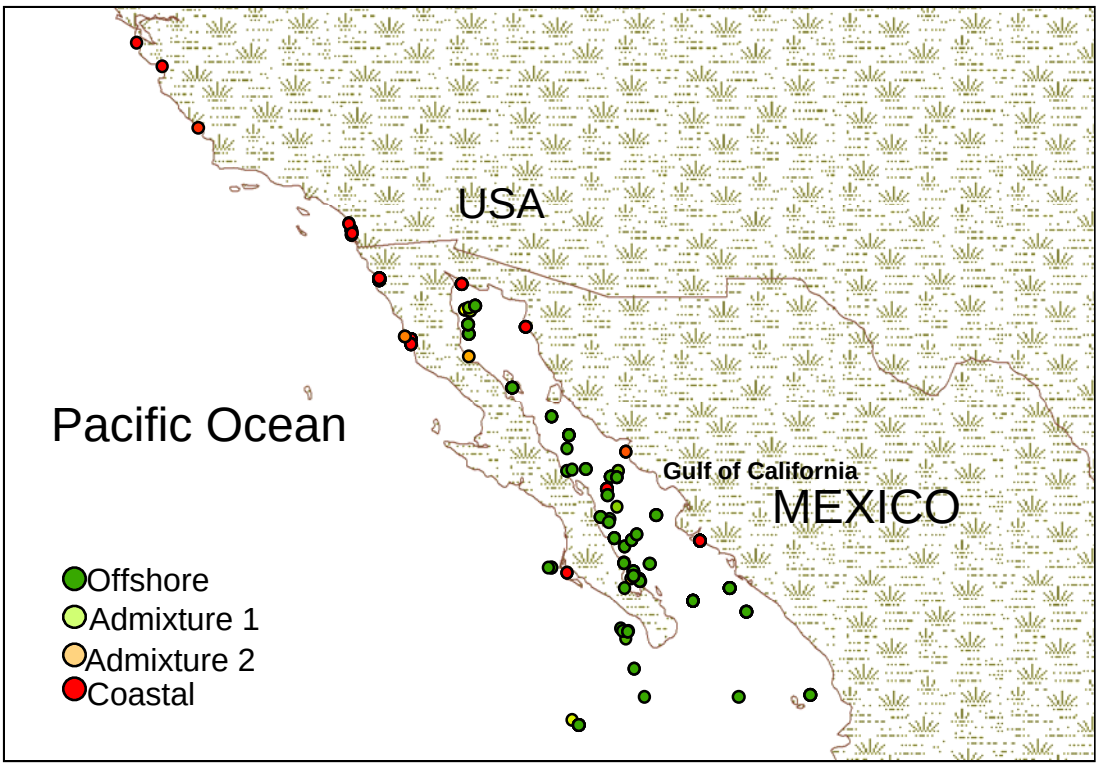

Figure 2. 5 Map of population membership of each dolphin individual, based on multi-locus genotype. The colour of the circles represent the posterior probability of each individual to belong to either offshore (green) or coastal ecotype (red); pale green and orange circles indicate some extent of admixture between ecotypes. Circles might represent more than one dolphin individual sampled in the same location.

2.4.3 Phylogenetic analyses

The reconstruction of the phylogenetic relationships among mtDNA haplotypes did not show evidence of a clear phylogeographic pattern. Both Neighbor-Joining (N-J) and Median-Joining network (MJN) methods were consistent, showing the close relationships among mtDNA haplotypes, indicating their recent divergence (Figure 2. 6 and Figure 2. 7). The MJN is most informative, as the NJ tree is dominated by polytomies (Figure 2. 6). The haplotypes found in dolphins from the NW showed fewer reticulations than those found in other regions (Figure 2. 7). The haplotype TTGC44 is shared with GC-SW offshore and the NGC populations, which supports the hypothesis of isolation between coastal populations from the GC and OP. This also suggests that the NW population has diverged for a longer period of time than the populations within the GC. The haplotypes present in NGC bottlenose dolphins maintain a close relationship with the offshore type haplotypes, and appear to be derived from the most common offshore

haplotypes: TTGC02, TTGC07 and TTGC13. While Mainland haplotypes are also derived and closely related to GC-SW offshore, they are peripheral and only the most common are shared with other populations (Figure 2. 7). In the N-J reconstruction, there are few supported lineages, and the tree is dominated by an extensive polytomy that includes samples from all regions (Figure 2. 6). Three haplotypes from NGC are exceptional, forming a distinct lineage.

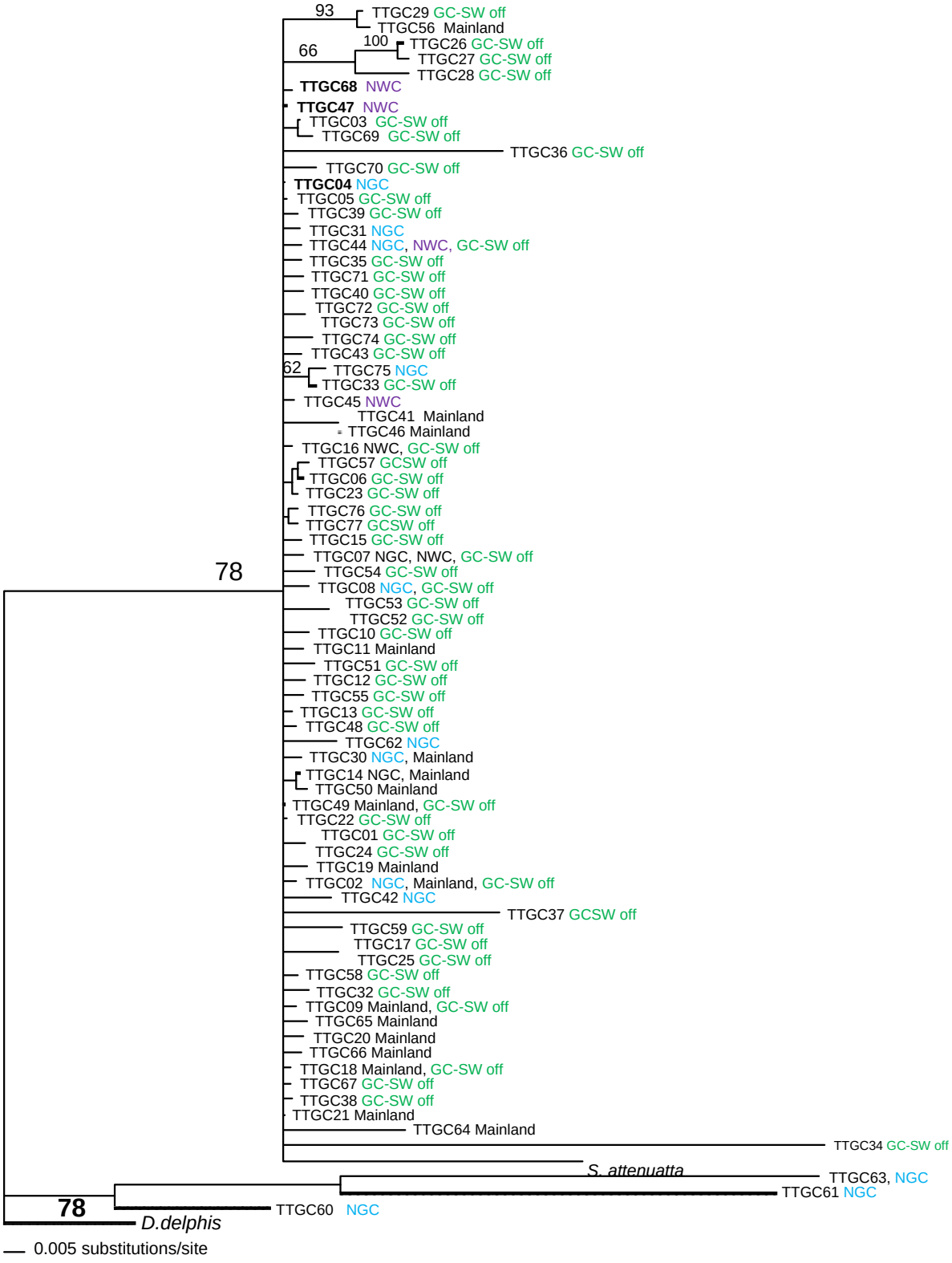

Figure 2. 6 Neighbor-Joining reconstruction of 77 mtDNA control region haplotypes of *Tursiops truncatus* estimated under TrN + I + G model of molecular evolution. *Stenella attenuatta* and *Delphinus delphis* rooted. Numbers along the branches indicate the boostrap support.

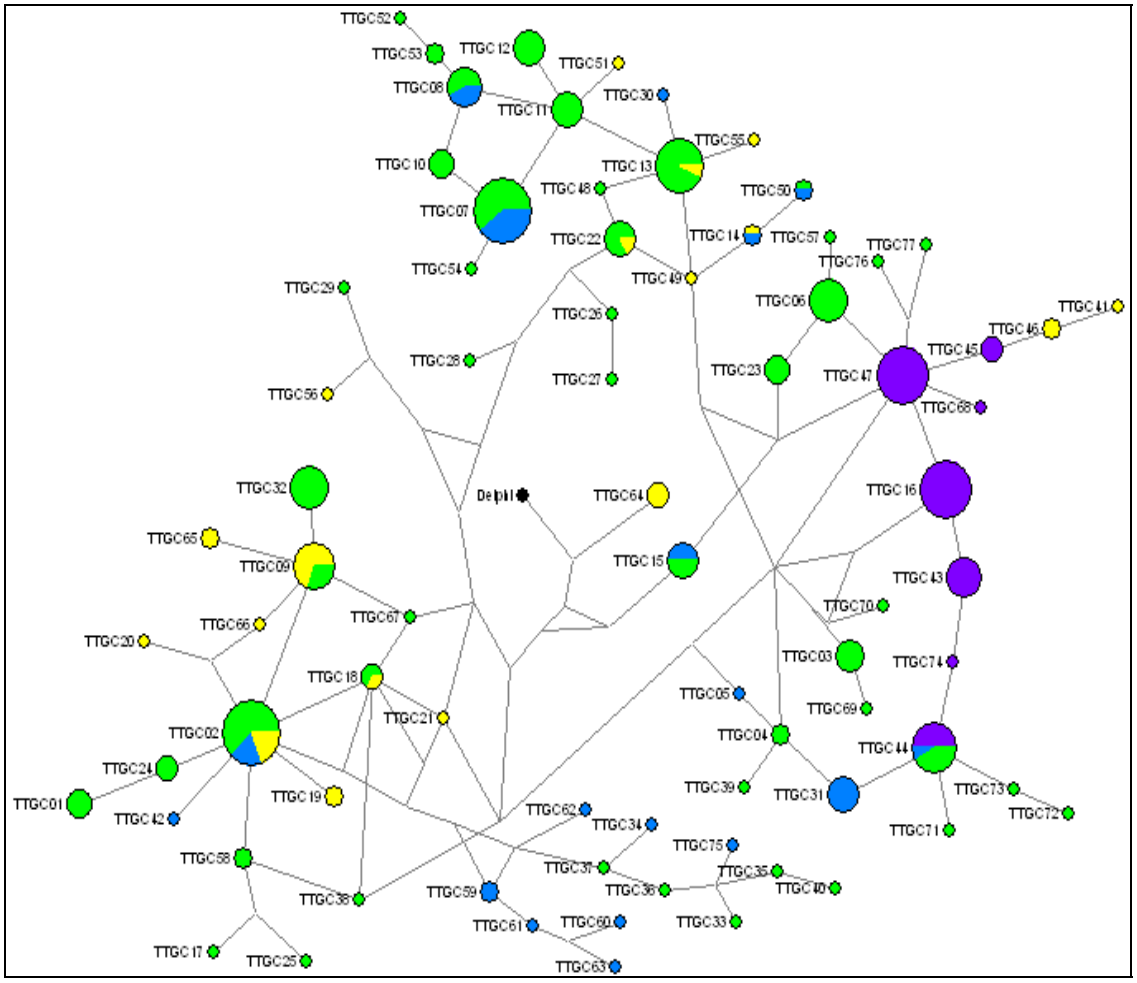

Figure 2. 7 Median joining network of the 77 mtDNA haplotypes sampled within the Gulf of California and western coast of Baja California. The circles represents mtDNA control region haplotypes, the size is proportional to the frequency of the haplotype in the whole dataset. Frequency in each population indicated by colour subdivisions; blue: Northern gulf, green: GC-SW offshore, yellow: Mainland and purple: North West coastal.

2.5 Discussion

2.5.1 Genetic differentiation between ecotypes

Enviromental and ecological factors, such as prey distribution and preference, are increasingly thought to contribute significantly to intra-specific genetic differentiation in mammalian species with high dispersal capabilities [e.g. cetaceans, (Hoelzel 1998), felids (McRae et al. 2005) canids (Sacks et al. 2004, Pilot et al. 2006, Muñoz-Fuentes et al. 2009)]. Bottlenose dolphin coastal and offshore ecotypes have been previously distinguished within the GC and PO by means of morphological, ecological and mtDNA molecular data (Defran and Weller 1999, Lowther 2006, Segura et al. 2006). In this study the molecular analyses of both mtDNA and microsatellite loci were consistent in supporting the significant differentiation between coastal and offshore ecotypes from both the GC and PO basins. This ecological pattern of differentiation seems to be also commonly recognized among other delphinid species [e.g. spotted dolphin, *Stenella attenuata*; (Douglas et al. 1984, Escorza-Treviño et al. 2005); spinner dolphin, *Stenella longirostris*; (Perrin and Gilpatrick 1994, Perryman and Westlake 1998, Perrin and Mesnick 2003); tucuxi, *Sotalia fluviatilis*; (Caballero et al. 2007); and killer whale, *Orcinus orca*; (Hoelzel et al. 1998a, 2007)]. In species with a wide geographic distribution over a variety of habitats, individual adaptation to specific environmental and ecological factors may be a result of reducing the migration between habitats (Hoekstra et al. 2005). The GC bottlenose dolphin ecotypes have shown marked habitat and prey preferences; the coastal bottlenose dolphin was mostly found in waters less than 60 m depth, and ^{13}C and ^{15}N stable isotope analyses indicated differences in prey choice with respect to offshore dolphins (Díaz-Gamboa 2003, Segura et al. 2006). Along the coast of California and Baja California offshore dolphins were usually found further than 4 km from shore (Lowther 2006) while coastal dolphins seem to follow a narrow alongshore corridor less than 1 km wide (Guzón 2002, Morteo et al. 2004). This study supports the hypothesis that ecological preferences of the bottlenose dolphin are diminishing the encounters among conspecifics of distinct ecotypes and leading to genetic differentiation of evolutionary significance.

2.5.2 Population differentiation

The analyses of genetic differentiation revealed an intricate pattern of population structure among sampled groups of bottlenose dolphins. At the fine geographic scale, fixation indices for both mtDNA and microsatellite data were consistent in showing significant pairwise genetic differences among coastal populations and the panmictic offshore population from the GC and SW (Table 2. 8 and Table 2. 9). Moreover, the strong differentiation among the coastal populations between the GC (NGC and Mainland samples) and the NW (composed by samples from California, Ensenada and San Quintín); suggests the isolation of the GC from the PO bottlenose dolphin populations.

The pattern of population differentiation supports the hypothesis of strong habitat fidelity in both sexes. No evidence of sex-biased dispersal was found as documented in the Gulf of Mexico (Rosel et al. 2009) and the North Atlantic (Natoli et al. 2005); (but see Krutzen *et al*., 2004b, Moller and Beheregaray, 2004). Moreover, the NW dolphins bear private mtDNA haplotypes that were not sampled in the gulf; this could be a result of a limited sampling effort or could indicate that the NW populations have not experienced recent gene flow as the levels of genetic diversity are also lower than the rest of the populations analyzed. The existing biogeographic conditions within the GC are believed to have persisted since the end of the Pleistocene (10 000 years ago; (Durham and Allison 1960), thus the Baja California peninsula as a land mass barrier, and geographical distance, are the possible factors driving the isolation of bottlenose dolphins from these basins, given that sample localities are separated by hundreds of kilometres (for instance, NGC and Mainland populations are separated by approximately 1200km). Several taxa have shown disjunct populations between PO and GC (Stepien et al. 2001, Bernardi et al. 2003, Sandoval-Castillo et al. 2004, Schramm et al. 2009). Walker (1960) reported that the vast majority of the fish species found in both locations exhibit morphological differences between the GC and PO. Recently, molecular analyses have revealed reciprocal monophyly of mitochondrial lineages from the GC and OP populations in several bony (Stepien et al. 2001, Bernardi et al. 2003) and cartilaginous-fish species (Sandoval-Castillo et al. 2004), which indicates no recent gene flow. Similarly, in species capable of long-distance travelling such as the California sea lion

(*Zalophus californianus californianus*), mtDNA markers revealed strong differentiation between the PO and the GC (Schramm et al. 2009). Furthermore, population studies conducted along the western coast of Baja California and the Southern California Bight (SCB) have suggested that dolphins from these areas may represent a single population, ranging from Santa Barbara to Ensenada (Defran and Weller 1999). The photo-identification evidence also suggested that bottlenose dolphins from San Quintín, locality south of Ensenada, have a restricted geographic range (Morteo et al. 2004). However, the genetic analyses conducted in this study did not show evidence of significant differentiation among the three NW sampled sites (California, Ensenada and San Quintín). Dolphins from the SCB exhibit an apparent lack of site fidelity and the ability to conduct long-shore movements, up to 600 Km, in response to prey abundance. The variability of the California current along the coast of California and Baja California creates a patchy and unpredictable pattern of prey distribution and abundance, therefore a wide foraging range of the SCB bottlenose dolphins promotes potential gene flow among neighbouring conspecifics.

On the other hand, mesoscale oceanographic structures such as dynamic eddies around Punta Eugenia, halfway down the peninsula (see Chapter 1), may impose a boundary to marine species distribution [for instance, California sea lions; (Schramm et al. 2009)]. These ecological boundaries might be particularly important for prey species and indirectly prevent long-distance travel restricting dolphin gene flow along the coast of Baja California. This might also explain the limiting southward movements of the NW bottlenose dolphins along the west coast of Baja California. This is also clear in the map of spatial analysis of genetic differentiation (Figure 2. 5), where NW dolphins showed a high probability of belonging to cluster 1 and to be highly differentiated from SW and most of the dolphins within the GC, which comprised cluster 2. The extent of genetic differentiation between PO and GC bottlenose dolphin populations was lower than the differentiation found between the Atlantic Ocean and the Gulf of Mexico [$\Phi st= 0.257$, $p<0.001$ compared to 0.702, $p<0.001$; (Natoli et al. 2004)]. This is possibly due to a recent divergence between the OP and GC basins, as supported by the phylogenetic reconstructions (NJ tree, Figure 2. 6 and star-shaped MJN, Figure 2. 7) and the low estimates of nucleotide diversity (Table 2. 1).

Conversely, a significant amount of gene flow appears to be taking place between the offshore ecotype from the GC and WC as indicated by low and non significant fixation indices and the fact that population assignment test pooled offshore dolphins in the same cluster. A recent study on the feeding ecology of teutophagus cetaceans within the GC, revealed that the occurrence of offshore bottlenose dolphins coincided in space and time with that of its preferred prey, the jumbo squid *Dosidicus gigas*, and dolphins were not present in the absence of squid (Díaz-Gamboa 2009). This suggests that movements of offshore bottlenose dolphins across the GC, and potentially outside the gulf, may be coupled to the migratory behavior of its prey favoring large scale gene flow and the existence of a large panmictic population. Departures from HW equilibrium in certain samples might suggest either sampling effects or some level of sub-structuring in this population, however given the available data it was not possible to distinguish further population boundaries.

At a smaller geographic scale, three populations could be distinguished within the gulf: NGC, Mainland and the GC-WC offshore population. These groups partially coincide with the population subdivision reported by Vidal-Hernández (1993), based on skull measurements, where specimens from northern region were differentiated from those from the south GC and Mainland (Figure 2.2). Likewise, a number of oceanographic and ecological studies have consistently subdivided the GC into four or more regions given the particularities of the oceanographic, topographic and climatic conditions (Álvarez-Borrego 1983, Santamaria-del-Angel et al. 1994). Ecological conditions may influence the phenotypic variability of dolphins (e.g. Perrin 1984, Ross and Cockcroft 1990, Hoelzel 1998, Hoelzel et al. 1998b, Viaud-Martínez et al. 2008) and can also influence their genetic structure, local adaptation and lineage evolution (Via 2002). The widest distance across the GC is 200 Km, a distance that bottlenose dolphins could travel daily, however, deep water oceanographic dynamics could represent a barrier to dispersal for coastal bottlenose dolphins, preventing them reaching their conspecifics on either the western or eastern coast of the gulf. Walker (1960) observed that the habitat discontinuity along the GC determines the zoogeographic pattern of a number of species of fish; moreover, he found that populations on both the eastern and western coasts of the entrance of the gulf were different. It is possible the habitat discontinuity along the coast

does not affect dolphin movements directly; however, it might influence dolphin transit indirectly by affecting their prey distribution, as documented for other cetacean species and bottlenose dolphins elsewhere (Defran and Weller 1999, Díaz-Gamboa 2003, Torres and Read 2009). In addition, capture-recapture studies (based on photo-ID), have shown a certain level of residency of coastal dolphins along the eastern and western shores of the GC [mainland: Bahía Kino, Sonora (Ballance 1990); Bahía Santa María, Sinaloa (Reza-García 2001); Bahía Banderas, Nayarit- Jalisco (Rodríguez Vázquez 2008); and Baja California: Bahía de La Paz (Rojo-Arreola et al. 2001, Salinas-Zacarías and Aureoles-Gamboa 2002)]. On the other hand, individual movements of up to hundreds of kilometers have also been recorded (Reza-García 2001). Long term studies in Florida have revealed dolphin movements out of their natal habitat or population and their interaction with neighboring populations (Sarasota Bay, Fazioli et al. 2006), notwithstanding the significant genetic differentiation among those interacting populations (Sellas et al. 2005).

Furthermore, in upper gulf, Silber *et al.* (1994) reported the occurrence of bottlenose dolphins all year round, with some seasonal movements along Baja California and Sonora coastline, which suggests a remarkable level of habitat fidelity. The distinctiveness of populations residing in the NGC has been noticed in several taxa (Walker 1960, Correa-Sandoval and Rodriguez-Cortes 1998, Riginos and Nachman 2001, Lin et al. 2009, Schramm et al. 2009), suggesting that this region is a well defined bioregion possibly delimited by the abrupt change in the temperature and bathymetry at the sills of the Midriff Island, at least in normal climate conditions. Both sea surface temperature (SST) and depth are oceanographic factors that modify cetaceans travelling routes, as recorded in bottlenose dolphins equipped with radio-transmitters in the Atlantic Ocean (Wells et al. 1999). In the Upper Gulf, different cetacean species use the habitat differentially. For example, *T. truncatus* preferably inhabits coastal shallow waters of 15-21ºC SST, with high turbidity and is the only cetacean that used to venture in the Colorado River; while common dolphins occurred in blue and deeper waters (Silber 1994). Habitat differences appeared to influence the feeding behaviours exhibited in bottlenose dolphins (Torres et al. 2003, Rosel et al. 2009, Torres and Read 2009); for instance a feeding strategy known as intentional beaching has been observed in

bottlenose dolphins from the Colorado River. This relates to the shallower waters (< 50m depth), smoother continental slope and sand-mud bottom, that make the habitat suitable for this feeding strategy. Recent studies have suggested the matrilineal transmission of foraging specialization (Krutzen et al. 2005, Sargeant et al. 2005, Weiss 2006, Mann et al. 2008) which could suggest a strategy whereby dolphins that learn to specialize in one type of feeding strategy tend to seek or stay in habitats that match their specialization, rather than change behaviour (Rosel et al. 2009).

This study supports the idea that bottlenose dolphins, despite their dispersal capabilities, exhibits fine-scale population differentiation as documented elsewhere (Sellas et al. 2005, Bilgmann et al. 2007, Moller and Harcourt 2007, Rosel et al. 2009, Tezanos-Pinto et al. 2009, Urian et al. 2009). Taking together the demographic, ecological and the genetic evidence provided in this study strongly support the hypothesis that bottlenose dolphin movements are habitat dependent, which defines fine-scale population structure as observed elsewhere.

2.5.3 Genetic diversity and phylogenetic relationships

Overall, genetic diversity estimates were slightly higher in the offshore population than the coastal populations within the GC, consistent with previous findings (Segura et al. 2006) and elsewhere (Hoelzel et al. 1998b, Natoli et al. 2005, Rosel et al. 2009, Tezanos-Pinto et al. 2009). The higher level of gene diversity observed in offshore bottlenose dolphins could be due to a larger effective population size and a high gene flow among neighboring populations, as offshore dolphins tend to disperse in a wider range in groups of >100 individuals (Salinas-Zacarías and Aureoles-Gamboa 2002). The lack of differentiation between SW and GC offshore dolphins supports the hypothesis of high gene flow between these populations.

The NGC and Mainland coastal populations showed a relatively high genetic diversity, compared to the Atlantic populations, coastal ecotype h= 0.43 - 0.74 and offshore ecotype h= 0.73 - 0.94; (Natoli 2004), coastal and inshore bottlenose dolphins from the eastern North Atlantic and Gulf of Mexico h= 0.49 to 0.76, (Rosel et al. 2009). This would be consistent with the recent divergence between coastal and offshore ecotypes within the GC, resulting in a retention of ancestral polymorphism, as suggested

by the low nucleotide diversity levels and the phylogenetic reconstructions (poorly resolved Neighbour-Joining tree; Figure 2. 6, and star shaped MJN; Figure 2. 7). It is likely that dolphins from the open ocean colonized the GC basin gradually; this hypothesis is supported by the fact that the most common haplotypes are maintained in both coastal and offshore populations. Conversely, the haplotypes found in the NWC populations appear to be highly differentiated from those found in offshore bottlenose dolphins and coastal populations within the Gulf. The close relationship between the haplotype TTGC44 (common in NWC and offshore populations and also found in NGC population), and TTGC30 (the most common in NGC), supports the hypothesis that offshore dolphins are founders of coastal populations that gradually colonized the PO coast and more recently the GC, as indicated by the partial separation of the NWC haplotypes within the MJN (Figure 2. 7).

The incomplete lineage sorting could also be affected by occasional events of introgression. It has been hypothesized that cetaceans can extend their normal distribution range during extreme conditions; an increase in the abundance of several species of cetaceans has been recorded along the Canal de Ballenas, northern GC, during El Niño event (Breese and Tershy 1993), and along the coast of California (Wells et al. 1990), which potentially increases contact among regional populations.

Consistent with expectations, analysis of the genetic diversity of bottlenose dolphins conducted elsewhere shows lower genetic diversity in more isolated populations (Rosel et al. 2009), however, the NGC, the most isolated and differentiated population in this study, showed a relatively high level of genetic diversity. In contrast, the NW population, located along the open coast of California and Baja California, exhibited the lowest genetic diversity (Table 2. 1). This apparent paradox may be due to the sampling localities in the NW group being the southernmost limit in the distribution of the SCB population and the lack of gene flow from offshore parapatric populations. In the particular case of the NGC population, the high levels of gene diversity observed and the close relationship with the offshore population, as indicated by the non significant Φ_{st} value, may be the result of a recent colonization of the northern region of the gulf. Under this scenario, ancestral and shared polymorphisms are expected to occur between the NGC and offshore bottlenose dolphin populations. This is evident in the analysis of

population membership, where most NGC dolphins showed the highest posterior probabilities to belong to the offshore ecotype (Figure 2. 5) and the number of shared haplotypes (Table 2. 2).

2.5.4 Conservation and management implications

This study revealed a complex pattern of fine-scale population structure for the bottlenose dolphin *Tursiops truncatus* in the GC and the WC of Baja California. The GC has been recognized as a priority for conservation and management actions worldwide, given the outstanding levels of biodiversity present in this marginal sea. Genetic diversity of the GC bottlenose dolphins appears to be higher than other regions in the distribution of the species, making the gulf an important reservoir of diversity for the species. Effective conservation depends on accurate information about stock boundaries, abundance and habitat requirements. Thus, the distinction of demographically independent stocks of evolutionary significance is mandatory for such a strategic region of biological and economic importance in Mexico.

This study provides new evidence pointing to the distinction of coastal and offshore bottlenose dolphin ecotypes based on bi-parentally inherited microsatellite loci, which strongly supports the recognition of these ecotypes as demographically independent stocks with evolutionary significance, as previously suggested (Segura et al. 2006).

Habitat dependent genetic structure was also a relevant finding of this study, consistent with earlier studies (e.g. Natoli et al. 2005). Our data revealed the existence of at least four stocks, which has an important long-term implication for the delimitation of geographical regions in need of protection. For instance, a number of studies consistently support the isolation of the northern "Alto Golfo de California" (NGC) and the need for this to be considered as a critical habitat for a number of species. Fortunately, Mexican authorities are currently conducting conservation and management actions in this region. This study supports previous evidence that micro-evolutionary processes are taking place in this region, creating and maintaining high levels of biodiversity, besides the significant numbers of endemic species. For instance, the Vaquita, *Phocoena sinus,* and totoaba, *Cynoscion macdonaldi*, are endangered endemic species currently under management

actions for population recovery. The presence of these species and the consistent population isolation of several taxa have provided evidence for designating the northern gulf as the current Vaquita Refuge Area, where law enforcement has significantly restricted fishing operations, enhanced the use of alternative fishing gear to reduce entangling cetaceans, turtles, and sharks among other by-catch species (Jaramillo-Legorreta 2008).

Currently, there is increasing pressure to develop the coastal areas, such as marinas, resorts and shrimp farms, which increase the eutrophication and modify the coastal area. It has been shown that a large extension of mangroves along the GC coastal area has been drastically reduced due to anthropogenic activities; thereby losing the ecological service mangrove forests provide for the functioning of the coastal ecosystem and the gulf, by maintaining high levels of productivity (Aburto-Oropeza et al. 2008). Therefore, the destruction of the coastal area might compromise the continuation and survival of species that are habitat dependent. Here we provide evidence supporting the hypothesis that bottlenose dolphin are habitat dependent species by showing fine scale population structure, probably due to foraging preferences. This study has an immediate impact on the conservation and management of this species and the region by providing data to the Mexican federal authorities so that they can effectively implement and enforce official norms regulating the protection of dolphins and this ecologically and economically important region for the country.

2.6 Conclusions

The bottlenose dolphin populations within the Gulf of California and the western coast of Baja California showed a pattern of fine-scale genetic structure, similar to that seen for this species in other regions. High levels of genetic structure were observed between bottlenose dolphin coastal and offshore ecotypes consistent with previous findings in the study area and elsewhere.

Genetic differentiation was strong between the Gulf of California and northwestern coastal populations, suggesting low gene flow between coastal populations inside and outside of the gulf, and reinforcing our understanding that habitat

specialization is an important driver in the evolution of population structure in the bottlenose dolphin.

The close phylogenetic relationship among haplotypes from offshore and coastal bottlenose dolphin populations within the gulf is consistent with the hypothesis of gradual colonization of the offshore ecotype into the coastal habitat across the length of the gulf. The analysis of molecular variance between coastal northern gulf and the offshore populations provides evidence of the closer relationship of the northernmost coastal population and the offshore bottlenose dolphin ecotype.

This study provides valuable knowledge of bottlenose dolphin genetic diversity, which can ultimately encourage effective conservation both through the identification of local populations in need of separate management, as well as the identification of general processes that may explain population structure in similar environments.

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Chapter 3

Population genetic structure of the long-beaked common dolphin, *Delphinus capensis*, within the Gulf of California and western coast of Baja California, Mexico.

3.1 Abstract

The common dolphins, *Delphinus spp.,* are one of the most common cetacean species in the Eastern Tropical Pacific and Gulf of California (GC). However, data to evaluate their status is inadequate, according to the IUCN-Red List. Recently, genetic analyses, supported by phenotypic analyses, have revealed a low level of intraspecific differentiation of the genus elsewhere. In this study, we investigated the genetic structure of long-beaked common dolphins, *Delphinus capensis*, at a fine geographic scale. Individuals from different regions of the GC and the west coast of Baja California (WCBC) were genotyped using mitochondrial DNA control region sequences and 18 microsatellite loci. The results suggest low levels of genetic differentiation between GC and WCBC populations for both markers, though microsatellite DNA loci showed a stronger pattern. The low magnitude of population structure limited the statistical power of the Bayesian methods used in the study to detect individual clusters. The pattern of population structure observed in this species resembles that seen for other cetacean species in Pacific Ocean and Gulf of California. This result reinforces our understanding that habitat specialization is an important driver in the evolution of population structure in the common dolphin. The GC study provides the potential to understand this process in greater detail, given the various environmental gradients defined within the Gulf.

3.2 Introduction

The assessment and understanding of population structure in marine mammals is challenging because of their high potential for long distance movements and the absence of obvious boundaries to dispersal in the oceans, although many species of cetacean have shown strong population structure (Chivers et al. 2002, Hayano et al. 2004, Natoli et al. 2004, Escorza-Treviño et al. 2005, Adams and Rosel 2006, Oremus et al. 2007).

Common dolphins, genus *Delphinus*, are distributed worldwide in temperate and tropical waters of the Pacific Ocean, Atlantic Ocean, Mediterranean and Black Sea (Perrin 2002). This genus harbours a high degree of phenotypic polymorphism that had led historically to the description of nearly 30 nominal species throughout its distributional range; although, only *D. delphis* (Linnaeus 1758) was widely recognized (Hershkovitz 1966). The recognition of the Pacific Ocean long-beaked common dolphin as a second species in the genus (*D. bairdii*, Dall 1873) was later promoted (Banks and Brownell 1969); this species is now known as *D. capensis* (Rice 1998). Beak length emerged as a key phenotypic character defining local populations and putative species (Heyning and Perrin 1994). The short-beaked form, *D. delphis*, shows a worldwide distribution, while long-beaked forms show discrete distributions in the Pacific Ocean, South Africa and Indo-Pacific Ocean, including China and the Middle East (Perrin et al. 2009). However, recent genetic evidence suggested that the long-beaked form has evolved independently in different regions (Natoli *et al*. 2006 and Chapter 4).

In the last decade, studies conducted independently in different areas of the world have revealed a high phenotypic variation of the genus *Delphinus*, for instance, in the Pacific Ocean (Heyning and Perrin 1994, Pompa-Mancilla 2004), in the Atlantic Ocean (Murphy et al. 2006, Murphy and Rogan 2006) and in the Indo-Pacific Ocean (Jefferson and van Waerebeek 2002). However, common dolphins worldwide appear to be little genetically structured, despite their high levels of genetic diversity (Natoli et al. 2006, Amaral et al. 2007, Natoli et al. 2008, Viricel et al. 2008, Mirimin et al. 2009). With an exception of the South Australian and Tasmanian common dolphins that have been shown to be strongly genetically differentiated (Bilgmann et al. 2008).

The long-beaked, *D. capensis*, and short-beaked, *D. delphis*, common dolphin forms are sympatric in the Pacific Ocean (PO) and Gulf of California (GC). *D. delphis* preferentially inhabits offshore waters; although it has been suggested that its abundance and distribution changes with oceanographic conditions inter-annually and seasonally north-South as well as inshore-offshore (Forney and Barlow 1998). *D. capensis* typically occurs in shallower and warmer water closer to the coast than *D. delphis* (Heyning and Perrin 1994, Barbosa 2006). The evaluation of demographic isolation of *D. delphis* populations in the Eastern Tropical Pacific (ETP) revealed populations that are genetically distinguishable and that the population boundaries correspond to habitat boundaries (Chivers *et al.* 2005). Analyses of skull measurements, from stranded and by-catch specimens from within the GC and west coast of Baja California (WCBC), also revealed significant differentiation between these *D. delphis* populations (Pompa-Mancilla 2004).

Overall, studies in the PO and the GC are concentrated on *D. delphis* and little is known about *D. capensis* ecology and population genetics, despite of its abundance. Recent estimates of abundance of *D. capensis* in waters off California, Oregon and Washington are 15, 334 individuals (Barlow and Forney 2007), Mexican Exclusive Economic Zone (EEZ) along the PO 55, 000 individuals and within the GC 69,000 individuals (Gerrodette and Palacios 1996).

This study assessed the genetic diversity and population structure of *D. capensis* within the GC and western coast of Baja California, towards a better understanding of the evolution of population genetic structure of cetacean species in this region. The high habitat diversity present in the study area allowed testing the hypothesis that common dolphins within the GC and PO are genetically structured in association with local habitat differences. The long-beaked common dolphin normally occurs in large groups year round within the Gulf of California. Thus little or no gene flow is expected between the gulf and western coast of Baja California long-beaked common dolphin populations. Unexpectedly, a modest level of population genetic structure was estimated among the GC and PO long-beaked common dolphin populations. These results are valuable to the designation of management units of common dolphins and improve conservation action in the region, as the GC and adjacent waters are major fishing areas in Mexico.

3.3 Methods

3.3.1 Sample collection and DNA extraction and purification

Skin biopsy samples were collected from two different regions across the length of the Gulf of California and two along the western coast of Baja California (Figure 3. 1). The collection of skin biopsy samples and DNA extraction and purification was conducted as described in Chapter 2.

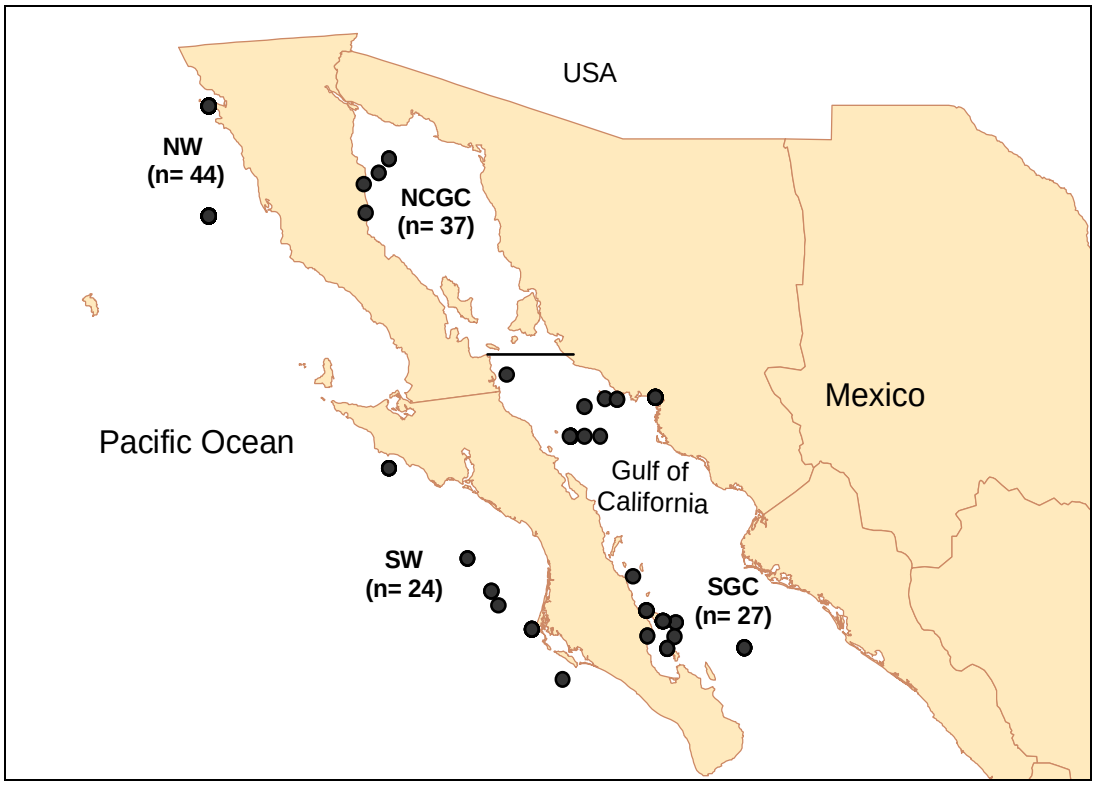

Figure 3. 1 Geographic distribution of *Delphinus capensis* samples and sample sizes analyzed.

3.3.2 Mitochondrial DNA (mtDNA) analyses

A fragment of 778bp of the mtDNA control region was amplified using the Polymerase Chain Reaction (PCR) and universal primers (mtcr F 5' TTC CCC GGT GTA AAC C 3' and mtcr R 5' ATT TTC AGT GTC TTG CTT T 3'). The PCR reactions were performed in 25µL volume with the following conditions: 10mM Tris-HCl, 50mM KCl, 2.5mM $MgCl_2$, 0.25mM each dNTP, 0.12µM each primer, 1.25 unit of *Taq* DNA polymerase (NEB, UK), and approximately 50ng of genomic DNA. The thermo-cycling

profile began with a hot start denaturation step of 5 min at 95ºC, following by 36 cycles of 45 sec at 51ºC, 1 min at 72ºC and 45 sec at 94ºC; and a final elongation step of 10 min at 72ºC. PCR products were verified using agarose gel electrophoresis. Positive reactions were purified with spin columns (QIAGEN, UK) and sequenced in an automatic sequencer (ABI 3730 Gene Analyzer, Applied Biosystems).

Sequences were checked with the software CHROMASlite (Technelysiun Pty. Ltd.) to verify base calling and aligned with CLUSTAL X (Jeanmougin et al. 1998). The evolutionary model that best fit the mtDNA sequences variation was tested with program MODELTEST 3.7 (Posada and Crandall 1998) and used in further analyses. Unique haplotypes were identified using DNAsp version 3 (Rozas and Rozas 1999). The indices of genetic diversity: haplotype diversity (H) and nucleotide diversity (π) and the fixation indices to assess the extent of genetic differentiation among regional populations (F_{st} and Φ_{st}), were estimated using ARLEQUIN (Schneider et al. 2000). A Mantel test was run to correlate genetic distances, linear $F_{st}/(1- F_{st})$ (Rousset 1997), with swimming straight-line geographic distances among regions as performed in ARLEQUIN (Schneider et al. 2000). The phylogenetic relationships among the mtDNA haplotypes were represented as a median-joining network rooted with *Tursiops* and were calculated using the program NETWORK 4.5.1.0 (Bandelt et al. 1999).

3.3.3 Microsatellite analyses

Eighteen microsatellite DNA loci, derived from bottlenose dolphin, orca, humpback whale and common dolphin, were amplified using two separate multiplex 8µL polymerase chain reaction (PCR) using Multiplex Kits (QIAGEN, UK). One multiplex reaction amplified the loci D08, KWM1b, KWM2a, KWM2b, KWM12a and TexVet5 with the following conditions 15 min at 95ºC, 40 cycles of 90ºC sec at 50ºC, 1 min at 72ºC, 30 sec at 94ºC following by 90 sec at 50 ºC and 30 min at 60ºC. The rest of the loci: AAT44, Dde09, Dde59, Dde65, Dde66, Dde69, Dde70, Dde72, Dde84, EV14, EV37Mn and TexVet9 were amplified in a second reaction with the following conditions15 min at 95ºC, 40 cycles of 90ºC sec at 7ºC, 1 min at 72ºC, 30 sec at 94ºC following by 90 sec at 57 ºC and 30 min at 60ºC.

Genotypes across all loci were scored using the software STRand 2.3.106 (Hughes 1998) and tested for the presence of allelic dropout, stuttering errors and null alleles using the program MICRO-CHECKER (Oosterhout 2004). A scan for loci under selection was carried out across all loci using the programs Lositan (Antao et al. 2008) and Bayescan (Foll and Gaggiotti 2008). Bi-parental genetic diversity (estimated as observed heterozygosity (*Ho*) and expected heterozygosity (*He)*, deviation from Hardy-Weinberg equilibrium, and regional differences in allele frequencies (*Fst*) were all computed in ARLEQUIN 2.0 (Schneider et al. 2000) to assess the regional differentiation among populations. Allelic richness, number of alleles per locus and sex-biased dispersal were tested by the estimations of F_{IS}, F_{ST}, relatedness, mean assignment index and variance of assignment indices were also estimated using FSTAT 2.9.3 (Goudet 2002). A Mantel test was used to correlate genetic distance defined as F_{st} /(1- F_{st}) and swimming straight-line geographic distances among regions, for both mtDNA using ARLEQUIN and microsatellite data using ISOLDE extension of GENEPOP.

A Bayesian clustering assignment method to estimate population structure was performed as implemented in STRUCTURE (Pritchard et al. 2000), whereby population clusters (K= number of cluster populations), with the minimum deviations from Hardy-Weinberg and linkage equilibrium were detected without *a priori* individual assignment to populations. This program uses the Markov chain Monte Carlo (MCMC) method to estimate the posterior probability $P(X \mid K)$, of the data to fit each hypothetical number of clusters (K). Five independent runs for each number of populations (k= 1 – 5) were performed using the correlated allele frequency and admixture models with 1,000,000 repetitions and a burn-in of 500,000. Population structure was inferred by the modal value of ΔK, which correspond to the rate of change in the likelihood of K and proved to be the optimal estimation for genetic population subdivision (Evanno et al. 2005). An additional Bayesian method to test for individual assignment was performed using microsatellite genotypic and geographical data as implemented in GENELAND (Guillot et al. 2005); this program test for the most likely number of populations using MCMC and estimates the posterior probability of individual membership to each population. Four independent runs were performed to simulate number of populations (K) after 100 000 MCMC iterations.

3.3.4 Sex determination

Sex was determined by amplifying fragments of the gene Zfy/x and SRY. The PCR reactions were performed in 10μL volumes consisting of 10mM Tris-HCl, 50mM KCl, 2.5mM MgCl2, 0.25mM each dNTP, 1 μM each primer: P15EZ: 5' ATA ATC ACA TGG AGA GCC ACA AGC T 3', P23EZ: 5' GCA CTT CTT TGG TAT CTG AGA AAG T 3', Sry-Y53-3c: 5' CCC ATG AAC GCA TTC ATT GTG TGG 3' and Sry- Y53-3d: 5' ATT TTA GCC TTC CGA CGA GGT CGA TA 3', 1.25 U of Klear Taq DNA polymerase (KBiosciences, USA) and approximately 50ng of genomic DNA. The thermo cycling profile consisted of a hot start denaturation step of 15 min at 95ºC, followed by 36 cycles of 45 sec at 60ºC, 1 min at 72ºC and 45 sec at 94ºC and a final elongation step of 10 min at 72ºC. PCR products were verified using agarose gel electrophoresis. The band for the gene Zfy/x appeared between 400 and 500 bp in both sexes, while the male/SRY band appeared at 200 bp in males only.

3.4 Results

3.4.1 Genetic diversity

We amplified fragments of 776bp of the mtDNA control region for a total of 132 individuals. Individuals were grouped into four putative populations based on the geographic region where they were sampled. We indentified 53 haplotypes defined by 50 segregating sites Table 3. 1. The average haplotype and nucleotide diversities estimated by region ranged from 0.92 to 0.967 and 0.017 to 0.018, respectively (Table 3. 2). Tajima's *D* values were all non-significant; conversely Fu's *Fs* values were large, negative and highly significant for all populations, suggesting possible population expansion (Table 3. 2).

Table 3. 1 Variable sites among the 53 mtDNA control region haplotypes found and the absolute frequency in each population

Haplotype	11111112 2233333333 3333344444 4455555555 6666677777 1704566660 4613444555 5678812444 5501335568 1566702337 1744512679 0293057012 8033647578 1390258922 3901324084	NW (n= 44)	SW (n= 24)	SGC (n= 27)	NCGC (n= 37)
Hap_47	CAAAAACTGC TTTACCGTCC TGACCTTAAT CTTTTCGTGT TAGGAGAATT	1			1
Hap_49	....A.	1	3	5	5
Hap_51	.... .A....C... ....A. .G........	1	1		
Hap_52	....C... ....A.			1	1
Hap_53	....C... ....A. ......G...			1	
Hap_54	....G......C ....A.		1	1	
Hap_55	....T.....C ......A.A. ...A......		1		1
Hap_56	.... .A.T.....C ......A.A. ...A......	5			
Hap_57	....T...... ......A.A. ...A......				1
Hap_58	....T...... ....A.	1			
Hap_59	.........T ....C.T ...T..C... ....C...A.	1			
Hap_60	....G....T ....T...... ....A.		2		
Hap_61	....G....T ...G...... ...T...... ....A.	1			
Hap_62	....G....T ...G...... ...T...... .....T..A.	1			
Hap_63	.........T ...G...... ...T...... .....T..A.		1		
Hap_64	.........T ...G...... ...T...... ....A. ...A....G.				2
Hap_66	.........T ...G...... ...T...... ....A.	4	1	2	1
Hap_67	.........T ...G...... C..T...... ....A.	1	3		1
Hap_69	.........T ....T...... ....A.	1			
Hap_70	.........T ....T...... .G......A.	1			
Hap_71	.........T ....T.C.... ....A.			1	
Hap_72	.G.......T ....T...... ....A.				1
Hap_73	.........T ....T...... ....A.		1	1	2
Hap_74	.........T ....T...... ....A. C.........			1	1
Hap_75	.........T ....T...G.. ....A. C.........			1	
Hap_76	.........T ....T...... ....AC				1
Hap_77	.........T .C........ C..T...... ....A. ...A......	1			
Hap_78	.......C.T .... C..T...... ..C....CA. ...A......	1			
Hap_79	.........T ....T.....C ..C.....A. ..AA......		1		
Hap_80	......T..T ..C......T ...T...... ..C.....AC			1	
Hap_82	.........T ....T...... ..C.....A. ....C..	1			
Hap_83	.........T ....T...... ..C.....A.				1
Hap_84	...G.....T ....G...T. ...T...... ....A.	7	1	2	
Hap_86	.........T ....T. ...T...... ....A. .....A....			1	
Hap_87	T........T ....T. ...T...... ....A. .....A....	1			
Hap_88	.........T ....T...... ....A. ....GA....	2	2		
Hap_91	.........T ....T...... ....A. .....A....	1			
Hap_92	.........T ....T...... .....TA.A.	1			1
Hap_93	.....G...T C....TA... ...T...... .....T..AC ...A....AC		1	2	1
Hap_94	.....G..AT .....TA.T. ...T...... T....T..AC ...A....AC		1	1	
Hap_95	.....G...T .....TA.T. ...T....G. .....T..AC ...A....AC	1		1	1
Hap_96	..G..G...T .....TA.T. ...T...... .....T..AC ...A....AC				1
Hap_97	.....G...T .....TA.T. ...TT..... .....T..AC ...A....AC			1	
Hap_98	.....G...T .....TA.T. ...T...... .....T..AC ...A....AC	1	2	1	10
Hap_99	.....G...T .....TA.T. ...T...... ...C.T..AC ...A....AC	1			
Hap_100	.....G...T ....TTA.T. ...T...... .....T..AC ...A....AC	4	1	2	
Hap_101	.....G...T ....TTA... ...T...... .....T..AC ...A....AC	2	1	1	
Hap_102	.........T ....TTA.T. ...T...... .....T..AC ...A....AC				1
Hap_103	.....G...T .....TA.T. ...T...... .....T..A. ...A....AC				1
Hap_105	.....G...T .....TA.T. ...T...... .....TA.A.				1
Hap_106	.....G...T .....T..T. ...T...... ....AC ....AC				1
Hap_107	.........T ....TTA.T. ...T...... ....A.				1

Table 3. 2 Haplotype and nucleotide diversity, *Tajima's D* and *Fu's F*s values estimated by population.

Population	Acronym	n	*H*	π	*Tajima's D*	*Fu's Fs*
Northwest Baja Coast	NWC	44	0.952	0.018	-0.864^{ns}	-9.152^{s***}
Southwest Baja Coast	SWC	24	0.967	0.017	-0.49^{ns}	-5.457^{s**}
South Gulf of California	SGC	27	0.959	0.018	-0.422^{ns}	-6.4^{s***}
North-central Gulf of California	NCGC	37	0.92	0.018	-0.113^{ns}	-9.095^{s***}

*p<0.05, **p<0.01, ***p < 0.001, ns: non significant, s: significant

A total of 140 long-beaked common dolphins were genotyped for 18 microsatellite DNA loci. No evidence of allelic dropout or scoring errors due to stuttering was found across all loci, except for the locus D08. This locus was eliminated from the dataset as it consistently showed errors across all populations. The presence of null alleles was detected in some loci, but their frequencies were not high or consistent across all populations (Table 3. 3). The examination for loci under natural selection conducted in BayeScan identified evidence for positive selection in the locus Tex Vet 9, and it was therefore not included in further analyses. A total of 16 microsatellite loci were used in the rest of the analyses. The number of alleles per locus ranged from 2 to 14. Heterozygosity estimates per locus and population are shown in Table 3. 3. Departure from HW equilibrium was detected only in locus Tex Vet 5 in the NW population. The exclusion of this locus did not change the results; therefore it was included in the analyses.

Table 3. 3 Genetic diversity at microsatellite loci for each population. No alleles: number of different alleles per locus, Ho: observed heterozygosity, He: expected heterozygosity, H-W: significance for deviation from H-W estimated by population. Nulls freq: frequency of null alleles per locus when applicable.

Locus		Pacific Coast		Gulf of California	
	Population	NW	SW	SGC	NCGC
AAT	No alleles	10	10	9	11
	Ho	0.715	0.913	0.757	0.913
	He	0.861	0.833	0.863	0.832
	H-W	0.026	0.239	0.036	0.239
	Nulls freq				
Dde09	No alleles	5	6	6	7
	Ho	0.792	0.652	0.781	0.652
	He	0.779	0.767	0.769	0.767
	H-W	0.958	0.245	0.228	0.244
	Nulls freq				
Dde59	No alleles	5	9	7	6
	Ho	0.682	0.783	0.788	0.783
	He	0.747	0.841	0.834	0.841
	H-W	0.038	0.284	0.068	0.283
	Nulls freq				
Dde65	No alleles	6	5	7	6
	Ho	0.636	0.826	0.697	0.826
	He	0.071	0.747	0.791	0.747
	H-W	0.014	0.727	0.347	0.727
	Nulls freq				
Dde66	No alleles	11	10	11	8
	Ho	0.698	0.913	0.757	0.913
	He	0.81	0.852	0.844	0.852
	H-W	0.105	0.614	0.432	0.614
	Nulls freq				
Dde69	No alleles	6	5	7	5
	Ho	0.763	0.714	0.633	0.714
	He	0.708	0.659	0.648	0.659
	H-W	0.009	0.419	0.259	0.419
	Nulls freq				
Dde70	No alleles	13	13	12	12
	Ho	0.933	0.826	0.939	0.826
	He	0.874	0.854	0.91	0.854
	H-W	0.999	0.623	0.408	0.623
	Nulls freq				
Dde72	No alleles	9	9	7	8
	Ho	0.841	0.869	0.812	0.869
	He	0.842	0.879	0.823	0.879
	H-W	0.057	0.339	0.306	0.339
	Nulls freq				
Dde84	No alleles	9	8	8	10
	Ho	0.717	0.652	0.727	0.652
	He	0.724	0.761	0.829	0.761
	H-W	0.366	0.041	0.388	0.041
	Nulls freq				
EV14	No alleles	14	12	13	14
	Ho	0.869	0.956	0.909	0.956
	He	0.902	0.888	0.901	0.888
	H-W	0.618	0.414	0.723	0.414
	Nulls freq				0.065

continued…

EV37	No alleles	8	8	10	12
	Ho	0.692	0.609	0.697	0.609
	He	0.687	0.784	0.818	0.784
	H-W	0.547	0.055	0.012	0.055
	Nulls freq				0.106
TexVet9	No alleles				
	Ho	0.064	0.087	0.091	0.087
	He	0.083	0.208	0.117	0.208
	H-W	1	0.051	1	0.051
	Nulls freq				
KWM12a	No alleles	8	8	8	11
	Ho	0.723	0.869	0.636	0.869
	He	0.756	0.8	0.816	0.8
	H-W	0.756	0.889	0.041	0.889
	Nulls freq			0.084	0.098
KWM1b	No alleles	3	3	2	2
	Ho	0.456	0.217	0.333	0.217
	He	0.436	0.371	0.379	0.371
	H-W	0.76	0.166	0.65	0.166
	Nulls freq				
KWM2a	No alleles	13	12	12	14
	Ho	0.684	0.956	0.969	0.956
	He	0.862	0.891	0.878	0.891
	H-W	0.004	0.342	0.777	0.342
	Nulls freq	0.089			0.091
KWM2b	No alleles	5	4	5	6
	Ho	0.581	0.739	0.697	0.739
	He	0.656	0.659	0.685	0.659
	H-W	0.886	1	0.489	1
	Nulls freq				
TexVet5	No alleles	11	8	9	12
	Ho	0.682	0.636	0.727	0.636
	He	0.864	0.792	0.859	0.792
	H-W	**<0.001**	0.177	0.153	0.177
	Nulls freq	0.091			0.152

3.4.2 Population structure

A number of shared mtDNA control region haplotypes were found among the four regions generating estimates of genetic differentiation of F_{st} = 0.022, p< 0.001 and Φ_{st}= 0.014, p = 0.121, over all populations. The maximum number of individuals sharing a haplotype was nine and the dataset contains a number of singletons (n=32). Population pairwise comparisons were estimated for both fixation indices using mtDNA control region data (Table 3. 4). Estimates of fixation indices were in general low and not statistically significant, except for the NCGC population (Table 3. 4).

Table 3. 4 Genetic differentiation based on mitochondrial DNA control region fixation indices. Below the diagonal Φst and above diagonal Fst pair-wise comparisons.

Population	NW (n= 44)	SW (n= 24)	SGC (n= 26)	NCGC (n= 40)
NW		0.012*	0.011	0.051**
SW	-0.012		-0.009	0.012*
SG	0.011	-0.016		0.022*
NCGC	0.047*	0.024	-0.001	

p < 0.05 * > p <0.008** after Bonferroni correction.

The population differentiation pattern based on microsatellite data was somewhat different from the findings based on mtDNA. Significant population differentiation was detected between the Gulf of California and the Pacific coast populations (Table 3. 5). However, Bayesian clustering analyses run in STRUCTURE failed in detect any signal of population structure. The highest likelihood that best explained the variability observed among microsatellite loci was at K=1, consistent for several independent runs. The modal value of K estimated as ΔK, rate of change in Ln(K), also indicated the lack of population differentiation (K=1). Equally, independent runs to simulate the number of populations using both geographical and genetic data, performed in GENELAND consistently indicated the presence of only one population (Figure 3. 3 and Table 3. 6).

Table 3. 5 Microsatellite Fst pair-wise comparisons based on 16 loci. p-values upper diagonal p= 0.008 after Bonferroni correction, Fst overall loci an d populations = 0.007, p<0.001.

	NW (n = 50)	SW (n = 23)	SGC (n =29)	NCGC (n= 36)
NW		0.739	<0.001	<0.001
SW	-0.002		0.036	<0.001
SGC	0.009*	0.016**		0.468
NCGC	0.011**	0.008*	0.001	

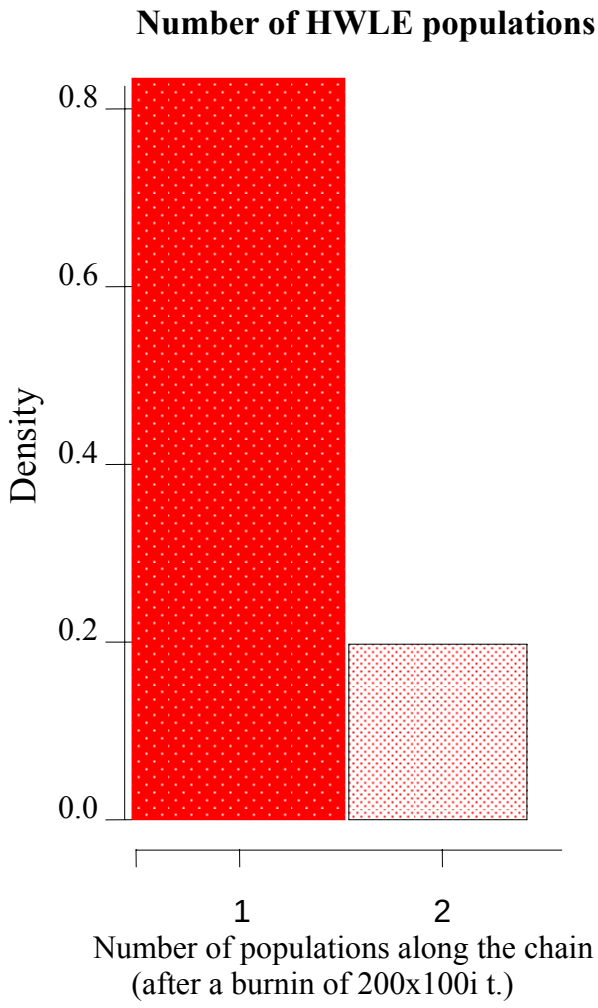

Figure 3. 2 Modal number of populations (K) simulated from posterior distribution (run 4). HWLE: populations under Hardy-Weinberg and Linkage equilibrium.

Table 3. 6 Multiple-runs computations for inferring the number of populations performed using GENELAND.

Run	*Number of populations*	Average log posterior probability
1	1 (69.49%)	-7141.989
2	1(64.59%)	-7100.367
3	1(67.89%)	-7149.115
4	1(72.69%)	-7187.675

The Mantel test performed for mtDNA and microsatellite data did not show a significant correlation of genetic distance to geographical distance (p = 0.083 and p = 0.081, respectively).

Testing sex-biased dispersal revealed lower estimated values of F_{st} or Relatedness for males than for females; however values were marginally not significant (Table 3. 7). Overall, the sex-biased dispersal test was unable to detect any sex-biased difference in the dispersal behaviour of males and females, though a larger sample size may have supported evidence for a male bias.

Table 3. 7 Statistical test for sex-biased dispersal between males and females over all populations. n = number of individual tested, *Ho*: observed heterozygosity; *He*: expected heterozygosity; F_{IS}: inbreeding coefficient; F_{ST}: fixation index, *R*: relatedness coefficient, *AIc*: mean corrected assignment index, *vAIc*: variance of the corrected assignment index *AIc*.

	n	F_{is}	F_{st}	Relatedness	*Ho*	*He*	*AIc*	*vAIc*
Females	61	0.059	0.017	0.032	0.682	0.725	-0.101	17.28
Males	66	0.051	0.004	0.008	0.687	0.723	0.092	21.399
p-values		0.701	**0.056**	**0.057**	0.792	0.871	0.803	0.383

3.4.3 Phylogeographic analysis

The Median-Joining network (MJN) of the mtDNA haplotypes did not show evidence of a clear phylogeographic pattern, although haplotypes from the NWC seem to concentrate in one region of the network. The MJN star-shape showed the close relationships among mtDNA haplotypes, indicating their recent divergence and rapid expansion. The haplotypes shared among the four populations appeared mostly at the center of the stars, while haplotypes found in just one population have a more external position (Figure 3. 3). Also, the MJN showed fewer reticulations among haplotypes found in dolphins from the western coast than those found in GC populations, indicating that perhaps the haplotypes found in the GC are older than those found in the PO.

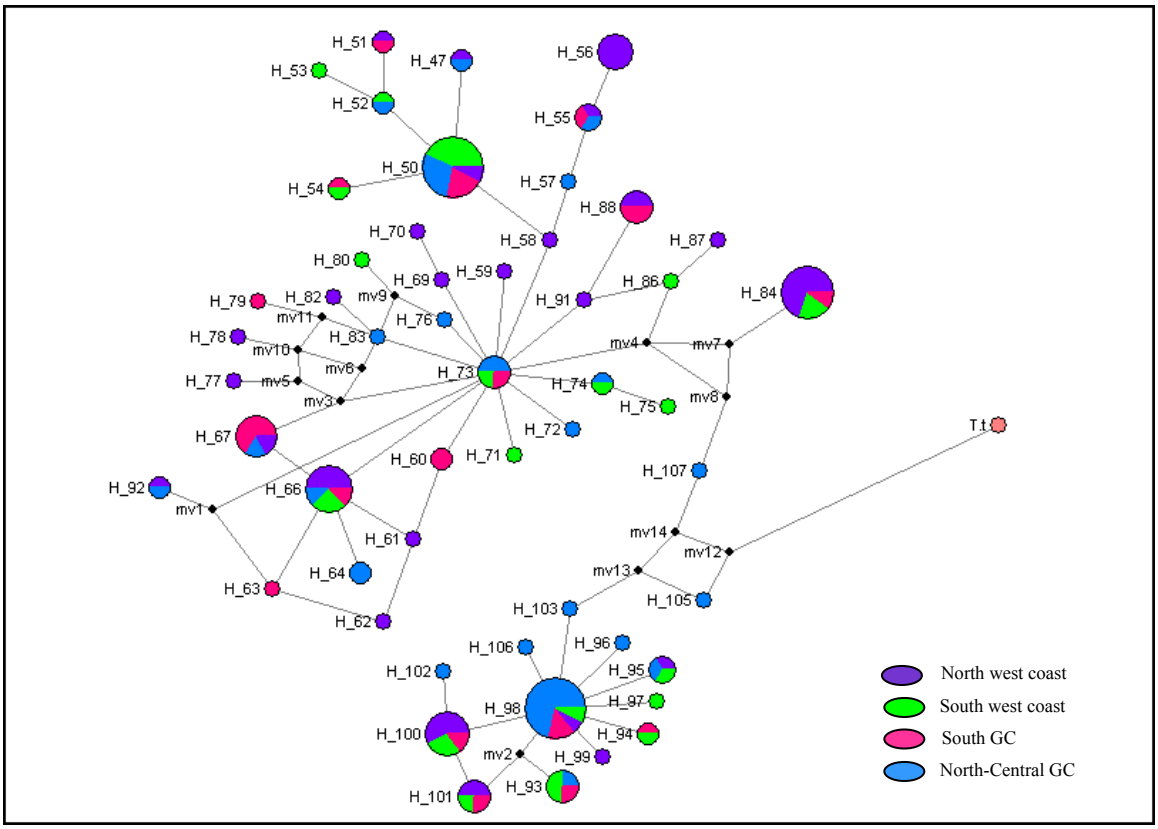

Figure 3. 3 Median-neighbour joining network estimated for phylogenetic relationships among mtDNA control region haplotypes, *Tursiops truncatus* rooted. Size of the circles is proportional to haplotype frequency. Color code represents the geographic region where haplotype was found.

3.5 Discussion

3.5.1 Genetic diversity

Estimates of genetic diversity were high for all regions, based on both mtDNA and microsatellite loci. The high values of observed heterozygosity (*Ho*) at 16 microsatellite loci, mtDNA haplotype diversity and number of singletons are indicative of a large population size (see estimates of female Ne, Table 11 Chapter 4) , which is consistent with the estimates of abundance for the PO (N= 55,000 individuals) and GC (N= 69,000 individuals) (Gerrodette and Palacios 1996). On the other hand, the low values of nucleotide diversity revealed the existence of many close phylogenetically related mtDNA haplotypes. Similar patterns of genetic diversity, high haplotype and low nucleotide diversities, are typical of large populations that have recently expanded rapidly from small populations, which allow the retention of new mutations without sufficient time for the accumulation of large differentiations among haplotypes [e.g. Atlantic herring, (Hauser et al. 2001) and Dover Pacific sole (Stepien 1999)]. The signal of population expansion was also confirmed by the Tajima's *D* values that were all negative, although not statistically significant; and the large, negative and highly significant values of Fu's *Fs* estimated for all populations (Table 3. 2).

3.5.2 Genetic structure

Population genetic structure was estimated over all regions; F_{st} values for both mtDNA and microsatellites were relatively low but highly significant. Pairwise comparisons for both markers consistently showed differentiation between the North-Central Gulf of California (NCGC) and the rest of the populations. However, differentiation between PO and GC populations were only evident for microsatellite loci. The inability to show significant population differentiation albeit the similar values in both nuclear and mtDNA fixation indices has been noticed in other species populations showing high haplotypes diversities; e.g. gray whales (Alter et al. 2009) and pelagic fishes, (Hauser et al. 2001). In such cases of high diversity, where many individuals carry a different haplotype and haplotypes are very closely related, the analyses of population genetic structure based haplotype frequency are complicated and not appropriate (Stepien

1999). As discussed above, *D. capensis* populations showed high levels of haplotype diversity and low levels of nucleotide diversity, but also the high number of singletons, which may explain the lack of resolution in mtDNA population structure analyses. In addition, multi-locus data are in general known to have much greater power to detect low levels of population structure, (Ryman et al. 2006).

Conversely, a low magnitude of population differentiation at microsatellite loci compromises the accuracy to detect clusters using linkage and Hardy Weinberg Equilibrium (Pritchard et al. 2000, Corander et al. 2003). An evaluation of the Bayesian clustering, as implemented in STRUCTURE, using simulated data detect a good performance when population differentiation are F_{st}= 0.02-0.03 or higher (Latch et al. 2006). Therefore, the estimates of $F_{st} < 0.02$ in this study, were inadequate for STRUCTURE (Pritchard et al. 2000) and GENELAND (Guillot et al. 2005) to detect differences between GC and PO common dolphin populations; although, it is likely that differences between basins do exist given the evidence of biogeographic distinction of the GC and PO (Bernardi et al. 2003, Sandoval-Castillo et al. 2004, Lin et al. 2009, Schramm et al. 2009). For example, the shovelnose guitarfish GC and PO populations are historically isolated, as revealed by lineage sorting and lack of gene flow (Sandoval-Castillo et al. 2004).

The pattern of genetic population structure found in *D. capensis* indicated restricted gene flow between PO and GC populations and a high dispersal range of individuals of both sexes within basins. The long-beaked common dolphin, *D. capensis,* occurs across the length of the GC during spring and mostly in the midriff islands and central region during autumn (Díaz-Gamboa 2009). In the west coast of Baja California *D. capensis* also occurs year round with an increase in its abundance during summer (Valles Jiménez 1998). This ample distribution in time and space was suggested to be a consequence of a more opportunistic diet biased toward fish prey than bottlenose dolphins, that feed preferably on giant squid (Díaz-Gamboa 2009). *D capensis* feed in coastal waters principally on sardines (*Sardinops sagax*), Pacific mackerel (*Scomber japonicus*), small schools of anchovies (*Engraulis mordax*), squid (*Loligo opalescens*), giant squid (*Dosidicus gigas*) and occasionally herring (*Harengula thrissina*) threadfin herring (*Opisthonema libertate*) and hake (*Merluccius productus*) (Gallo Reynoso 1991,

Díaz-Gamboa 2009). Regardless of the wide distribution observed in *D. capensis* within the GC and PO, high densities of dolphins were found to be associated to habitats with high chlorophyll concentrations (Díaz-Gamboa 2009), which were coincident with the maximum in sardine capture, indicating a suitable ecosystem to hold a number of marine mammals or any other top predators (Breese and Tershy 1993, Mercuri 2007). Even though common dolphins are known as opportunistic feeders, they seem to move in response to prey migration or seasonal fluctuations, as documented elsewhere, for instance in the Mediterranean Sea, South Africa and South Australia common dolphin high dispersal rates were found to be predicted by sardine distribution (Cockcroft 1990, Cañadas et al. 2002, Bilgmann et al. 2008, Cañadas and Hammond 2008).

The GC and the western coast of Baja California are highly variable ecosystems, where oceanographic conditions change seasonally, providing a variety of habitats that vary in time and space (Santamaría-del Ángel et al. 1994, Logerwell 2001, Soto-Mardones et al. 2004, Lluch-Cota et al. 2007, Mercuri 2007). Likewise, giant squid distribution changes seasonally north and south; during spring giant squid are found from the south of the midriff islands to the mouth of the GC while they are present in the north region only in Autumn (Díaz-Gamboa 2009). Sardine abundance also shows seasonal changes; within GC sardines are abundant in the central region during spring, migrate to the south for hatching during winter and then migrate to the north in summer for feeding (Sokolov 1974). Along the west coast of Baja sardines occur mostly year round, having maximum abundance peaks in Autumn in Ensenada (north west coast) and during Spring in Punta Eugenia (south west coast) (Félix-Uraga and Garcia-Franco 2004). The presence and abundance of potential common dolphin prey year round in the GC support the hypothesis of a resident population *D. capensis* inhabiting the gulf, with relatively low gene flow between the GC and PO, but high dispersal within basins mediated by prey abundance fluctuations.

The partial isolation of the GC and PO *D. capensis* populations is likely to be associated with habitat preferences related to prey abundance rather than isolation by distance as no correlation was found between genetic and geographical distances (Mantel test for both mtDNA and microsatellite data). Similarly, in South Australia, habitat differences (colder in Tasmania), in association with different prey abundance, seem to

explain the lack of gene flow between South Australian and Tasmanian common dolphin populations (Bilgmann et al. 2008). The oceanographic features in both GC and PO systems are distinct, for instance the average sea surface temperatures (SST) in the north GC range from 14 to 30 °C and 20- 30 °C in the south GC (Álvarez-Borrego 1983, Lluch-Cota et al. 2007), while in the PO SST are typically below 20 °C (Félix-Uraga et al. 1996). These two systems are distinguished as two biogeographic provinces which have distinct characteristic habitats, flora and fauna (Durham and Allison 1960). Indeed, genetic molecular analyses have revealed the isolation and genetic differentiation of fish populations within the GC e.g. blennioid fish (Riginos and Nachman 2001, Lin et al. 2009), cartilaginous fish (Sandoval-Castillo et al. 2004) and sardines (Ríos Vargas 2007). Thus the signal of population structure observed in this study supports the understanding that habitat specialization is an important driver in the evolution of the common dolphin population structure within the GC and PO as observed in *D. delphis* from South Australia and Tasmania (Bilgmann et al. 2008).

3.5.3 Phylogeography

The MJN showed a complex relationship among mtDNA haplotypes. The most common haplotypes exhibited several interconnections suggesting a certain degree of homoplasy and close relationships among ancestral haplotypes, as observed in other dolphin populations elsewhere e.g. *Stenella frontalis* in the Atlantic (Adams and Rosel 2006) and *D. delphis* in the Atlantic Ocean (Viricel et al. 2008). The star-shaped phylogenies are indicative of rapid and recent divergence of *D. capensis* in the region, but also the number of ancestral haplotypes (stars) suggests that more than one maternal lineage founded these populations, as all populations shared the ancestral haplotypes. The derived and less frequent haplotypes are mostly found in only one population as expected in highly structured populations, however, this interpretation is limited as most of the non-shared haplotypes are singletons. A larger sample size is needed in order to increase the representation of those apparently private haplotypes and the power of the molecular analyses and interpretations.

3.5.4 Conservation implications

Long-beaked common dolphins *D. capensis* are the most common cetacean within the GC and western coast of Baja California (WCBC). However, little is known about the species; for instance according to IUCN-Red List the knowledge to evaluate their vulnerability status is inadequate, which harms the current management actions and the preservation of the outstanding regional biodiversity. For instance, the GC is a unique and exceptionally productive ecosystem; which holds 33 out of the 39 cetacean species that inhabit Mexican waters.

D. capensis occurs year round within the GC and WCBC (Valles Jiménez 1998, Barbosa 2006, Díaz-Gamboa 2009), and likewise records of stranded animals were year round with an increment during the main fishing season (Gallo-Reynoso 2004, Bravo et al. 2005, Mercuri 2007). The GC and the WCBC have become the major fishing areas in Mexico due to the high productivity of these regions. Unfortunately, fishing and other anthropogenic activities in the region have adversely affected the cetacean community. For instance, the major cause of mortality of the endemic porpoise Vaquita, *Phocena sinus*, is the interaction with fishing nets (Rojas-Bracho et al. 2006, Jaramillo-Legorreta et al. 2007). These observations strongly suggest that *D. capensis,* alongside many other marine mammals, are also threatened by fishing activities within the GC and WCBC (Gallo-Reynoso 2004). However, there is not an accurate assessment of the real impact of fishing or any other anthropogenic activity on *D. capensis* populations, as this is limited by the lack of information currently available on the status of the species in the region.

The analyses of the genetic structure and diversity conducted here, suggest the presence of at least two differentiated management stocks, one in the GC and the other in the WCBC. Despite the potential for gene flow in the species the results of this study suggest that the evolution of *D. capensis* population structure is habitat dependant. Therefore, given the distinct oceanographic characteristics of the two basins, it is strongly recommended that the GC and WCBC *D. capensis* populations should be considered as two different management units in any current and future management actions.

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Chapter 4

Phylogeography and evolution of the short and long-beaked forms of common dolphins in the genus *Delphinus*

4.1 Abstract

Dolphins in the genus *Delphinus* exhibit high levels of morphological variation across their wide geographic distribution. The extent of intraspecific variation has vexed the taxonomic status of the genus. More than 20 nominal species of the genus *Delphinus* have been described, but only *Delphinus delphis* reached a full species taxonomic status until the mid 1990's. Further revision of the morphological variation of common dolphins from the Eastern Tropical Pacific (ETP) revealed the existence of the short-beaked and long-beaked forms of common dolphins. This pattern of differentiation has been identified in other regions of the world. However, the taxonomic relation between long-beaked common dolphins from the ETP and elsewhere is still unresolved.

This study has evaluated the genetic differentiation between long-beaked and short-beaked common dolphin from the Gulf of California and Pacific Ocean off Baja California and California, using both microsatellite and mitochondrial DNA control region sequences. The results supported the strong and significant differentiation between forms, although some extent of introgression between forms was found. Ecological and morphological divergence among common dolphin populations appears to be associated with changes in the paleoceanographic conditions of the region such that reciprocal monophyly between the sympatric *D. delphis* and *D. capensis* forms has evolved within the Holocene timeframe

4.2 Introduction

The apparent lack of geographic boundaries in the marine environment has challenged our understanding of how speciation or diversification occurs in the sea. Nevertheless, the application of molecular tools in phylogeography has revealed high levels of cryptic biodiversity and cases of ongoing or recent speciation in different taxa e.g. from sessile invertebrates (Landry et al. 2003), fish (Bernardi et al. 2003, Pondella et al. 2005, Lin et al. 2009) and even highly mobile animals such as marine mammals (Natoli et al. 2004). Diversification events in marine organisms may be driven by a variety of mechanisms, including vicariance events and the consequent reduction in dispersal, as proposed for divergent fish species following the formation of the Gulf of California, which isolate this basin from the Pacific Ocean (Jacobs et al. 2004), and local habitat dependence (Hoelzel 1998). Known examples of resource specialization provide clear evidence for intraspecific differential niche use; e.g., the killer whale, *Orcinus orca*, fish and marine mammal feeders (Hoelzel et al. 1998a, Foote et al. 2009). However, this would only lead to genetic structure among populations, if it also promotes a reduction in gene flow, for example through assortative mating or physical separation within local environments, or by strong local adaptation (Hoelzel 1998).

Among cetacean species there are a number of examples of differentiation between populations based on apparent differential habitat dependence. For example, in the eastern tropical Pacific (ETP) the pan-tropical spotted dolphin (*Stenella attenuata*) is subdivided into two subspecies, the coastal spotted dolphin (*S. attenuata graffmani*) and the offshore spotted dolphin (*S. a. attenuata*), (Perrin et al. 1994). Analyses of the mtDNA control region and microsatellite DNA loci suggested genetic differentiation between coastal and offshore forms and among coastal population along the ETP coast, consistent with morphological evidence (Escorza-Treviño et al. 2005).

In the genus *Sotalia,* two different ecotypes can be distinguished based on their morphological differentiation, previously considered nominal species (Rice 1998). Revisions of the taxonomic status of the genus *Sotalia* recognized only one full species, *S. fluvitalis*, which include the two ecotypes as subspecies *S. fluvitalis fluvitalis* (riverine ecotype) and *S. fluvitalis guianensis* (coastal ecotype), (Rice 1998). However, a more recent study based on multi-loci genetic divergence and phylogenetic patterns, together

with the morphological and biogeographical evidence, strongly supports the recognition of these two *Sotalia* ecotypes as full species (Caballero et al. 2007).

In waters off South Africa, China and the Indo-Pacific Ocean the bottlenose dolphin, *Tursiops sp*, coastal and offshore ecotypes exhibit a significant degree of phenotypic differentiation (Ross and Cockcroft 1990, Wang 2000b, Wang 2000a). Molecular evidence, based on mtDNA control region sequences supported the distinction of the Chinese coastal bottlenose dolphin as a different species, *T. aduncus* (Wang et al. 1999), which supported the clarification of the taxonomic status of the Indo-Pacific bottlenose dolphin to full species status (Rice 1998).

In general, the taxonomic status of cetacean species has been controversial, given the high level of intra-specific phenotypic and genetic polymorphism and the recent radiation of the group. Many of the phylogenetic relationships among delphinid species in particular remain unresolved (LeDuc et al. 1999, May-Collado and Agnarsson 2006). A recent analysis using amplified fragment length polymorphism (AFLP) provided some further resolution between closely related delphinid species, although deep internal relationships were still not well supported (Kingston et al. 2009).

For the genus *Delphinus* in particular, morphological variation across their wide geographic distribution has complicated the assignment of alpha taxonomy. More than 20 nominal species of the genus *Delphinus* had been historically described (Hershkovitz 1966), but only *Delphinus delphis* reached a full species taxonomic status until the mid 1990's (Evans 1994). Further revision of the morphological variation of common dolphins from the ETP revealed the existence of the long-beaked form of common dolphin (Banks and Brownell 1969). Later revision of the morphological differences between these forms supported the recognition of *D. capensis*, (first named as *D. bairdii*, Dall 1873), as a distinct species in the genus. Analyses of mitochondrial DNA control region and nuclear AFLP makers provided evidence to designate the long-beaked common dolphin form as a different species *D. capensis* (Rosel et al. 1994, Kingston and Rosel 2004, Kingston et al. 2009). The same pattern of morphological differentiation was identified in other regions of the world, and a third morphotype identified with an even longer rostrum and greater number of teeth. This morphotype was identified in the Indian Ocean and waters off China, and has been proposed to be a subspecies of *D.*

capensis, *D. capensis tropicalis* (Jefferson and van Waerebeek 2002). This subspecies is distributed as follows: Red Sea, Gulf of Aden, waters off Somalia; and the Arabian Peninsula, Gulf of Oman and the Persian Gulf, Pakistan, India, Sri Lanka, South and East China and Southern Japan, (Perrin *et al.* 2009). The lack genetic differentiation of short and long- beaked common dolphins, elsewhere, for instance in the Atlantic Ocean, Argentina, Mauritania and South Africa did not support the status of the long beaked common dolphin as a different species at a global scale, but suggests convergence of morphotype evolution in different regions (Natoli et al. 2006). Therefore, the taxonomic relationship between long-beaked common dolphin populations is still unclear (Natoli et al. 2006, Perrin et al. 2009).

In the Atlantic Ocean, short-beaked common dolphins, *D. delphis*, show a complex pattern of population differentiation. The comparisons of skull measurements revealed some degree of latitudinal variation, and evidence that short-beaked common dolphins from the eastern North Atlantic are larger than California dolphins (Murphy et al. 2006). Moreover, significant genetic differentiation was found between eastern and western regions of the Atlantic Ocean, but not among localities in the eastern North Atlantic, even over a range of up to 1000km (Natoli et al. 2006, Amaral et al. 2007, Mirimin et al. 2009). Even the differentiation between the east and west was relatively weak, with first generation migrants (as identified by microsatellite genetic analyses) suggesting possible trans-Atlantic migration (Mirimin et al. 2009). Although, differentiation was found between a population off Greece and samples from elsewhere in the Mediterranean and eastern North Atlantic (Natoli et al. 2008)

The short-beaked common dolphins are the most common odontocete in the ETP (Gerrodette and Palacios 1996), whereas long-beaked common dolphin are the most common within the Gulf of California (Hansen 1990, Breese and Tershy 1993, Gerrodette and Palacios 1996, Díaz-Gamboa 2009). For instance, the estimated abundance along the California Current System for the short-beaked was 352,069 individuals (234,430-489,826), while the long beaked was 21,902 individuals (4833-43,765) (Barlow and Forney 2007). However, studies of the genus *Delphinus* are limited within the GC and western coast of Baja California. The extent of polymorphism exhibited among common dolphins in the ETP led to the designation of five management

units based on their distribution and body length, three of them within Mexican waters: 1) the Northern common dolphin, 2) Baja- Neritic common dolphin, 3) Central common dolphin, 4) Guerrero common dolphin, and 5) Southern common dolphin (Perrin *et al*. 1985) (Figure 4. 1). The long-beaked form was included in the Baja-Neritic common, while the short-beaked as Northern common dolphin (Smith 1979 in (Perrin et al. 1985).

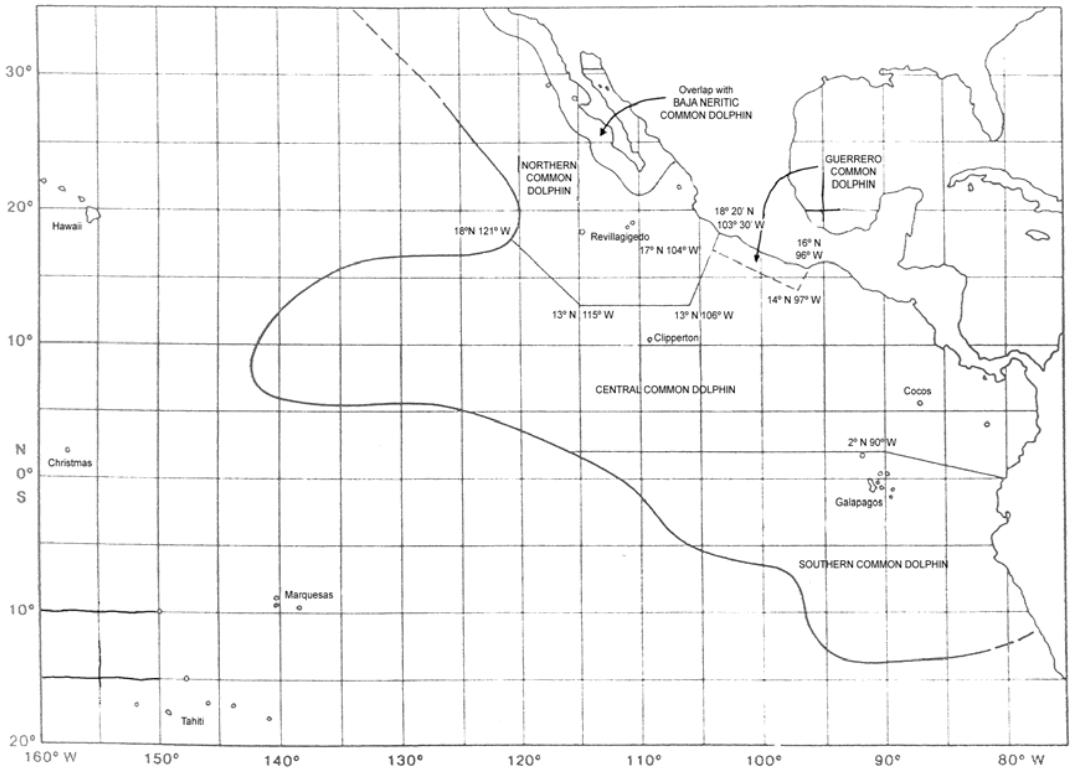

Figure 4. 1 Distribution of the common dolphin management units proposed by Perrin et al. 1985.

A more recent and comprehensive morphological analysis evidenced the presence of the two sympatric forms of common dolphin: short-beaked *D. delphis* and long-beaked *D. bairdii (*now *D. capensis)*, with clearly distinct features (*D. delphis* total length ranges 172- 201cm in males and 164-193 in females and 200 Kg weight, while *D. capensis* ranges 202-235 cm males, 193-224 females and 235 kg weight) (Heyning and Perrin 1994). The two sympatric forms differ in the ratio of rostral length and zygomatic width *D. delphis* ranges 1.21-1.47 and in *D. capensis* 1.52- 1.77 (Heyning and Perrin 1994). Figure 4. 2 and Figure 4. 3, illustrate the differences in coloration pattern and beak length between the two common dolphin forms.

Despite the fact that these two putative species occur in sympatry, the long-beaked form, *D. capensis,* typically occurs in shallower and warmer water close to the coast; whereas *D. delphis* is distributed from the coast to several kilometres offshore (Heyning and Perrin 1994).

As mentioned before, in the Pacific Ocean these putative species are genetically distinct (Rosel et al. 1994, Natoli et al. 2006, Kingston et al. 2009); however, their phylogenetic relationships are not well understood in the Pacific, neither is the alfa-taxonomy of the genus worldwide. This study test the hypothesis that the Pacific common dolphin short and long-beaked forms are genetically divergent at the species level as a result of local habitat changes occurred in the past. This study investigated the molecular phylogenetic relationships between the two Pacific short and long-beaked forms using both mitochondrial control region sequences and nuclear microsatellite loci. The results strongly support the divergence between the two *Delphinus* forms in the Pacific Ocean and suggested that this diversification event might result from geological changes in the environment during the Holocene.

Figure 4. 2 Long-beaked common dolphin, *D. capensis*. Photo by Iris Segura.

Figure 4. 3 Short-beaked common dolphin, *D. delphis.* Photo by Iris Segura.

4.3 Methods

4.3.1 Sample collection

Skin biopsy samples were collected from different regions across the length of the Gulf of California and the north-western coast of Baja California (Figure 4. 4). Sample collection was conducted as described in Chapter 1. The biopsy sample set (n= 120) was complemented with DNA samples (n= 166) that were obtained from the DNA tissue archive from SWFSC-NOAA (South West Fisheries Science Center of the National Oceanographic and Atmospheric Administration in the USA) (Figure 4. 4). Additional tooth samples were also obtained (n=72). Unfortunately, only 22 tooth samples were successfully analyzed because of the poor quality and quantity of DNA extracted. The total number of samples used for mitochondrial and microsatellite analyses are summarized in Table 4. 1.

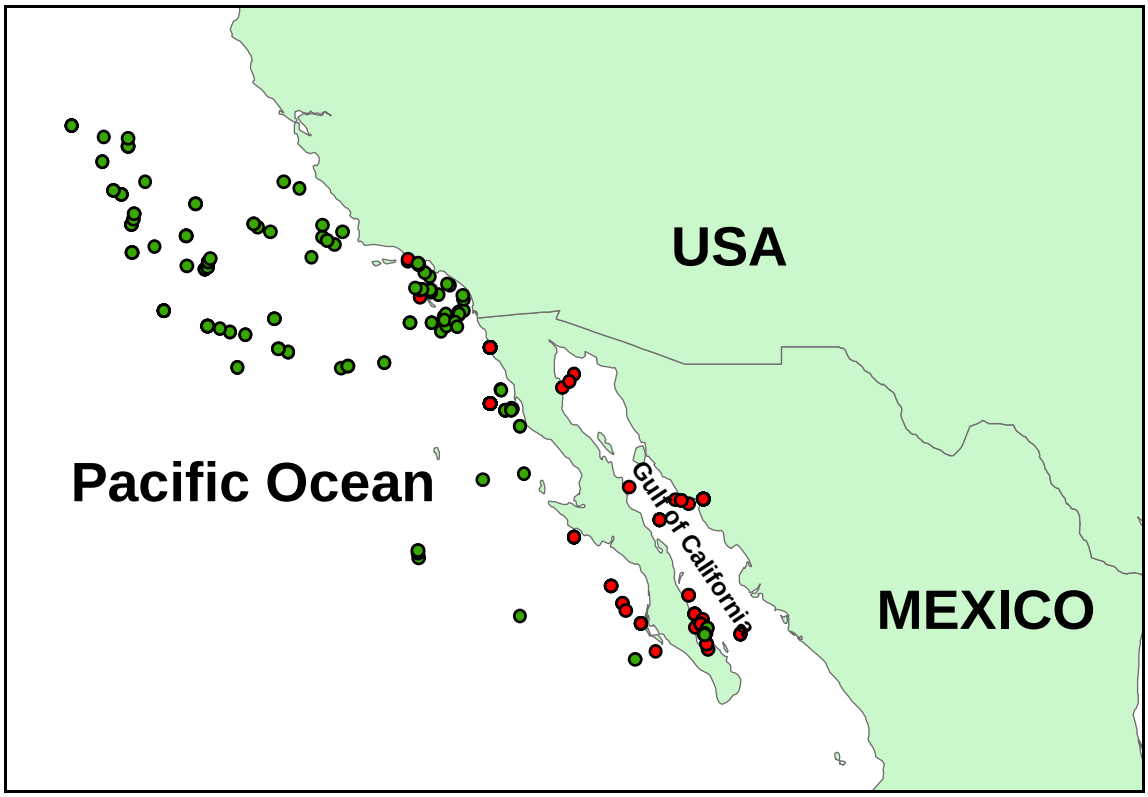

Figure 4. 4 Geographic location of individual samples used in this study. Long-beaked form, *D. capensis*: red circles, short-beaked form, *D. delphis* green circles. Circles might represent more than one dolphin individual sampled in the same location.

Table 4. 1 Number of individuals each putative species included in this study. Number of mtDNA control region sequences analyzed and number of individuals genotyped.

Population	*MtDNA*	Microsatellites
D. capensis	142	170
D. delphis	50	138

4.3.2 DNA extraction and purification

DNA was extracted from biopsies following the phenol-chloroform or salt saturation protocols described by Sambrook *et al*. (2001) and Aljanabi and Martínez (1997), respectively. Bone and tooth samples were processed in an ancient DNA facility in order to prevent cross contamination. DNA from bone and tooth samples was extracted by drilling the solid tissue down to a powder. In preparation for drilling, samples were treated with 10% bleach solution to remove any contaminating DNA that may have collected on the outer surface and rinsed with deionised water. The powder drilled from the outer layer was discarded. The rest of the powder was collected in tubes with 3mL of digestion buffer (0.425 M EDTA pH 8, 0.5% Sodium dodecyl sulphate, 0.05 M tris pH 8.5) and 0.5 mg/mL Proteinase K.The samples were incubated in a rotator overnight at 55 ºC. DNA was then extracted following the spin purification columns purification protocol (QIAGEN, UK).

4.3.3 Mitochondrial DNA (mtDNA) analyses

Fragments of about 776 base pairs (bp) from the mtDNA control region were amplified using the Polymerase Chain Reaction (PCR) and universal primers (mtcr F 5' TTC CCC GGT GTA AAC C 3' and mtcr R 5' ATT TTC AGT GTC TTG CTT T 3'). The PCR reactions were performed in 25µL volume with the following conditions: 10mM Tris-HCl, 50mM KCl, 2.5mM $MgCl_2$, 0.25mM each dNTP, 0.12µM each primer, 1.25 unit of *Taq* DNA polymerase (NEB, UK), and approximately 50ng of genomic DNA. The thermo cycling profile began with a hot start denaturation step of 5 min at 95ºC, followed by 36 cycles of 45 sec at 48ºC, 1 min at 72ºC and 45 sec at 94ºC; and a

final elongation step of 10 min at 72ºC. PCR products were verified using agarose gel electrophoresis scanning. Positive reactions were purified using purification spin columns (QIAGEN, UK) and following sequencing reaction, products were sequenced in an automatic sequencer (ABI 3730 Gene Analyzer, Applied Biosystems).

Sequences were checked with the software CHROMAS Lite (Technelysiun Pty. Ltd.) to verify base call and aligned using CLUSTAL X (Thompson *et al*. 1994).Unique haplotypes were identified using DNAsp version 3 (Rozas and Rozas 1999). The best evolutionary model that fit the mtDNA sequence variation observed was tested with MODELTEST 3.7 (Posada *et al.* 1998). The best evolutionary model suggested by MODELTEST 3.7 was used in all further analyses. The extent of genetic differentiation between the putative species (using fixation indexes F_{st} and Phi_{st}), haplotype diversity (h), nucleotide diversity (π), Tajima's D and Fu's F_s test of selective neutrality were estimated using ARLEQUIN (Excoffier *et al*. 1992). Evolutionary pair-wise sequence divergence was estimated between putative species in MEGA 4 (Tamura et al. 2004). Additional published sequences of short-beaked and long-beaked common dolphins from the Pacific Ocean (Rosel et al. 1994), South Australia and Tasmania (Bilgmann et al. 2008), eastern North Atlantic, South Africa and Mauritania (Natoli *et al.* 2006), were compared with those generated in this study.

Historical demographic expansion was investigated by examination of the distribution of pairwise differences between mtDNA control region sequences (mismatch distribution), (Rogers and Harpending 1992, Excoffier 2004). Multimodal distributions are generally expected in samples from populations at demographic equilibrium, and usually unimodal in populations that recently underwent a population expansion (Rogers and Harpending 1992, Excoffier 2004). The parameters of the demographic expansion $\boldsymbol{\theta_0}$, and $\boldsymbol{\theta_1}$ that correspond to mutation parameter before and after population growth; and $\boldsymbol{\tau}$, an index of time since expansion expressed in mutational time, were estimated by a generalized non-linear least square approach using ARLEQUIN (Schneider et al. 2000).

4.3.4 Microsatellite analyses

Sixteen bi-parental inherited microsatellite DNA loci were amplified by PCR using two separate primer multiplexes in 8µL using the Multiplex Kit (QUIAGEN, UK).

One multiplex reaction amplified the loci KWM1b, KWM2a, KWM2b, KWM12a and TexVet5 with the following conditions: 15 min at 95ºC, 40 cycles of 90ºC sec at 50ºC, 1 min at 72ºC, 30 sec at 94ºC following by 90 sec at 50 ºC and 30 min at 60ºC. The rest of the loci: AAT44, Dde09, Dde59, Dde65, Dde66, Dde69, Dde70, Dde72, Dde84, EV14 and, EV37Mn were amplified in a second reaction with the following conditions: 15 min at 95ºC, 40 cycles of 90ºC sec at 7ºC, 1 min at 72ºC, 30 sec at 94ºC following by 90 sec at 57 ºC and 30 min at 60ºC.

Genotypes across all loci were tested for the presence of allelic dropout and null alleles using the program MICRO-CHECKER (Van Oosterhout *et al*. 2004). Bi-parental genetic diversity (estimated as observed heterozygosity (*Ho*) and expected heterozygosity (*He)*), differentiation based on Wright's inbreeding coefficient (*Fst*) and deviation from Hardy-Weinberg equilibrium were all computed in ARLEQUIN 2.0 (Schneider *et al.* 2000) to compare between putative species. Allelic richness and number of alleles per loci and F_{IS} were also estimated using FSTAT 2.9.3 (Goudet 2002). Test for sex-biased dispersal between individuals of putative species was also performed using FSTAT 2.9.3 (Goudet 2002). The Bayesian clustering assignment method to estimate population structure was performed as implemented in STRUCTURE (Pritchard *et al.* 2000), whereby population clusters were detected without *a priori* assignment to populations and assuming the admixture model. Five independent runs for each number of populations (k= 1 - 5) were performed using the correlated allele frequency and admixture models with 1,000,000 repetitions and a burn-in of 500,000. Individual immigration or possible migration ancestry was tested in STRUCUTRE (Pritchard *et al.* 2000) by using the admixture model with prior population information of migration rates (v = 0.005 and 0.01) and testing 0< number of generations >2. Recent migration rates between putative species were estimated using Bayesian multilocus genotyping approach as implemented in BayesAss (Wilson and Rannala 2003). This approach allows the inferences of asymmetric migration rates and also individual assignments. The MCMC was run for 900,000 iterations, a burn-in of 3,000,000 iterations and migration delta values were tested as 0.01 and 0.005.

4.3.5 Isolation with migration (IMa)

A coalescent approach method as implemented in "Isolation and Migration" (Hey and Nielsen 2007) was performed to estimate marginal probability distributions for demographic parameters related to the diversification process between the two putative *Delphinus* species. The main parameters are: time since population-divergence ($T= t \mu$), asymmetric migration rates between putative species ($M_1= m_1/\mu$, $M_2= m_2/\mu$), and the two contemporary and one ancestral effective population sizes N_e, based on neutral population genetic diversity ($\theta= 4N_e\mu$). Estimates of marginal probabilities were scaled by mutation rate (μ) using the hypervariable region (HVR1; 340bp) and microsatellite data. The posterior estimates of the model were converted to demographic units, *i.e.* effective population sizes and divergence time in years using the appropriate mutation rate. For the mtDNA HVR1 was $\mu= 5 \times 10^{-7}$ substitution per site per million years, which is within the intervals of estimates based on ancient DNA datasets over several species (Lambert et al. 2002, Ho et al. 2005, de Bruyn et al. 2009), and for microsatellite data $\mu= 5 \times 10^{-4}$ per generation, which is considered as the average mutation rate over many species, including cetaceans (Estoup et al. 2002, Sun et al. 2009, Fontaine et al. 2010), and a generation time of 22.4 years (Taylor et al. 2007). To assure parameter convergence each run was carried out with burn-in 100,000 steps, 200 chains and the HKY substitution model for mitochondrial data and the stepwise mutation for microsatellites.

4.3.6 Phylogenetic analyses

A neighbour-joining phylogenetic reconstruction of mtDNA haplotypes was conducted in PAUP v 4.0 (Swofford 2002) and rooted with a homologous sequence from *Sotalia fluviatilis (*Genbank accession number EF027091) and *Tursiops truncatus*. An alternative representation as a median-joining network rooted with *Tursiops truncatus* was also generated using the program NETWORK 4.5.1.0 (Bandelt et al. 1999).

In addition, a Bayesian inference tree was estimated using MrBayes 3.1.2 (Ronquist and Huelsenbeck 2003), and a model of nucleotide substitution selected under the Akaike Information Criterion and maximum likelihood using MODELTEST 3.7 (Posada *et al.* 1998). The parameters of the substitution model derived from

MODELTEST 3.7 were fixed in the MCMC analysis. The model of substitution used was GTR+G+I, gamma shape parameter was fixed at α = 0.72 and the proportion of invariable sites was fixed at Pinvar = 0.73. Convergence was reached when the values for standard deviation of split frequencies fluctuated below 0.01. Two independent runs of 13740000 generations and a burn-in of 34350 generations were completed. All initial trees before convergence were discarded and the consensus tree and posterior probabilities for nodes were estimated from the remaining post-burn-in sampled trees.

4.4 Results

4.4.1 Genetic differentiation

A total of 193 samples were analyzed; 106 mtDNA control region haplotypes were identified showing 105 variable sites among 778 base pairs (bp). There was only one fixed difference between the long-beaked and short-beaked forms, a G/A transition in the nucleotide position 213 (Table 4. 2). The model of molecular evolution that best fit the mtDNA haplotype variation was Tamura-Nei (Tamura and Nei 1993) with a proportion of variable sites (*I*= 0.072) and among-site rate variation modelled with a gamma distribution (shape parameter α= 0.073), based on maximum likelihood and the Akaike information criterion.

Haplotype and nucleotide diversities were relatively high in both common dolphin putative species (Table 4. 3), however, the long-beaked form, showed lower diversity indices than the short-beaked form. This suggests that short-beaked common dolphin comprises a large population size or could also be related to a different demographic history, different life history, or some combination of different factors. No shared haplotypes were observed between the two putative species, which suggests no current female-mediated gene flow and that populations have been isolated for a certain period of time. Likewise, estimates of genetic differentiation revealed low but highly significant values for F_{st} (0.021, $p<0.001$) and Phi_{st} (0.466, $p<0.001$), which suggest restricted gene flow among the putative species.

Table 4. 2 Variable sites among mtDNA control region haplotypes, only the first 425 bp shown. Fixed mutation shown in the 213 bp in the long-beaked form haplotypes (Hap 47-107).

Haplotype	[11111111 1111111111 2222222222 2333333333 3333333333 3333333444] [1700444455 5566666677 0001224566 7001223333 3444455555 6677888012] [1704045845 6801236702 7893580028 5259261367 9015701289 0213136443]
DdHap_1	CATAGACTCA CTCACATATC ATAGTGTATT TCGTACCATC TCTTACCCTC GTTAGTCGCT
DdHap_3	..C..... ....T..... C...G..... ....A...T.
DdHap_4	..C..... ....T..... C...G..... ....A...T.
DdHap_5	..C..... ....T..... C...G....T ....A...T.
DdHap_6	..C..... ...G...... ..T..... C...G..... ......T... ....A...T.
DdHap_7	..C...T... ....T..... C......... ......T... ....A...T.
DdHap_8	..C..... ....T..... C....T.... ...C...... ....A...T.
DdHap_9	..C..... ....T......C C...G..... .T........ ....A...T.
DdHap_10	..C..... ....T......C C...G..... .T........ ....A...T.
DdHap_11	..C..... ....T..... CTA..... .T........ ....A...T.
DdHap_12	..C..... ....G.. ..T..... C......... .T........ ....A.....
DdHap_13	....T..... C......... .....T.... ....A...T.
DdHap_14	....T..... C......... .....T.... ....A...T.
DdHap_15	..C....C.. ..T..... ....A...T.C. C....TT... ....T.
DdHap_16	..C....C.. ..T..... ....A...T.C. C....TT... ....T.
DdHap_17	..C....C.. ..T..... ....A...T.C. C....TT... ....T.
DdHap_18	..C...T... ....T..... C.....T... C.....T... ....A...T.
DdHap_19	..C..... ....T ..T..... C......... .....TT... ....A...T.
DdHap_20	..C..... .... .CT..A.G.. C......... .....T.... ....A...T.
DdHap_21	..C..... ....T..... C.A..... ..C...T... ..C.A...T.
DdHap_22	..C..... ....T..... C.A..... .TC...T... ..C.A...T.
DdHap_23	..C..... ....T..... C.A..... ..C...T... ....A..AT.
DdHap_24	..C..... ....T..... C.A..... ..C..... A...A...T.
DdHap_25	..C..... ....T..... C.A..... C.C..... T...A...T.
DdHap_26	..C..... ....T.C..... C.A..... C.C..... T...A...T.
DdHap_27	..C..... ....C. ..T.....C. C.A..... .TC..... ....A...TC
DdHap_28	..C..... ....T.....C. C.A..... .TC..... ....A...T.
DdHap_29	..C..... .....G.... ..T.....C. C.A..... ..C..... ....A...T.
DdHap_30	..C..... .... G.T..... C.A..... CTC...T... A...A...T.
DdHap_31	..C..... .... G.T..... C.A..... CTC...T... A...A...T.
DdHap_32	..C.A.... ....T..... C.A..... .TC..... ....A...T.
DdHap_33	..C..... ....T..... C.A..... CTC.G.T..T ....A...T.
DdHap_34	..C..... ....T..... C......... ..C..... ....A.....
DdHap_35	..C..... ....T..... C.A..... .T........ ....A.....
DdHap_36	..C..... .C........ ..T..... C......... .TC..... .C..A.....
DdHap_37	.... TC........ ..T..... C......... .TA..T.... ....A...T.
DdHap_38	.... TC........ ..T..... C......... .TA..T.... A...A...T.
DdHap_39	.... T......... ..T..... C......... .TA..... .C.GA.....
DdHap_40	..C..... .... G.T.....C. C.A..... ..C...T... ....A.....
DdHap_41	..C..... .... G.T.....C. C.A..... ..C...T... ....A.....
DdHap_42	..C..... ....G.. G.T.....C. C......... ..C...T... ....A.....
DdHap_43	..C..... ....C.....C. C.A..... ..C...T... ....A...T.
DdHap_44	..C.....T. ....T..... C......... ..C...T... ....A...T.
DdHap_45	..C..... ....C..... CT........ ..C...T... ....A...T.
DdHap_46	..C..... ....G.. ..T..... C......... ..C..... ....A.....
DdHap_108	....G.. ..T..... C......... ..AC..... ....A...T.
DcHap_47	..C..... ....G.. ..CA..... C......... ..CCGT.... ....AC..T.
DcHap_49	..C..... ....G.. ..CA..... C......... ..CCGT.... ....AC..T.
DcHap_50	..C..... ....G.. ..CA..... C......... ..CCGT.... ....AC..T.
DcHap_51	..C..... ....G.. ..CA..... C......... ..CCGT.... A...AC..T.
DcHap_52	..C..... ....G.. ..CA..... C......... ..CCGT.... ....AC..T.
DcHap_53	..C..... ....G.. ..CA..... C......... ..CCGT.... ....AC..T.
DcHap_54	..C..... ....G.. ..CA..... C......... ..CCGT.... ...GAC..T.
DcHap_55	..C..... ....G.. ..CA..... C......... ..CCGT.... ....A...T.
DcHap_56	..C..... ....G.. ..CA..... C......... ..CCGT.... A...A...T.
DcHap_57	..C..... ....G.. ..CA..... C......... ..CCGT.... ....A...T.
DcHap_58	..C..... ....G.. ..CA..... C......... ..CCGT.... ....A...T.
DcHap_59	..C..... ....G.. ..TA..... C......... ..CCG..T.. ....A...T.
DcHap_60	..C......G ....G.. ..TA..... C......... ..CCGT.... ....A...T.
DcHap_61	..C......G ....G.. ..TA..... C......G.. ..CCGT.... ....A...T.
DcHap_62	..C......G ....G.. ..TA..... C......G.. ..CCGT.... ....A...T.
DcHap_63	..C..... ....G.. ..TA..... C......G.. ..CCGT.... ....A...T.
DcHap_64	..C..... ....G.. ..TA..... C......G.. ..CCGT.... ....A...T.

continue...

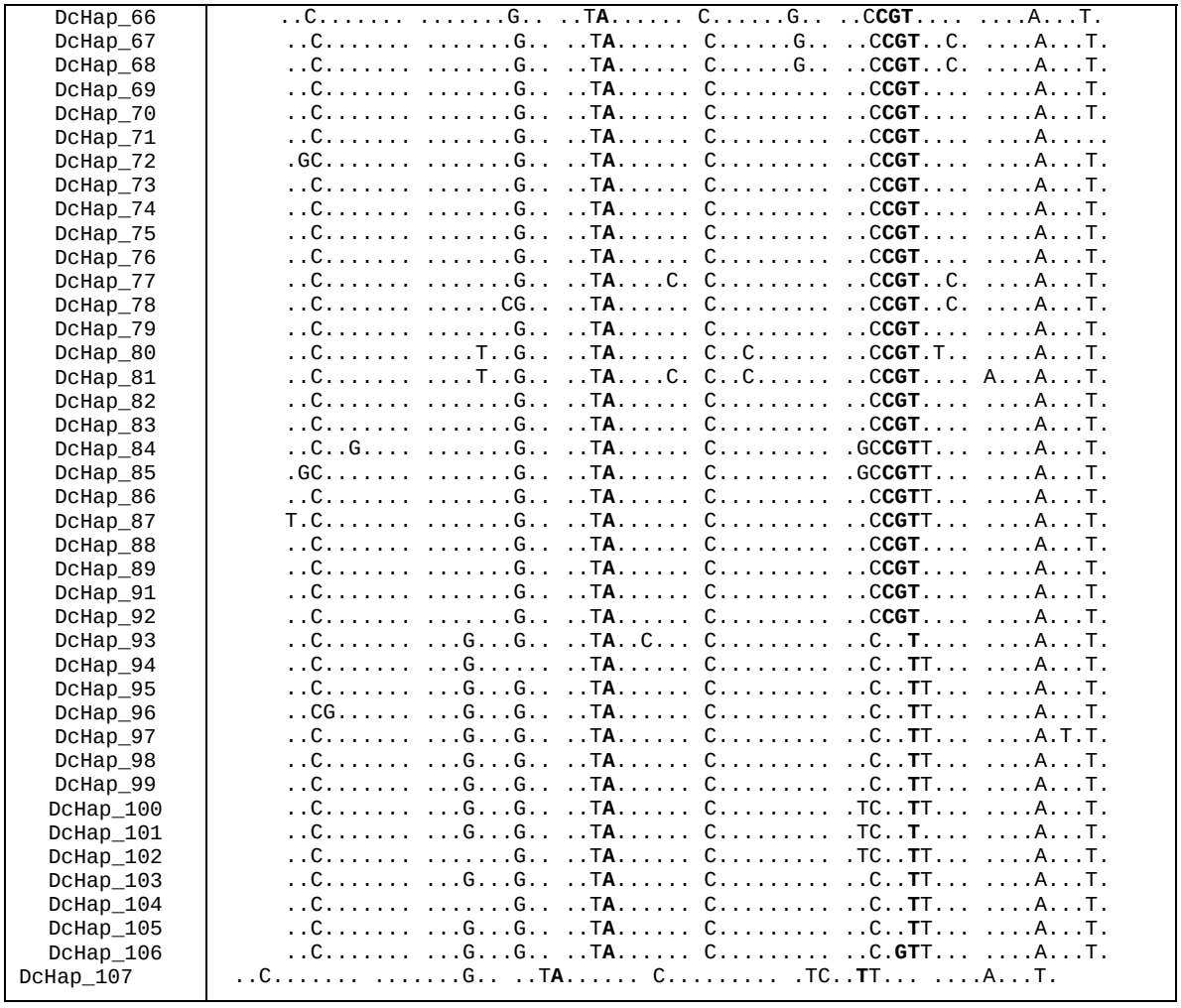

Table 4. 3 Mitochondrial DNA control region diversity indexes. H= Haplotype, π= nucleotide diversity, D= Tajima's D and F= Fu and Li F, ns= non significant.

	n	***H***	***π***	***D***	***F***
D. delphis Short-beaked	53	0.994	0.035	1.622 ^{ns}	-2.225 ^{ns}
D. capensis Long-beaked	138	0.965	0.019	1.278^{ns}	-1.187 ^{ns}

Estimates of evolutionary divergence over sequence pairs between *D. delphis* and *D. capensis* was 0.02, based on the pairwise analyses of the 107 mtDNA control region haplotypes using Tamura-Nei method and 1000 bootstrap replicates (Tamura et al. 2007).

Additional published mitochondrial control region haplotypes, fragments of 280bp length contain, (Table 4. 4) were aligned and compared to sequences derived in this study. The average estimate of evolutionary divergence between *D. capensis* and *D. delphis* sequences was equally divergent from the Pacific to comparisons against other short-beaked common dolphin populations from the North Atlantic, Tasmania and South Australia short-beaked populations and long-beaked from Mauritanian and South African populations (Table 4. 5).

Table 4. 4 Additional sequences used for comparison and evolutionary divergence analyses.

Population	Acronym	n	Reference
North Atlantic	NA	40	(Natoli et al. 2006)
Mauritania	MAU	6	(Natoli et al. 2006)
South Africa	SA	6	(Natoli et al. 2006)
South Australia	SAus	22	(Bilgmann et al. 2008)
Tasmania	TAS	13	(Bilgmann et al. 2008)
Pacific Ocean (*D. delphis*)	PO *sb*	15	(Rosel et al. 1994)
Pacific Ocean (*D.capensis*)	PO *lb*	10	(Rosel et al. 1994)

Table 4. 5 Estimates of evolutionary divergence based on mtDNA control region sequences of 280bp length. Evolutionary divergence below diagonal among common dolphin forms, population acronym and number of sequences analyzed in parenthesis.

Population	PO *sb* (n = 61)	**PO *lb*** (n= 71)	SAus (n= 22)	TAS (n= 13)	NA (n= 40)	SA (n= 6)	MAU (n= 6)
PO *sb*	--						
PO *lb*	**0.04**	--					
SAus	0.03	**0.04**	--				
TAS	0.02	**0.04**	0.02	--			
NA	0.03	**0.04**	0.03	0.02	--		
SA	0.03	**0.04**	0.03	0.03	0.03	--	
MAU	0.03	**0.04**	0.03	0.03	0.03	0.03	--

A total of 308 individuals were genotyped at 16 microsatellite loci, 138 *D. capensis* and 170 *D. delphis.* In general, no allele dropout or genotyping errors due to stuttering were found, except for loci EV14 and EV37Mn, which showed presence of null alleles in both *capensis* and *delphinus* sample. Also, there were few cases of departure from Hardy-Weinberg for the loci EV14, EV37Mn, KWM2a, KWM2b and TexVet5 (Table 4. 6). Although, there were no differences observed in the analyses, before or after the removal of these loci, so the 16 loci were used in further analyses (see Appendix). Overall, the two putative species show high levels of genetic polymorphism at nuclear microsatellite loci (Table 4. 7).

Table 4. 6 Genetic diversity at microsatellite loci. Number of alleles, He: expected heterozygosity, Ho: observed heterozygosity and H-W: H-W equilibrium test, *departure from H-WE

Locus	Population	*D.capensis*	*D. delphis*
AAT	No alleles	12	16
	Ho	0.825	0.835
	He	0.86	0.878
	H-W	0.115	0.175
Dde09	No alleles	7	8
	Ho	0.757	0.786
	He	0.785	0.799
	H-W	0.542	0.309
Dde59	No alleles	9	15
	Ho	0.736	0.806
	He	0.777	0.893
	H-W	0.057	0.076
Dde65	No alleles	8	10
	Ho	0.627	0.745
	He	0.728	0.802
	H-W	0.005	0.367
Dde66	No alleles	12	14
	Ho	0.729	0.774
	He	0.814	0.849
	H-W	0.035	0.15
Dde69	No alleles	9	8
	Ho	0.683	0.705
	He	0.681	0.723
	H-W	0.006	0.279
Dde70	No alleles	14	19
	Ho	0.887	0.928
	He	0.893	0.922
	H-W	0.821	0.439
Dde72	No alleles	9	14
	Ho	0.849	0.783
	He	0.836	0.871
	H-W	0.008	0.009
Dde84	No alleles	11	14
	Ho	0.706	0.84
	He	0.794	0.846
	H-W	0.267	0.144
EV14	No alleles	18	19
	Ho	0.867	0.853
	He	0.894	0.908
	H-W	**0.001***	**0.001***
EV37	No alleles	14	19
	Ho	0.646	0.421
	He	0.747	0.875
	H-W	0.016	**0**
KWM12a	No alleles	13	15
	Ho	0.671	0.783
	He	0.761	0.866
	H-W	0.029	0.043
KWM1b	No alleles	4	5
	Ho	0.316	0.312
	He	0.338	0.346
	H-W	0.605	0.086
KWM2a	No alleles	15	20
	Ho	0.808	0.796
	He	0.886	0.914
	H-W	0.036	**0.002***
KWM2b	No alleles	6	8
	Ho	0.644	0.629
	He	0.658	0.655
	H-W	**0.001***	0.862
TexVet5	No alleles	12	18
	Ho	0.649	0.777
	He	0.871	0.914
	H-W	**0***	**0.001***

Table 4. 7 Genetic diversity at microsatellite level. He= expected heterzygosity, Ho= observed heterozygosity, FIS= Inbreeding coefficient.

Genetic indices	*D. delphis*	*D.capensis*
Sample size	138	170
Number of alleles per locus (±s.d. across loci)	10.93 (±3.75)	14.25 (±5.17)
Allelic richness (±s.d. across loci)	10.63(±3.61)	13.09(±4.56)
He (±s.d. across loci)	0.816 (±0.14)	0.77 (±0.14)
Ho (±s.d. across loci)	0.743 (±0.16)	0.712(±0.14)
F_{IS}	0.088	0.073

The analyses of genetic differentiation based on microsatellite data also revealed significant genetic differentiation between *capensis* and *delphis* forms (F_{st} = 0.029, p<0.001 based on 16 microsatellite loci), consistent with mtDNA findings. The test for sex-biased dispersal provided no evidence in support of bias for either sex (Table 4. 8).

Table 4. 8 Statistical test for sex-biased dispersal between males and females over all populations. n = number of individual tested, *Ho*: observed heterozygosity; *He*:expected heterozygosity; F_{IS}: inbreeding coefficient; F_{ST}: fixation index, *R*: relatedness coefficient, *AIc*: mean corrected assignment index, *vAIc*: variance of the corrected assignment index *AIc*.

	n	F_{is}	F_{st}	Relatedness	*Ho*	*He*	*AIc*	*vAIc*
Females	116	0.076	0.034	0.062	0.688	0.745	0.287	19.405
Males	148	0.066	0.038	0.069	0.701	0.749	-0.225	19.619
p-values		0.44	0.55	0.51	0.3	0.34	0.34	0.96

Bayesian individual assignment implemented in STRUCTURE also strongly supported the differentiation between long and short-beaked common dolphin forms, the highest posterior probabilities were consistently found for K=2 (Table 4. 9, Figure 4. 5). Potential events of introgression were detected as indicated by some extent of admixture (Figure 4. 5).

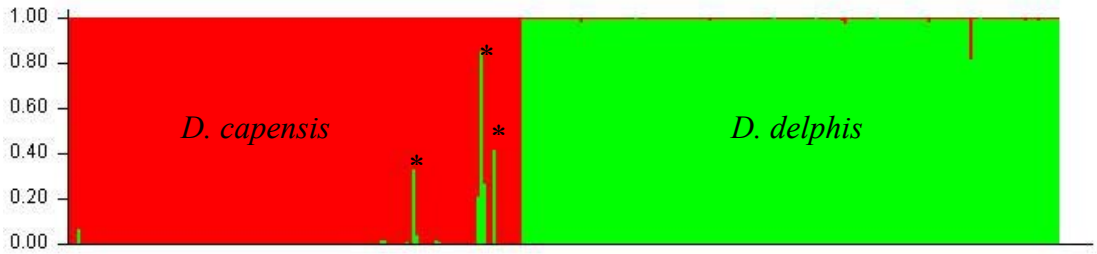

Figure 4. 5 Estimated proportion of the coefficient of admixture of each common dolphin individual, columns, based on multilocus genotype and *a prior* migration rate of 0.05. * Represents potential events of introgression

Table 4. 9 Number of putative populations (K) and their posterior probabilities [Ln P(D)] estimated by the Bayesian cluster analysis performed in STRUCTURE.

K	Ln P(D)	Var[LnP(D)]
1	-14931.7	94.3
2	**-14487.95**	282.8
3	-14573.57	796.12
4	-14746.9	1828.25
5	-14730.2	1644.27

4.4.2 Isolation and migration

The BayeAss estimates of recent migration rates between *D. delphis* and *D. capensis* were low, mean migration rate between putative species, estimated as proportion of migrants per generation was m = 0.166 (CI: 0.007 – 0.325), and asymmetric indicating more gene flow from *D. delphis* individuals into *D. capensis* populations (Table 4. 10).

Table 4. 10 Estimates of asymmetric migration between *D. delphis* and *D. capensis*, based on microsatellite loci. CI: confidence limit.

	Dc → Dd	***Dd → Dc***
Migration rate	0.006	0.023
CI	0.003 – 0.017	0.009 – 0.042

Population parameters estimated in IMa using the mtDNA HVR1 properly converged and gave consistent results for repeat runs (Table 4. 11 and Figure 4. 6). Estimates of contemporary female effective population sizes indicated that the *D. delphis* population size is considerably larger than *D. capensis* (Table 4. 11). The small ancestral population size is unexpected (since ancestral Ne is often inflated in the two population-one ancestor model), but consistent with recent population expansion for both species (Table 4. 11). Population expansion hypothesis was also supported by the analyses of mismatch distribution (Figure 4. 7 and Table 4. 8). Migration rates showed high probabilities at zero in both directions, consistent with the asymmetric estimates of migration conducted in BayesAss (Table 4. 10). The estimates for the time of divergence between long-beaked and short-beaked forms, based on the HVR1 sequence and estimated mutation rate of 5 $x10^{-7}$ substitution per site per year ($s.s.yr^{-1}$), suggested that the diversification of common dolphins began after the last glacial maximum (LGM) < 10,000 years ago (Table 4. 11). In comparison, when population parameters were estimated using a larger fragment of mtDNA control region (778bp) and slower mutation rate ($5x10^{-8}s.s.yr^{-1}$), derived from interspecific datasets (Hoelzel 1991), the divergence time was two orders of magnitude earlier, 125 508.136 years before present (YBP) (Table A. 2). Despite that divergence time estimations were inconsistent, the estimation of

effective population size and migration rates were consistent for both runs using the short and long mtDNA control region sequences (HVR1 and 778bp) (see Appendix A.2). Estimates for divergence time based on IMa runs using only microsatellite DNA data suggested a much more recent splitting time (Table 4. 12). This analysis also suggested ongoing migration and diminished contemporary effective population sizes. The ancestral Ne estimate, however, was large and consistent with the ancestral Ne estimate from the mtDNA analysis (since Ne is four times smaller for mtDNA compared to the nuclear genome).

Table 4. 11 Summary results of IMa, based on mtDNA control region sequences (340bp). t: time from divergence, Ne: estimated effective population size, ancestral Ne: ancestor effective population size (high posterior probability range-HPD90).

Parameter	*D. delphis*	*D. capensis*
t (years)	8321.25 (5363.75 – 11471.25)	
Ne	4946.53 (3090.83 – 8296.43)	794.09 (485.61 – 1256.81)
ancestral Ne	208.46 (6.02 – 977.25)	
m	0.022 (0.025 - 0.607)	0.022 (0.022- 1.057)

Table 4. 12 Summary results of IMa, based on microsatellite genotypes. t: time from divergence, Ne: estimated effective population size, ancestral Ne: effective population size of the ancestor, m: migration rate; (high posterior probability range-HPD90).

Parameter	*D. delphis*	*D. capensis*
t (years)	177 (99 - 669)	
Ne	41.167 (17.482 – 287.044)	12.854 (13.912 – 31.854)
ancestral Ne	825.039 (606.607 – 1280.324)	
m	18.056 (0.028 – 62.269)	168.666 (75.684 – 168.666)

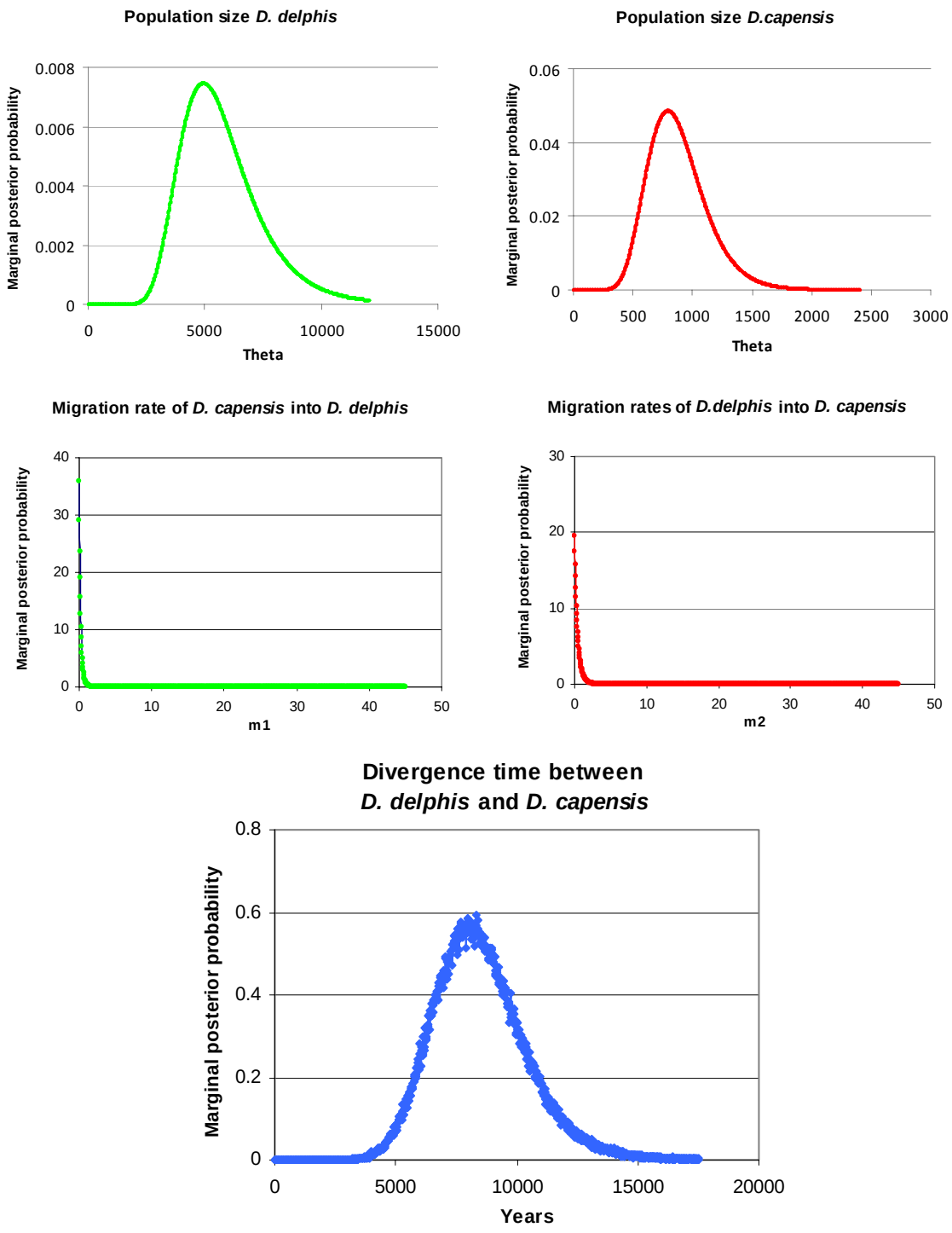

Figure 4. 6 Marginal posterior probability distribution of IMa model population parameters, based on Hypervarible mtDNA control region haplotypes (340bp).

4.4.3 Demographic history-Mismatch distribution

Mismatch distributions for *D. delphis* and *D. capensis* were unimodal, which suggests population expansion for both putative species. The time since the expansion began was estimated from $\tau = 2\mu t$, where μ is the mutation rate for the sequence analyzed (340bp HVR1, μ = 5x 10-7 s.s.yr-1) and t is the time since expansion began. The mismatch distribution of *D. delphis* suggested an earlier expansion than *D. capensis* (Figure 4. 7), although there is extensive overlap in the confidence limits for the two estimates (Table 4. 13).

Table 4. 13 Mismatch distribution parameter estimates under the model of sudden expansion. Confidence intervals shown within parenthesis, based on 1000 replicates.

Parameter	*D. delphis*	*D. capensis*
Tau	5.563 (3.533 - 8.075)	3.844 (0.855 - 8.749)
Time from expansion **years**	16,362 (10,391 – 23,750)	11,306 (2,515 – 25,732)
Theta 0	0.582 (0 - 2.892)	0.32 (0 – 3.992)
Theta 1	1657.5 (76.875 – 10,356.25)	10.695 (2.962 – 6,531.94)
SSD	0.002	0.003
p-value	0.274	0.695

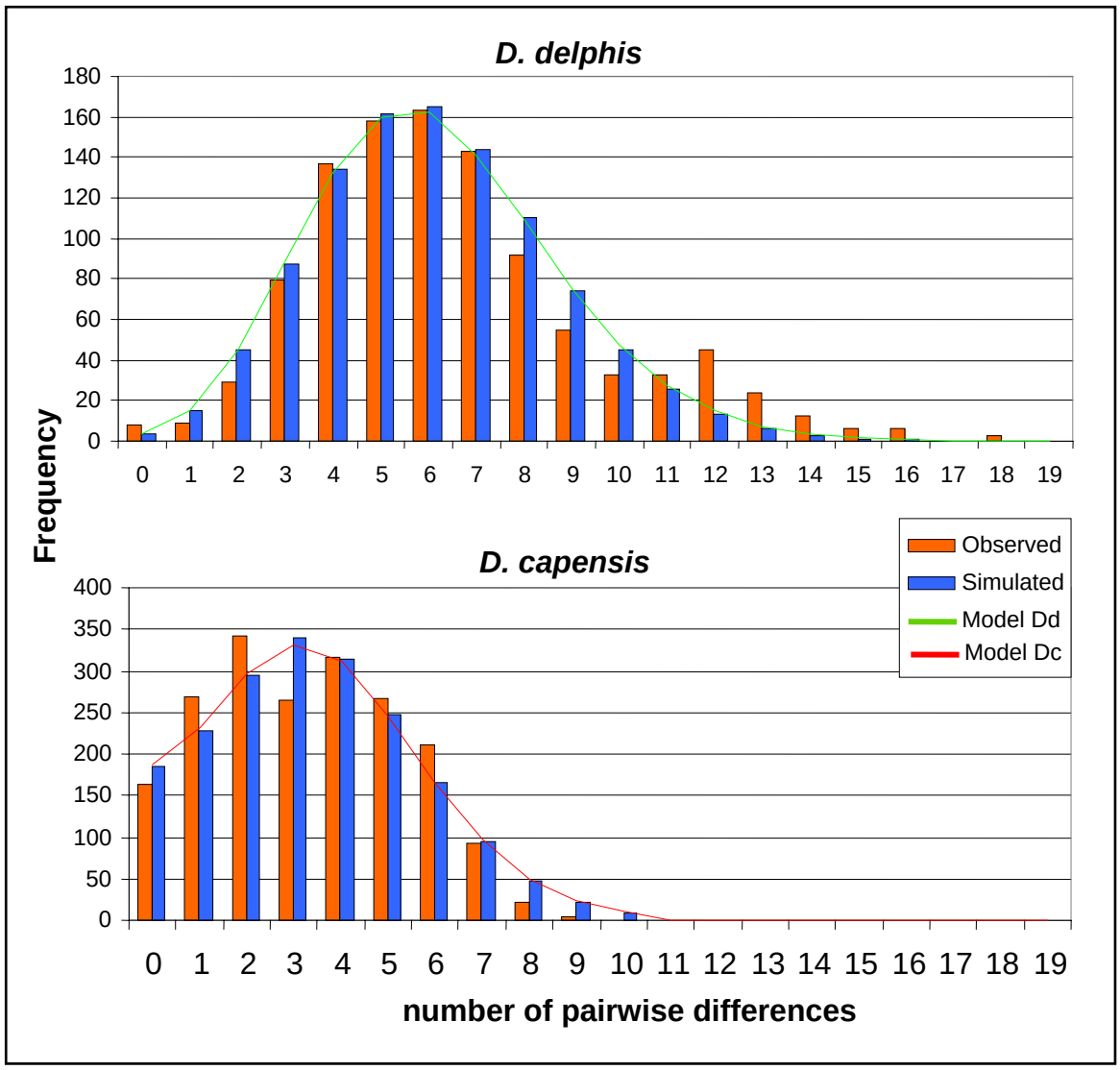

Figure 4. 7 Distribution of the number of pairwise differences (bars), and the expected mismatch distribution under the model of sudden expansion (solid line) of the HVRI of the mtDNA control region haploytpes.

4.4.4 Evidence of introgressive hybriditation

The presence of putative hybrids was detected based on incongruence among phenotypes and genetically distinctive traits (Table 4. 14). Phenotypic distinction was based on field observation and confirmation was only possible for skull specimens and based on the rostral length-zygomatic width ratio. The two common dolphin forms show a discrete range of the ratio of rostral length and zygomatic width, *D. delphis* ranges 1.21-

1.47 whereas, *D. capensis* 1.52- 1.77 (Heyning and Perrin 1994). Potential hybrids were identified as hybrids given the extent of admixture estimated from microsatellite genotypes in STRUCTURE, and the lack of correspondence with mtDNA control region haplotypes (Table 4. 14). Assignment to matrilineal lineage, mtDNA control region haplotype, was based on Median-joining network (MJN). Additional potential hybrids were detected as haplotypes derived from four skull specimens identified as *D. capensis,* based on their ratio of rostral length and zygomatic width, but placed within the *D. delphis* haplotype group in the (Figure 4. 11, haplotypes in blue). Unfortunately, the poor DNA quality for three samples did not allow the complete microsatellite genotyping of these individuals (specimen number 840429-10, 841100 and 180695-1). Haplotype 81 was also derived from a skull specimen identified as *D. capensis*, but no skull measurements nor microsatellite genotype were available for confirmation.

Table 4. 14 List of putative hybrids. Distinctive traits: phenotypic (field identification or ratio of rostral length and zigomatic width for skull specimens), genetic (mtDNA and microsatellite genotype), gender (F: female, M: male, U: unknown) and hybrid generation (G).

Individual **Haplotype ID**	**Gender**	**Phenotype** (rostral length zygomatic width ratio)	**Mt DNA**	**Mstat**	**G**
840429-6 **Hap89**	F	Dc (1.67)	Dd	Dd	F1
84042910 **Hap 68**	U	Dc (1.71)	Dd	U	U
841100 **Hap 104**	U	Dc (2.2)	Dd	U	U
180695-1 **Hap 85**	F	Dc (1.65)	Dd	U	U
16569 **Hap 81**	U	Dc (?)	Dd	U	U

4.4.5 Phylogenetic reconstruction

The Neighbour Joining, Bayesian inference and Median-joining network phylogenetic reconstruction methods clearly showed divergence between *D. capensis* and *D. delphis* haplotypes (Figure 4. 8, Figure 4. 9, Figure 4. 11). Although, the Bayesian tree highly supported the divergence of *D. capensis* (posterior probability = 84), the separation between *Tursiops* and *D. delphis* was not resolved. The Neighbour-Joining

tree marginally resolved reciprocal monophyly between the two putative species when only the samples from the ETP were included, bootstrap support = 54 (Figure 4. 8); however, the addition of haplotype sequences from other regions meant that bootstrap support was lost (Figure 4. 10). On the other hand, the Median-joining network (MJN) showed a strong divergence between *D. capensis* and *D. delphis*, and only one connection between the two haplotype clusters (Figure 4. 11), which may suggest a point of divergence between the two forms. The MJN also revealed complex reticulation at the centre of the *D. delphis* network. This suggests that there are many unsampled haplotypes, consistent with a large population and an origin separate from the local sample site. On the other hand, *D. capensis* showed a number of star-shaped haplotype clusters, which indicate rapid and recent expansion of the long-beaked form, but also a smaller population size than the short-beaked form (Figure 4. 11).

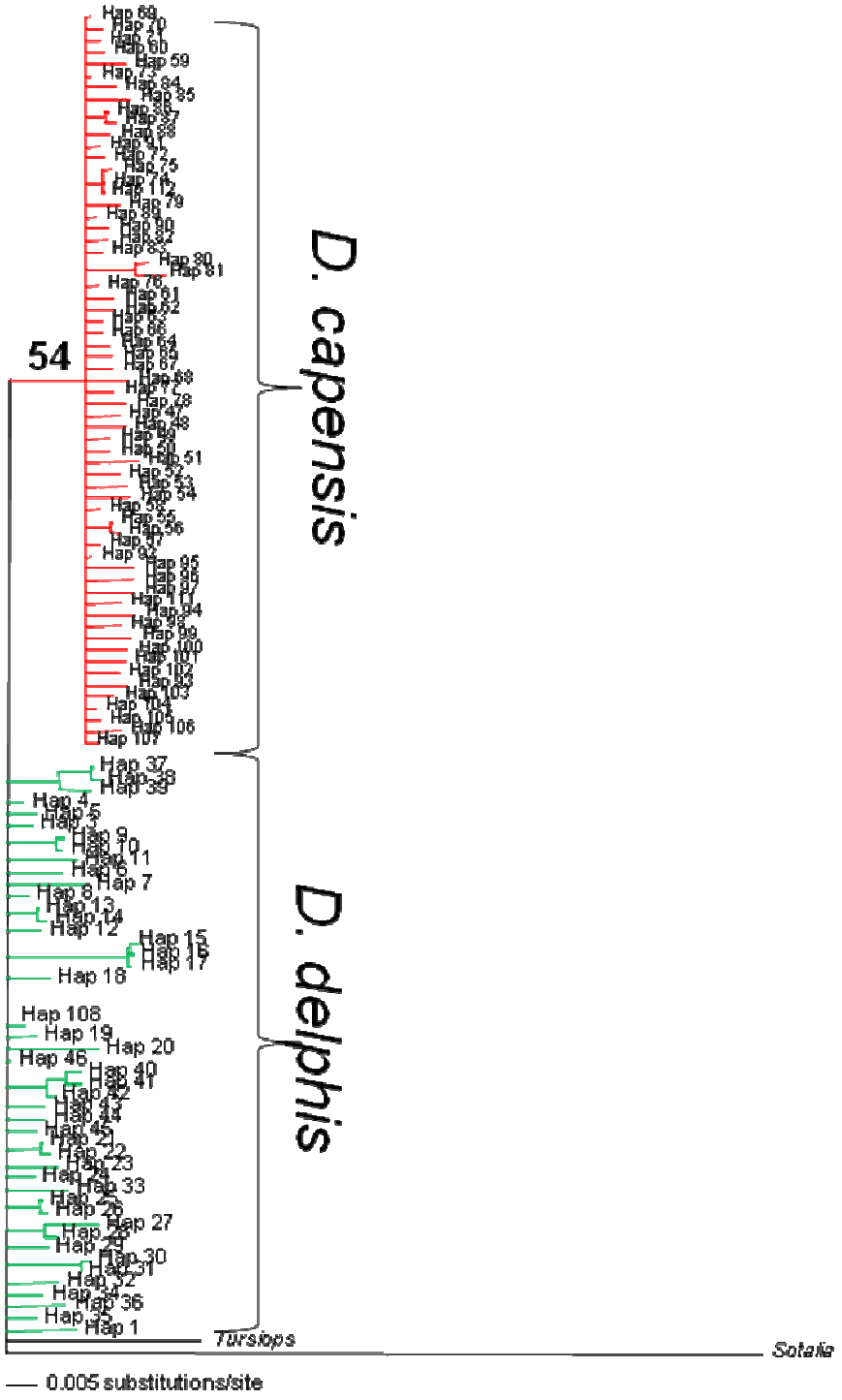

Figure 4. 8 Neighbor-Joining phylogenetic reconstruction of 107 mtDNA control region haplotypes of *Delphinus spp*. Consensus tree estimated under TrN + I + G model of molecular evolution.

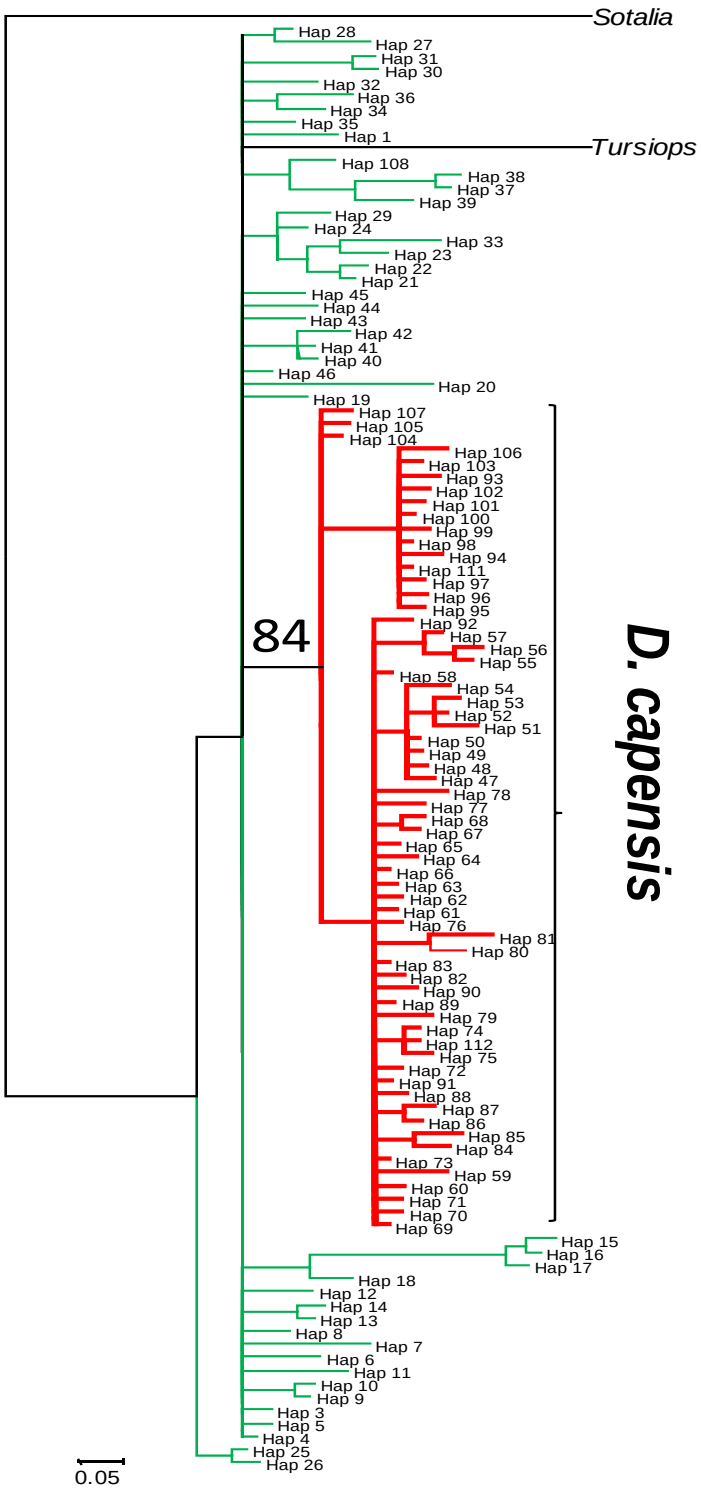

Figure 4. 9 Bayesian inference tree showing phylogenetic reconstruction of 107mtDNA control region haplotyes of Dspp. Consensus tree after 13740000 generations estimated under the GTR+G-I substitution model as implemented in MrBayes. Posterior probably of the node indicated along the branch. *Sotalia* and *Tursiops* were used as an outgroup.

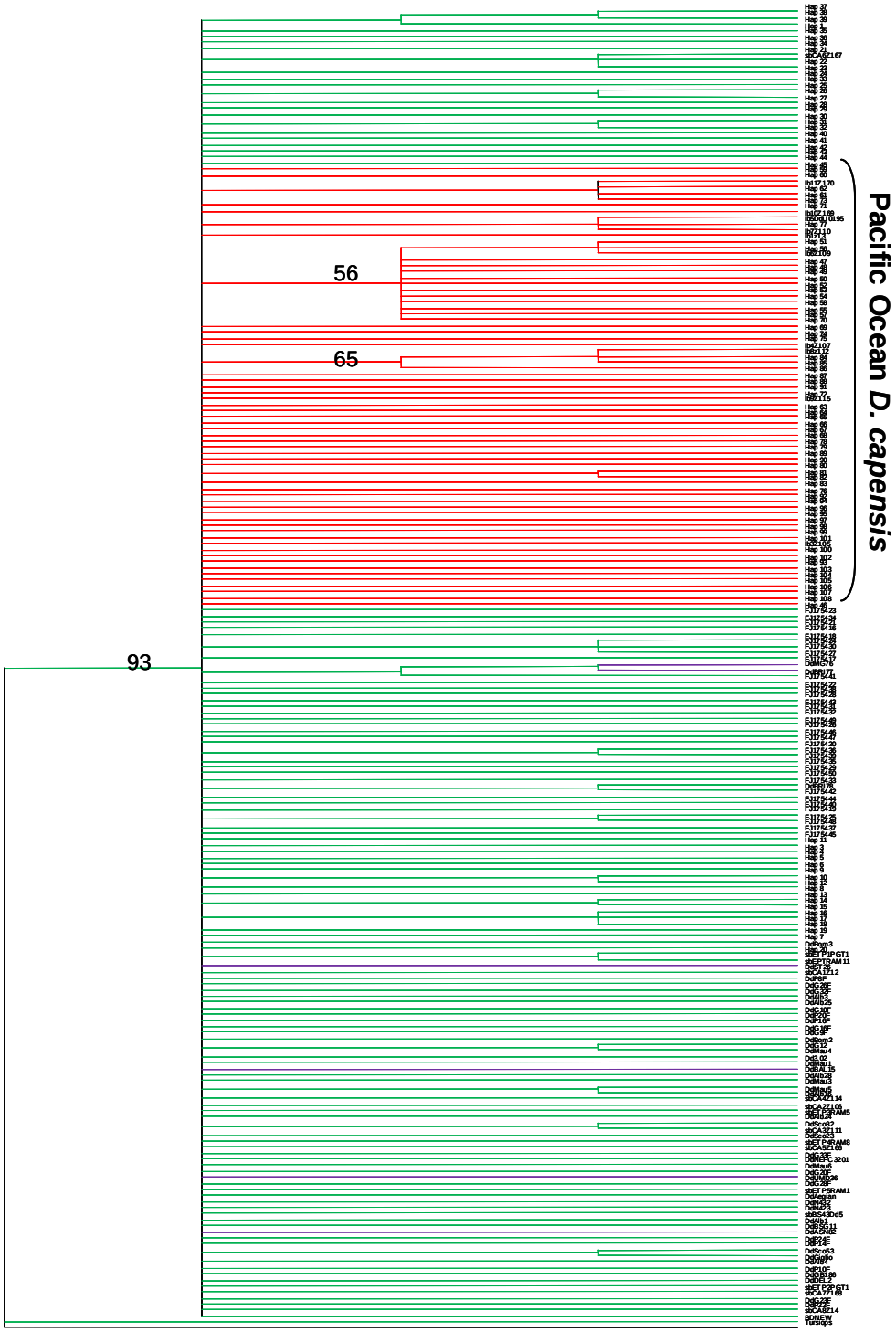

Figure 4. 10 Neighbor-Joining phylogenetic reconstruction of 219 mtDNA control region haplotypes of *Delphinus spp* worldwide. Consensus tree estimated under TrN + I + G model of molecular evolution. Bootstrap >50 shown along the branches. South African long-beaked dolphin indicated by purple branches.

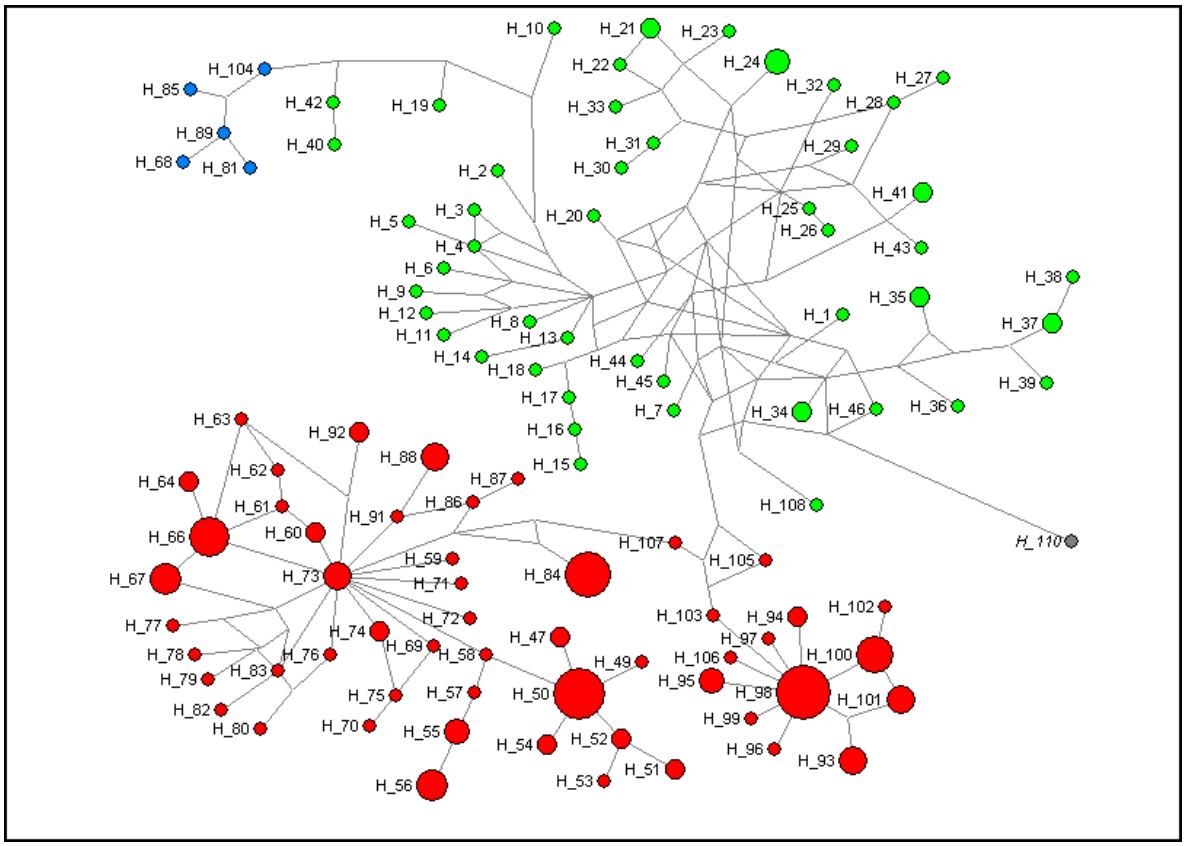

Figure 4. 11 Median-joining network of mtDNA control region haplotypes. Circle diameter proportional to haplotype frequency. Red circles correspond to *D. capensis*, green circles to *D. delphis*, blue circles: misplaced *D. capensis* haplotypes and in gray rooting haplotype (H110, corresponding to *Tursiops truncatus*).

4.5 Discussion

This study revealed significant genetic differentiation between long-beaked, *D. capensis* and short-beaked, *D. delphis*, common dolphins in the ETP, consistent with previous findings based on the mtDNA control region (Rosel et al. 1994, Natoli et al. 2006). Consistently, the Bayesian individual assignment test conducted in STRUCTURE and the extent of genetic differentiation as estimated by the fixation indices, based on mtDNA and microsatellite data, indicated little contemporary gene flow for either females or males, also suggested by the test for sex-biased dispersal (since this test is dependent on contemporary movement, and no significant differences were found).

Despite the significant differences found at both mitochondrial and microsatellite loci, evidence of introgression suggests some level of continuing gene flow. This is consistent with some observations in the ETP suggesting interbreeding among these two forms (Evans 1975), although these observations did not include the western coast of Baja California nor within the GC. In this study five putative hybrids were distinguished based on incomplete correspondence of phenotype, mtDNA and microsatellite genotype (Table 4. 14), and as mentioned above, the microsatellite DNA data suggest the possibility of ongoing male-mediated gene flow at some level. However, the data for maternal lineage divergence is strong, and suggestive of incipient speciation. Hybrids are in fact common for cetaceans, even between formally recognized species. For example, between the blue whale, *Balenoptera musculus* and the fin whale, *B. physalus* (Arnason et al. 1991) and harbour porpoise, *Phocoena phocoena*, and Dall's porpoise, *Phocoenoides dalli*. The latter showed divergence at cytochrome *b* mtDNA of 6.5% (Willis et al. 2004). Therefore among delphinid species for which lineage sorting is not always complete (LeDuc et al. 1999, Kingston et al. 2009), hybrid introgression is likely to take place (Kingston et al. 2009).

Furthermore, while the analysis based only on mtDNA in IMa suggested no female migration, the analyses based on bi-parentally inherited markers in BayesAss and IMa were inconsistent, but both suggested biased gene flow from *D. delphis* to *D. capensis* at non-zero levels. This leaves open the possibility of ongoing male-mediated gene flow. Recent studies have shown that divergence or even incipient speciation is possible even in the face of recurrent or continuous gene flow between divergent taxa

(Hey 2006, Niemiller et al. 2008). For instance, continuous or recurrent gene flow over secondary contact was suggested among three divergent forms of Tennessee cave salamander, *Gyrinophilus palleucus*, (Niemiller et al. 2008).

Common dolphins generally show high levels of genetic polymorphism and gene flow over large geographic areas in the Atlantic Ocean (Natoli et al. 2006, Amaral et al. 2007, Mirimin et al. 2009). In the Indian Ocean relatively little is known about their population genetics, but a population of the long-beaked morphotype along the coast of South Africa was differentiated from other populations of both forms in the Atlantic and Pacific (Natoli et al. 2006). Natoli et al. (2006) also illustrated that the only population that could be distinguished as a separate lineages for mtDNA control region sequences was the ETP Pacific population, as confirmed by the estimates of evolutionary divergence (Table 4. 5) and phylogenetic reconstruction of worldwide derived haplotypes (Figure 4. 10). Genetic differentiation was also reported elsewhere in the Pacific, for example from South Australia and Tasmania (Bilgmann et al. 2008). In general, there was no correspondence between morphotype and genotype at the global scale, and therefore no support for all long-beaked forms being conspecific (Natoli et al. 2006; Amaral et al. 2007). Instead there was evidence for the convergent evolution of morphotype for the near-shore, long-beaked form.

In the ETP, the coincident split at both microsatellite and mtDNA markers shows clear divergence between these two regional forms, originally distinguished based on morphological traits (Heyning and Perrin 1994). However, the use of morphological characters alone may underestimate the number of species, as traits may have parallel evolutionary histories and converge as a result of similar selective pressures (Yang and Rannala 2010). The estimates of evolutionary divergence reported here supports the hypothesis that the Pacific long-beaked common dolphin, *D. capensis*, underwent an independent local origin and evolution from other long-beaked populations, e.g. the South African long-beaked common dolphin (Natoli et al. 2006). At the same time, the ETP *capensis* form has evidently become more isolated and diverged further than similar morphotypes studied elsewhere in the world. Overall, indices of genetic diversity were high in both putative species. However, nucleotide diversity in the *capensis* form was

lower than in *delphis*, indicative of a recent radiation and consistent with the star-shaped phylogeny and estimates of recent time of divergence inferred in IMa.

The taxonomic status of the long-beaked common dolphin, *D. capensis*, is still uncertain, but available data suggest that only the ETP population qualifies as an incipient species. Analyses of mitochondrial DNA control region and nuclear makers (AFLP) provided strong evidence to recognize the ETP long-beaked common dolphin form as distinct from *D. delphis* (Rosel et al. 1994, Kingston and Rosel 2004, Kingston et al. 2009), while genetic data seemed to exclude the possibility of similar long-beaked morphotypes elsewhere in the world being conspecifics (e.g. Natoli et al. 2006). Here nuclear multi-locus microsatellite data confirm the genetic distinction between the sympatric short and long-beaked common dolphin forms in the ETP off the coast of California, Baja California and Gulf of California.

The MJN reconstruction of the phylogenetic relationships among mtDNA control region haplotypes showed two well-defined matrilineal clusters, however lineage structure differed for *D. capensis* compared to *D. delphis* samples. Within the *D. delphis* lineage there was extensive reticulation consistent with a large, poorly sampled population with a broad distribution. This, together with the high estimate of female Ne for this populations suggests that *D. delphis* represents the parent population from which *D. capensis* was founded. The *D. capensis* lineage is quite distinct, instead showing a series of star formations and a relatively small number of common haplotypes. This is suggestive of a population that is well represented by the sample set, and a recent expansion. The mismatch distribution of pairwise differences of the HVR1 sequences was also consistent suggesting recent expansion of both populations. Furthermore, the single branch linking the two lineages may suggest that the founding event that established the *D. capensis* population in the ETP happened over a relatively brief period of time. The Bayesian inference tree was not able to resolve the separation of the root lineage, *Tursiops,* as a different species. This might be result of the close phylogenetic relationship of the species on the genera *Tursiops, Delphinus* and *Stenella* (Le Duc et al. 1999). Nonetheless, the divergence between *D. delphis* and *D. capensis* showed high posterior probability (PP = 84) despite the fact these putative species are on the same genus. Overall, these results suggest historic isolation of the long and short-beaked

common dolphin form for enough time to allow complete lineage sorting of the two common dolphin putative species within the Pacific Ocean and Gulf of California.

The IMa analyses estimated a time of divergence (based on the mtDNA HVR1 region and a mutation rate of $5x10^{-7}$ s.s.yr^{-1}) of 8,321 YBP. Although the IMa analysis based on microsatellite DNA loci suggested a much more recent date, this could be related to ongoing male-mediated introgression (see above). The timing of this event depends on the application of an appropriate mutation rate, though mutation rates are quite variable among species and not well understood (Nabholz et al. 2008, Nabholz et al. 2009). However, there is substantial information available for the HVR1 mutation rates, thus the estimates of IMa shown here are based on the HVR1 (Figure 4. 6), using a mutation rate that falls within the intervals of those derived from ancient DNA in several species. For example: Adélie penguin, *Pysocelis adeliae,* $9.6\ x10^{-7}$ s.s.yr^{-1} (Lambert et al. 2002), in Southern elephant seal, *Mirounga leonina,* $9.8x10^{-7}$ s.s.yr^{-1} (de Bruyn et al. 2009), and in Steller sea lion, *Eumetopias jubatus,* 2.7x10-^{7} s.s.yr^{-1} (Phillips et al. 2009). On balance, an event post-dating the last glacial period seems likely, based on the molecular data.

The Gulf of California and western margin of California and Baja California have experienced active geological and paleoclimatic changes that have driven the evolution of several taxa (Riginos and Nachman 2001, Jacobs et al. 2004). During the Pleistocene-Holocene transition, ~15,000 YBP, warm anomalies of the sea surface temperatures (SST) occurred leading to the collapse of the California Current (Herbert et al. 2001). Geological evidence suggested a decline in productivity, as a result of the unfavorable conditions to coastal upwelling along the California and Baja California margin (Herbert et al. 2001). The typical conditions of California Current, as currently persist, were reestablished during the Holocene ~9,000 YBP; resulting in higher regional productivity and coastal upwelling reestablished in the Northeast Pacific (Ortiz et al. 2004). Changes in upwelling patterns in a geological time scale have been correlated to events of speciation, for instance in kelp species and consequently in abalone species in response to food resource divergence (Jacobs et al. 2004).

Divergence time estimated between *D. capensis* and *D. delphis* correspond to these changes in upwelling conditions during the Holocene. A possible interpretation is

that changes in productivity and fish communities along the coastal margin resulted when coastal upwelling and the California Current were restored ~9,000 YBP. As a consequence a favorable emerging habitat became available for the ancestral common dolphin population, thus a founder dolphin population may have begun to exploit this new habitat and become independent of the offshore source population. Provided that Holocene changes in paleoceanography also had an effect in the pelagic environment, high productivity and therefore food availability could have promoted a demographic expansion in the ancestral *D. delphis* population as well (mismatch distribution provided evidence of sudden expansion dated 10,391 – 23,750 YBP). It is plausible that the ancestral dolphin populations took advantage of emerging habitat reducing resource competition, and also reducing gene flow with the parental population, becoming genetically isolated. Similarly, differences between South Australia and South-eastern Tasmania common dolphins have been hypothesized that were influenced by the Pleistocene changes in the ecosystem and the emergence of the Bassian land-bridge (Bilgmann et al. 2008). There is evidence that population differentiation over short time frames might evolve in response to rapid optimal environmental changes, for example Southern elephant seals, *Mirounga leonina*, in Antarctica (de Bruyn et al. 2009).

Despite the fact that *D. capensis* and *D. delphis* may occur in sympatry and feed on similar trophic levels, as revealed by stable isotope signals (Díaz-Gamboa 2009); *D. capensis* generally prefer shallower and warmer waters than *D. delphis* (Perrin 2002). Foraging specialization has been strongly associated with habitat preferences that in turn may promote adaptation to contrasting environments, for instance coastal vs. offshore, and subsequent reproductive isolation and eventual speciation (Schluter 2001). Divergence between common dolphin putative species and between other pairs of dolphin divergent forms seem to be strongly and consistently associated to foraging (Hoelzel et al. 1998b, Segura et al. 2006). By inference, the beak length seems to be a plastic trait subject to local adaptation, and not useful on its own to delimit taxonomic and evolutionary units in delphinid species.

Considering the total evidence, i.e. strong genetic differentiation, morphological and ecological differences between common dolphin forms and the historic changes in local oceanography, this study provides an example of how biodiversity is generated and

maintained over time. These results favor the recommendation that *D. capensis* and *D. delphis* in the ETP should be considered separate management units and encourage the conservation actions taking place within the Gulf of California and Baja California in order to protect the local and unique biodiversity of this region.

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Chapter 5

General discussion

5.1 Discussion

This study has investigated the evolution of population genetic structure of two closely related cetacean species that play key roles as upper-level predators in the Gulf of California and western coast of Baja California. In general, our understanding of population genetic structure in cetaceans has been challenged by their wide distribution and dispersal patterns, but also by the complexity of their ecological habitat. In this study, the evolution of population genetic structure in bottlenose, *Tursiops truncatus*, and long-beaked common dolphins, *Delphinus capensis* is interpreted in the context of habitat diversity across the study area (Chapter 2 and Chapter 3). Overall, the results support the hypothesis that local habitat dependence promotes population differentiation in the absence of physical boundaries to dispersal in these highly mobile species. This type of differentiation among ecotypes has been well documented in these two species (and in other delphinids; see below), but this study provides an unusual insight into the conditions that lead to incipient speciation in these groups. Ecological and morphological divergence among common dolphin populations appears to be associated with changes in the paleoceanographic conditions of the region such that reciprocal monophyly between the sympatric *D. delphis* and *D. capensis* forms has evolved within the Holocene timeframe (Chapter 4).

5.1.1 Evolution of population differentiation in bottlenose and common dolphin

In terrestrial mammals, population genetic structuring is sometimes more apparently a consequence of habitat discontinuity, due for example to barriers to gene flow imposed by mountains or rivers. For instance, three populations of chimpanzee, (*Pan troglodytes*), separated by rivers were found to be genetically differentiated (Becquet et al. 2007). It is possible that this type of small scale boundary may exist in the marine environment (beyond the obvious boundaries imposed by land mass), but remain unrecognised. However, even in terrestrial environments habitat preference has been proposed to act as barrier to gene flow in species that inhabit continuous habitats, such as the mountain gorilla (*Gorilla beringei beringei*) for which female choice for dispersal appears to be mediated by natal habitat preference (Guschanski et al. 2008).

Conversely, in the marine environment the apparent lack of habitat discontinuities disguised the recognition of population structure, especially in animals capable of long excursions, such as cetaceans. Cetacean species show great variation in genetic structure, which has been associated with historical factors, such as colonisations and changes in the marine environment; and current factors, such as resource specialization, social structure and aspects of life history and demography, or a combination of factors (Hoelzel 1998, Natoli et al. 2004, Hoelzel et al. 2007, Moller et al. 2007, Wiszniewski et al. 2010). Resource specialization can result in a narrow range of prey choices that consequently might restrict individual dispersal to habitats where those food items are available. Alternatively, specialists may focus on the same prey in different patterns of distribution. Thus, foraging specialization is strongly associated with habitat preferences that in turn may promote adaptation to contrasting environments and subsequent reproductive isolation and eventual speciation (Schluter 2001). Resource specialization (foraging and habitat), has resulted in genetic structuring among populations of several taxa, for example: analyses of mtDNA cytochrome b and microsatellite DNA loci revealed two divergent allopatric lineages consistent with ecological differences in habitat type for the tungara frog *Physalaemus pustulosus,* (Prohl et al. 2010). In Eastern Europe, wolf populations display non-random spatial genetic structure patterns which were correlated with habitat type and diet composition (Pilot et al. 2006). Likewise, in cetacean species resource specialization has been proposed to promote population divergence (Hoelzel 1998). For instance, in the killer whale, *Orcinus orca*, fish (resident) and marine mammal (transient) foraging specialists are genetically differentiated (Hoelzel et al. 1998a, Hoelzel et al. 2007).

This study found evidence of strong genetic differentiation in both bottlenose and common dolphin populations in the absence of physical barriers. The comparison of the patterns of population genetic differentiation found here for bottlenose and common dolphins supports the hypothesis of local habitat dependence and resource specialization at both the population and putative species level.

Both genera, *Delphinus* and *Tursiops*, have world-wide distributions and are poorly defined at the species level. Throughout their range there is a tendency for coastal populations to diverge morphotypically from pelagic populations. The most prominent

feature is the length of the beak, which some have suggested is associated with differences in prey choice and prey acquisition (Walker 1981, Heyning and Perrin 1994, Díaz-Gamboa 2003, Pompa-Mancilla 2004). However, phylogenetic studies provide equivocal classification with respect to alpha taxonomy, and there is evidence for convergent evolution of these morphotypes in different parts of the world (Natoli 2004, Natoli et al. 2006). While there are important differences with respect to population structure in detail (such as the much greater degree of population subdivision for *Tursiops* in the Atlantic compared to *Delphinus*), this theme associated with habitat specialisation in coastal and pelagic environments is common to both, and in fact to a number of other delphinid species. For example, the Eastern Tropical Pacific spotted dolphin, *Stenella attenuatta*, (Escorza-Treviño et al. 2005), Dall's porpoise, *Phocoenoides dalli*, (Escorza-Treviño et al. 2004), killer whale morphotypes, *Orcinus orca*, (Foote et al. 2009), and Tucuxi, *Sotalia fluviatilis*, (Caballero et al. 2007). Understanding why some populations diverge further than others when the mechanism seems similar is a major unanswered question. The comparisons presented here contribute to resolving this issue because they provide the opportunity to compare each genus in the same local, ecologically substructured habitat. Furthermore, results provide the best example of incipient speciation for either taxa, represented by the local population of *D. capensis*, as previously proposed (Rosel et al. 1994, Natoli et al. 2006).

Bottlenose dolphin genetic structure has been shown to be highly dependent on the type of environment the population inhabits. Little differentiation has been found in large pelagic populations over broad geographic areas (Hoelzel et al. 1998b, Natoli et al. 2005, Querouil et al. 2007), whereas in coastal populations considerable structure is found, and local adaptation to different ecological conditions may be leading to high site fidelity, especially in complex coastal margins (Parsons et al. 2006, Bilgmann et al. 2007, Moller et al. 2007, Rosel et al. 2009). In contrast, the common dolphin more typically inhabits pelagic habitats and shows lower levels of population structure; findings in the Atlantic common dolphin showed high levels of gene flow on each side of the ocean basin (Natoli et al. 2006, Mirimin et al. 2009). The dispersion of offshore bottlenose and common dolphin populations, has been linked to seasonal movement of prey species (Querouil et al. 2007, Bilgmann et al. 2008, Cañadas and Hammond 2008, Tezanos-Pinto et al. 2009),

and given that the open ocean provides few options for hiding from predators and dispersed resources, individuals form large groups that may confer advantages associated with reduced predation risk and increase foraging opportunities (Ballance 2002, Bearzi et al. 2009).

Social structure is also influenced by feeding ecology and by habitat; together these factors have a large impact on the patterns of dispersal and therefore gene flow among populations. Coastal bottlenose dolphins exhibit a fission-fusion society, where individuals may form strong relationships of variable duration (Connor et al. 2001, Connor 2002). Common dolphins show a fluid social structure with some aggregations by age and sex consisting of randomly related individuals (Neumann et al. 2002, Bruno et al. 2004, Viricel et al. 2008). Evidence that habitat variation may influence social structure in cetacean species has been noticed for spinner dolphins (Karczmarski et al. 2005), and for common dolphins in coastal habitat where they seem to exhibit social structure similar to the fission-fusion structure seen in bottlenose dolphins (Bruno et al. 2004).

The Gulf of California and western coast of Baja California provide a great variety of habitats with distinct oceanographic, topographic and climatic conditions (Álvarez-Borrego 1983, Santamaría-del Ángel et al. 1994). Nonetheless, the pattern of genetic structure observed in bottlenose and common dolphins are different. Fine-geographic scale structure was detected in coastal bottlenose dolphins, which matched the habitat discontinuities that consistently subdivided the Gulf of California into bioregions (Álvarez-Borrego 1983, Santamaría-del Ángel et al. 1994; Chapter 2). This result suggests that gene flow among bottlenose dolphin coastal populations might be restricted by local dependence on diverse ecological conditions, such as distinct prey items, as proposed for this species elsewhere, for instance, in the Mediterranean Sea, North Atlantic, Gulf of Mexico (Natoli et al. 2004, Rosel et al. 2009) and South Pacific Ocean (Hoelzel et al. 1998b, Bilgmann et al. 2007, Rosel et al. 2009, Torres and Read 2009, Wiszniewski et al. 2010).

Conversely, the long-beaked common dolphin genetic structure did not reflect the habitat heterogeneity of the region to the same extent. However, it was differentiated into at least two distinct stocks, one within the gulf and other inhabiting the western margin of

Baja California (Chapter 3). This division represents two major biogeographic regions based on their distinct oceanographic characteristics and complete lineage sorting in several taxa; for example: fish species (Stepien et al. 2001, Bernardi et al. 2003, Sandoval-Castillo et al. 2004, Lin et al. 2009). This pattern of differentiation for long-beaked common dolphin from the Pacific and gulf basins is in contrast to the findings in the Atlantic common dolphin where high levels of gene flow were documented over a wider geographic area (Natoli et al. 2006, Mirimin et al. 2009).

The difference in foraging specialization between coastal and offshore populations of both bottlenose and common dolphins is reflected in the pattern of genetic structure observed at a broader geographic scale. Offshore bottlenose dolphins from Pacific Ocean and Gulf of California may consist of a single population stock as genetic analyses revealed high levels of admixture between these two basins (Chapter 2). There is some indication that this may be the case in the North Atlantic, though few relevant comparisons were possible (Hoelzel et al. 1998b). As mentioned above, common dolphins are more typically pelagic and show little structure in the North Atlantic, while the long-beaked common dolphin prefers to inhabit coastal waters (Barbosa 2006). Thus, population structure in long-beaked common dolphin populations will be influenced by coastal processes rather than pelagic. However, the nature of this influence in coastal habitat may depend on differences in prey choice between the two species. Unfortunately too little is known about this to develop this idea further.

The large extent of genetic distinction of the long-beaked, *D. capensis* in the ETP, is in contrast to that extent of genetic differentiation between bottlenose dolphin ecotypes (Chapter 2) and among long and short-beaked common dolphin forms elsewhere (Natoli et al. 2006, Almaral et al. 2010). Divergence between *D. capensis* and *D. delphis* in the ETP may be consequence of paleoceanographic changes in marine productivity that occurred during the Holocene (Herbert et al. 2001), possibly linked to the emergence of a coastal favorable habitat (Chapter 4). Environmental changes at the geological scale have resulted in ecological-morphological divergence of the ancestral phenotypes in other ETP species, for example in divergent species of anchovies in the Pacific Ocean (Grant et al. 2010). In particular, proposed fluctuations in the upwelling process, which in turn could result in changes in the marine ecosystem (Herbert et al. 2001), have been suggested to

have promoted the evolution of several taxa (Riginos and Nachman 2001, Bernardi et al. 2003, Jacobs et al. 2004, Bernardi and Lape 2005, Pondella et al. 2005, Lin et al. 2009, Schramm et al. 2009). This raises the question of why these changes should have affected common and bottlenose dolphins differently. A plausible explanation is the difference in the degree of prey specialization between bottlenose and common dolphins. Common dolphins may have a more opportunistic diet than bottlenose dolphins (Díaz-Gamboa 2009). Thus, common dolphins may have been more likely to take advantage of Holocene fluctuations in prey items or abundance leading to emerging habitat (Chapter 4). Overall, no reciprocal monophyly was observed in the bottlenose dolphin for the study populations (though this has been documented elsewhere between nearshore and offshore forms; Hoelzel et al. 1998b, Natoli et al. 2005), which suggests ongoing gene flow preventing linage sorting or a more recent divergence between ecotypes.

5.1.2 Conservation implications

The Gulf of California is currently the focus of many conservation actions. This study will have an immediate impact in the conservation and management of these delphinid species in Mexico, particularly by determining the local segregation of regional dolphin populations. This information is needed by Mexican federal authorities to create, implement and enforce official norms regulating the protection and capture of dolphins in the country. It was decreed in 2002 that future live captures of dolphins in Mexico, for exhibition purposes, will be conditional on population and environmental assessments conducted by scientific institutions (DOF, 2002). However, the number of stocks that occur along the Pacific coast and within the Gulf of California have not been addressed. These results provide an assessment of the distribution of management units of these delphinid species within the study area (Table 5. 1). Therefore, an accurate assessment of the impact of mortality and live capture can be accomplished.

Genetic data strongly favours the differentiation of the GC as a reservoir of unique biodiversity. The extent of genetic and ecological partitioning in both bottlenose and common dolphin highlight the importance of resource specialization in the evolution of reproductive barriers among sympatric and parapatric populations (Hoelzel 1998), and supports the hypothesis of local habitat dependence. Instead of being divided solely by

evident boundaries (but including this division to some extent, either side of the Baja California peninsula), these populations are divided by behaviour, and are therefore cryptic to the usual designations of stock boundaries based on geography. This is why the genetic data are essential to the assessment of management stocks in these species. In spite of their high mobility, their diversity is partitioned and requires regional management on that basis.

Common dolphins from the study area showed a partially supported reciprocal monophyly, and a significant level of differentiation between *D. delphis* and *D. capensis*. However, reciprocal monophyly is not a strict signal of evolutionary divergence (Chivers et al. 2005), especially for intrinsically non-monophyletic families, such as Delphinidae (LeDuc et al. 1999, Kingston and Rosel 2004, Kingston et al. 2009). Whether such genetic divergence represent a speciation event is still controversial (Natoli et al. 2006, Bilgmann et al. 2008), however it is clear that these populations need to managed separately for the purposes of conservation.

Table 5. 1 List of management units proposed in this study and supporting evidence. Based on the hierarchical phylogeographic approach for stock designation (Dizon et al. 1992).

Population	Distribution	Population response	Phenotypic evidence	Genetic evidence
Bottlenose dolphins				
Coastal-offshore	Sympatric, but habitat preferences	Socio-demographic differences	Strongly differentiated	Strongly differentiated
Northern gulf	isolated	Resident, as indicated by its year round occurrence	Limited information	Strongly differentiated
Common dolphin				
Short and long-beaked putative species	Sympatric, but habitat preference	Large population size, but limited demographic information	Strongly differentiated	Strongly differentiated
Gulf of California *D. capensis*	Habitat discontinuity showed habitat preference	Larger population size within the gulf, compare to Pacific Ocean	No evidence	differentiated

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Appendix

6.1 Summary of the results from analyses based on 12 microsatellite loci.

All estimated and test performed for microsatellite data were also performed excluding loci EV14, EV37Mn, KWM2a, KWM2b and TexVet5, which showed departure from HWE. The results were similar to those estimates based on 16 loci. In this section shows the estimates and results based on 12 loci. Fixation index F_{st} = 0.023, p< 0.001.

Table A. 1 Statistical test for sex-biased dispersal between males and females over all populations. n = number of individual tested, *Ho*: observed heterozygosity; *He*:expected heterozygosity; F_{IS}: inbreeding coefficient; F_{ST}: fixation index, *R*: relatedness coefficient, *AIc*: mean corrected assignment index, *vAIc*: variance of the corrected assignment index *AIc*. Based on 12 microsatellite loci.

	n	F_{is}	F_{st}	Relatedness	*Ho*	*Hs*	*AIc*	*vAIc*
Females	116	0.077	0.036	0.064	0.713	0.773	0.157	11.135
Males	148	0.056	0.033	0.06	0.734	0.777	-0.123	11.99
p-values		0.25	0.66	0.76	0.27	0.49	0.54	0.62

Table A. 2 Summary results of IMa, based on mtDNA control region sequences (778bp). t: time from divergence, Ne: estimated effective population size, ancestral Ne: ancestor effective population size, m: migration rates; (high posterior probability range-HPD90).

Parameter	*D. delphis*	*D. capensis*
t (years)	125 508.136 (88 987.5 - 165462.5)	
Ne	120 046.80 (87 363.94 – 139 600.67)	23 639.23 (16 306.53 – 34 324.028)
ancestral Ne	7381.59 (2362.76 – 19 179.09)	
m	0.013 (0.001-0.036)	0.013 (0.001- 0.047)