Chapter I General Introduction

1.1 Evolution and Speciation

The evolutionary theory is a unification of concepts that provides a coherent explanation for the origin and continuation of the diversity and complexity of life. The core of this theory is that organisms have descended with modification from a common ancestor (Darwin 1859). Evolution occurs as an adaptive process mainly driven by natural selection on existent genetic variation (Huxley 1942). Genetic variation is affected by several evolutionary forces, such as mutation, gene flow, genetic drift and selection. Thus, genetic variation underpins biological diversity and also contains information of evolutionary history at all levels of biological hierarchy (Avise 2004).

Recent technological and theoretical advances have increased the potential of researchers for correctly analysing and interpreting the information content of DNA. This includes genome wide sampling of individuals and populations (Nielsen 2005; Luikart *et al.* 2003) to the use of more rigorous statistical methods for genetic inferences (Hey 2006; Knowles 2009). Consequently, modern genetic approaches can be used to address a range of questions about natural processes that have important impact on biology; from individual reproductive behaviour to biogeographic patterns (Avise 2004; Avise 2009). In fact, genetic tools have facilitated the rapid accumulation of molecular information necessary for the better understanding of complex and controversial issues, such as the origin of the species – a topic widely considered as the "mystery of mysteries".

Speciation is one of the most important and less understood processes in nature. Most biologist agree that species are fundamental biological units for several ecological and evolutionary process (De Queiroz 2007). However, contentious and unresolved debates still exist about the definition, delimitation and the origin of species (De Queiroz 2007 Wiens 2007). These debates persist mainly because of the continuity of the speciation process that make it difficult to establish limits between the starting and ending points of the process (De Queiroz 2007; Via 2009). Despite these controversies and the several alternative species definitions, the biological species concept has been the most accepted and used species concept in biological studies (Coyne & Orr 2004 ;De Queiroz 2007). Under this concept the species are delimited by intrinsic reproductive isolation between groups of organisms and the speciation occurs through the accumulation of ecological and genetic divergences that create barriers to gene flow between two populations (Mayr 1942). Hence, the study of patterns of reproductive isolation and the mechanisms responsible is fundamental to understand the speciation process.

Historically, the origin of the species has been explained based on the geographic arrangement of populations undergoing the speciation process (Coyne & Orr 2004). Even though several geographic modes of speciation have been proposed, the most accepted are allopatric, parapatric and sympatric (Endler 1977; Howard & Berlocher 1998a; Coyne & Orr 2004). In allopatric speciation, the divergence happens because of genetic drift and selection acting on populations isolated by geographic barriers (Mayr 1942). In parapatric speciation, reproductive isolation evolves gradually by divergent selection in adjacent populations found in different habitats (Gavrilets *et al.* 2000). Sympatric speciation also occurs by divergent natural selection but in this case there is complete absence of geographic barriers (Dieckmann & Doebeli 1999). Although this categorization of the process has been widely accepted, some

have argued that a classification focusing on mechanisms driving reproductive isolation would be more functional and productive (De Queiroz 2007). Four modes of speciation have been proposed according to initial causes of reproductive isolation: speciation by divergence under uniform selection, speciation by genetic drift, polyploidy speciation and ecological speciation (Schluter 2001; Schluter 2009; Via 2001; Via 2009). The speciation by divergence under uniform selection implicates allopatric or parapatric populations under similar selection pressures but with different advantageous mutations. Speciation by genetic drift requires only allopatric populations and time for genetic divergence to take place. The polyploid speciation occurs when chromosomal rearrangements or hybridization cause genetic incompatibilities. Finally, in ecological speciation reproductive isolation takes place due to divergent selection on ecological resource use or sexual selection and can occur between allopatric, sympatric or parapatric populations (Howard & Berlocher 1998a; Coyne & Orr 2004). These classifications indicate that studies of spatial patterns of population structure and the identification of barriers for gene flow are essential to understand the speciation process.

Speciation research has been dominated by a retrospective approach, focused on the analysis of reproductive isolation between already considered species. Certainly, this approach has contributed to identify different barriers to gene flow that isolate species (Howard & Berlocher 1998a; Coyne & Orr 2004). However, it has several limitations, especially regarding the analysis of mechanisms causing the evolution of barriers to gene flow (Via & West 2008; Via 2009). In fact, a retrospective analysis cannot distinguish between the genetic changes responsible for the initial isolation and the genetic differences accumulated after the isolation, making it easy to confuse between consequences and causes of speciation (Coyne & Orr 2004). Since divergent populations, races or ecotypes are early stages on a continuum, the barrier that partially isolated them must be equivalent to those that produce the initial isolation

of sister species in similar evolutionary conditions. A population level analysis of the ecology and genetics of partial reproductive isolation could contribute to the clarification of the real causes of reproductive isolation, their mechanisms and barriers (Via & West 2008; Via 2009). Therefore, the analysis of genetic differences between sister species and populations would allow us to integrate information about the early stages and the end products of reproductive isolation and, consequently, produce a most complete understanding of the speciation process.

1.2 Elasmobranchs

1.2.1 Evolution, Zoogeography, Ecology and Taxonomy

In an evolutionary perspective, the elasmobranchs have been a success story. They have shown long term diversity, numerous adaptive radiations and sophisticated morphological, ecological and behavioural specializations. These characteristics have promoted their high resilience to mass extinction in the last 400 million years and underpin their current biological diversity. Nowadays, elasmobranches are the second most diverse group of marine vertebrates; their taxonomic, morphological, ecological and behavioural diversity is overcome only for that of Osteichthyes (Compagno 1990).

There are several hundred species of fossil elasmobranchs described. They represent only a small fraction of the group diversity over the last 400 million years (Zangerl 1981; Cappetta 2004; Ginter *et al.* 2010). During that time elasmobranch diversity increased and decreased several times, such as recoveries from mass extinctions during the Permian-Triassic and Cretaceous-Tertiary transitions (Zangerl 1981; Cappetta 2004; Ginter *et al.* 2010). This

evolutionary vigour and flexibility has allowed sharks and rays to exploit new habitats and opportunities presented during the Cenozoic.

The contemporary elasmobranchs have colonized almost all aquatic ecosystems. Even though, only a few species (~5%) are found in rivers and lakes (Compagno 1995; Martin 2005) with the group being predominantly marine. They are found in most marine habitats; from the polar zone in the Arctic and Antarctic regions to the tropical zone in Equatorial regions; from the coastal zone to the pelagic realm, and from the water surface to the abyssal zone up to three thousand meters depth (Compagno 1990; Musick *et al.* 2004; Compagno 2005). Nonetheless, most species occur in coastal areas, from the intertidal to 200m depth of tropical and subtropical regions (Compagno 1990; Compagno 2005).

In these environments, elasmobranchs have a key ecological role. They are apex predators that consume a wide range of prey, from minute zooplankton to huge whales, interacting with almost all marine animals (Stevens *et al.* 2000; Wetherbee & Cortés 2004). As efficient wide-ranging predators, elasmobranchs contribute to the shaping of community structure by impacting the population sizes of prey and competitor species, and also play a role in the transfer of energy between trophic levels and habitats (Wetherbee & Cortés 2004). Changes in the abundance of elasmobranchs could have strong top-down ecological effects in the marine ecosystem (Stevens *et al.* 2000).

Elasmobranchs are in general relatively high mobile animals, but because of their niche diversity also show a range of mobility potential (Compagno 1990; Musick et al 2004; Frisk 2010). Thei vagility tends to be lowest in demersal, higher in benthopelagic and highest in

pelagic species, being also lower in coastal than in oceanic species. Vagility has been associated with species diversity, with poor dispersers accounting for the highest diversity of elasmobranch species (Musick *et al.* 2004).

There are currently more than 1,100 described living elasmobranch species, with approximately 10 new species are described annually (Compagno 2005; Last 2007). In addition, many cryptic species of sharks, rays and skates are expected to exist due to the inherent difficulties in studying their ecology and behaviour. The high species diversity in elasmobranchs contrasts with some of their characteristics. In addition to relatively high vagility (Compagno 1990; Musick *et al.* 2004), elasmobranchs show low molecular evolutionary rate (Martin 1999). High evolutionary rates allow accumulation of variation, enabling genetic differentiation in short timeframes. Some authors have reported a positive correlation between molecular evolutionary rates and the genetic diversity within a population and speciation rates within a taxon (Barraclough & Savolainen 2001). In addition, the effect of physical barriers to gene flow is relative to organism mobility. Vagility has been actually inversely correlated with taxonomic diversity, speciation rate, and genetic differentiation (Rosenblatt & Waples 1986; Chenoweth et al. 1998; Musick *et al.* 2004).

Despite their ecological importance and evolutionary success, there are significant gaps in our knowledge of evolutionary history and relationship in several groups of sharks and rays. For instance, population genetic and phylogenetic studies are relatively rare in elasmobranchs (Beheregaray 2008; Heist 2004a). The vast majority of phylogenetic studies in elasmobranchs are on higher taxonomic levels (e.g. Bernardi & Powers 1992; Naylor 1992; Martin 1993; Dunn & Morrissey 1995; Naylor *et al.* 1997; Arnason *et al.* 2001; Martin 2001; Douady *et al.* 2003; Dunn *et al.* 2003; Winchell *et al.* 2004; Iglésias *et al.* 2005), but at family or bellow

level has rarely been analysed (López *et al.* 2006; Human *et al.* 2006; Cavalcanti 2007; Corrigan & Beheregaray 2009; Straube *et al.* 2010; Stelbrink *et al.* 2010; Vélez-Zuazo & Agnarsson 2011). Despite the bloom of genetic population works in elasmobranchs during the last two years, the number of paper relative to other organism remind limited.

1.2.2 Fisheries and management

Although elasmobranchs have been considered historically as a resource of low economic value, the last decades have seen a major expansion of shark, ray and skate fisheries (Bonfil 1994; Stevens *et al.* 2000; Clarke *et al.* 2005). This expansion has been attributed to three main factors: (1) the escalating human demand for food, (2) the lucrative markets for fins in Asia, and (3) the collapse of preferred traditional fisheries (Fowler *et al.* 2005; Clarke *et al.* 2005). Thus, nowadays sharks and relatives are target species by artisanal, industrial and recreational fisheries. Between 2000 and 2007, more than 5,760,000 tonnes of landed chondrichthyan fishes were reported (FAO 2011). Assuming an average weight of 20kg per individual (Bonfil 1994; Stevens *et al.* 2000), the global reported catches represent over 280 million elasmobranchs in an eight year period. Moreover, because of inaccurate records and unreported landing, the actual fisheries-related elasmobranchs mortality is thought to be up to two times higher (Bonfil 1994; Stevens *et al.* 2000). Most elasmobranchs are long-lived animals with slow growth, late maturation and low fecundity; these characteristics make them extremely susceptible to overexploitation (Cailliet *et al.* 2005). In fact, many elasmobranch fisheries have collapsed; some species have been extirpated or are considered as threatened (Walker 1998; Camhi *et al.* 1998; Casey & Myers 1998; Dulvy & Reynolds 2002; Dulvy *et al.* 2008; Dulvy & Forrest 2010). The elasmobranch overfishing has produced major economical and ecologically negative effects (Stevens *et al.* 2000; Myers *et al.* 2007). Many elasmobranch fisheries are not regulated because of the lack of basic biological information

needed to implement adequate management (Camhi *et al.* 1998; Fowler & Cavanagh 2005). Therefore, studies that improve our knowledge about elasmobranch ecology, population structure and life-history are priorities in management policies. This is especially true in economically-poor regions with high species diversity, such as the Gulf of California in Mexico.

Elasmobranchs have been an important food resource in Mexico for centuries and their exploitation can be traced back to pre-Hispanic cultures (Applegate *et al.* 1993). However, commercial fisheries of elasmobranchs in Mexico have not started until the end of the 19th century, when shark fins were first exported to Asia. Since that time, following a global pattern, elasmobranch fisheries in Mexico have increased in size and extension (Bonfil 1994). In fact, in the last decades Mexico has been ranked as one of the ten largest fishing countries of elasmobranchs in the world (FAO 2011; Clarke *et al.* 2005). Until 2007, Mexico lacked an elasmobranch management and conservation regulation. Although the implementation of such regulation, known as NOM-29-PEC, represents a significant improvement to shark management in Mexico, the legislation lacks species-specific guidelines and does not account for stock structure, geographic variation in population demography, and patterns of connectivity. The lack of sustainable management of the elasmobranch fishery in Mexico has been reflected in a decline of catches in the Gulf of Mexico (Bonfil 1994; Castillo-Geniz *et al.* 1998), in the central Pacific coast (Pérez Jiménez *et al.* 2005), and in the Gulf of California (Elizalde-Hernandez personal communication). The latter region produces more than 60% of the national elasmobranchs catch (INAPESCA 2010).

1.3 The Gulf of California and the Baja California Peninsula

The Gulf of California, also known as the Sea of Cortez, is a fascinating and important marine ecosystem. It ranks amongst the five most productive and biodiverse marine ecosystems in the world (Alvarez-Borreo 1983) and supports significant fisheries and tourism activities that are important to the national economy (Enríquez-Andrade *et al.* 2005). In addition, the Gulf of California shows ecological and geological characteristics that are key to study microevolutionary processes (Bernardi *et al.* 2003; Jacobs *et al.* 2004).

1.3.1 Geological History

Even though that geologists originally considered that the Baja California Peninsula was formed as the product of simple detachment from the mainland, recent studies indicate a much more complex formation process. This process involves the interactions of plate drift, volcano activity and hydrostatic buoyancy. The formation of the Gulf of California and the Baja California Peninsula started around 12.5 million years ago (Mya), when the southern region of a proto-peninsula rotated westwardly and away from the mainland, and a temporary proto-gulf was produced in the current upper gulf region (Hausback 1984). At that time the Cape Region was a series of volcanic islands located some kilometres south from its actual position and the proto-gulf was less than half of its actual size (Fig 1.1; Gastil *et al.* 1975). By the late Miocene (~7.5 Mya) the proto-gulf extended up to the current island regions, the proto-peninsula was separated from the mainland by a channel, while the southern portions of the peninsula existed as an archipelago (Fig 1.2; Grismer 1994). Around 5.5-6 Mya tectonic interactions moved the proto-peninsula some 300 km northwest and the Gulf of California was permanently formed, with the southern peninsula still remained as an archipelago (Fig. 1.3; Hausback 1984). By 4 Mya the peninsula was almost completely connected except for

the cape region. During late Pliocene (~ 3 Mya) a mid-peninsula was temporarily reformed (F. 1.4; Ochoa-Landin *et al.* 2000) but by the end of the Pleistocene the Baja California Peninsula was completely formed. In 1997, Upton and Murphy used phylogenetic evidence to propose the re-occurrence of the mid-peninsula seaway around 1 Mya. Geological evidence supports the existence of a mid-peninsula seaway, but by 3 Mya it was closed. The final conformation of the Baja California Peninsula allowed the gradual establishment of the present environmental conditions of the Gulf (Ochoa-Landin *et al.* 2000).

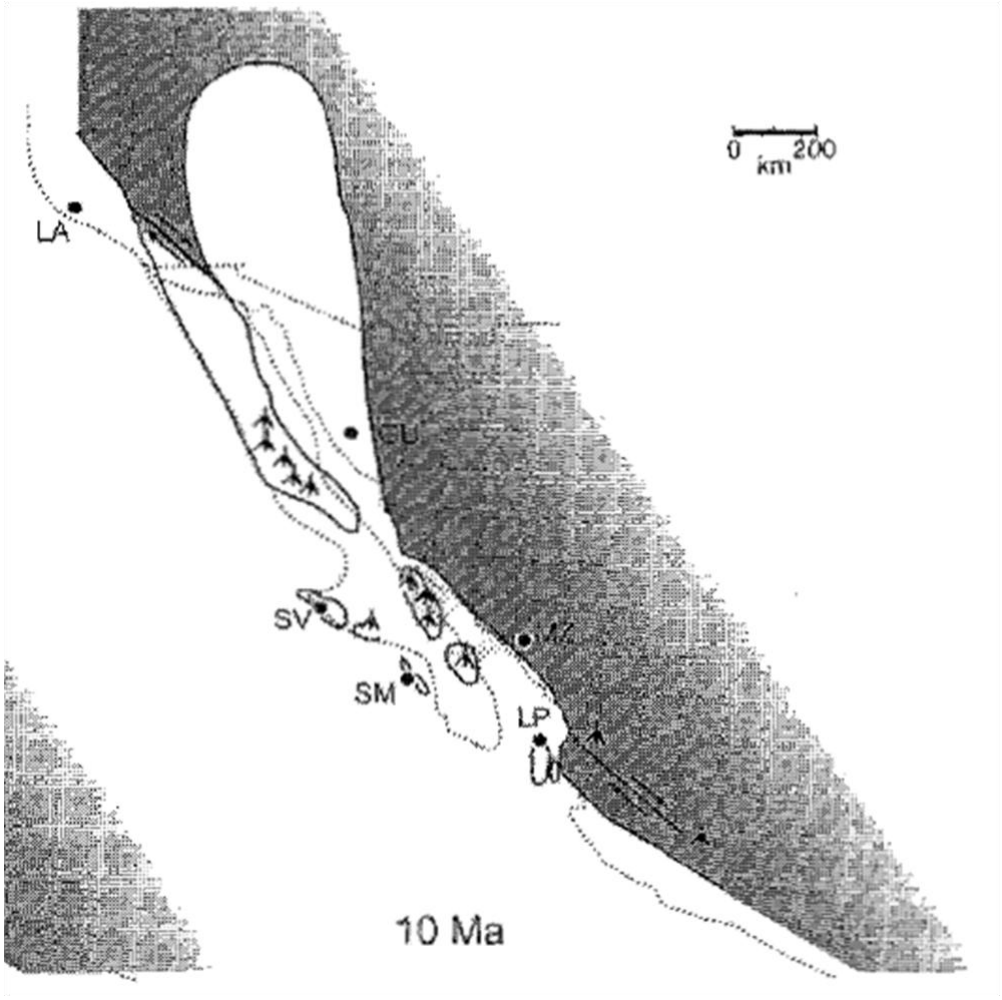

Fig. 1.1. Paleogeographic reconstruction of western North America for the Mid-Miocene. The stripped areas symbolize the temporary gulf and the dashed line represents the contemporary coast shoreline (from Murphy & Aguirre-Leon 2002)

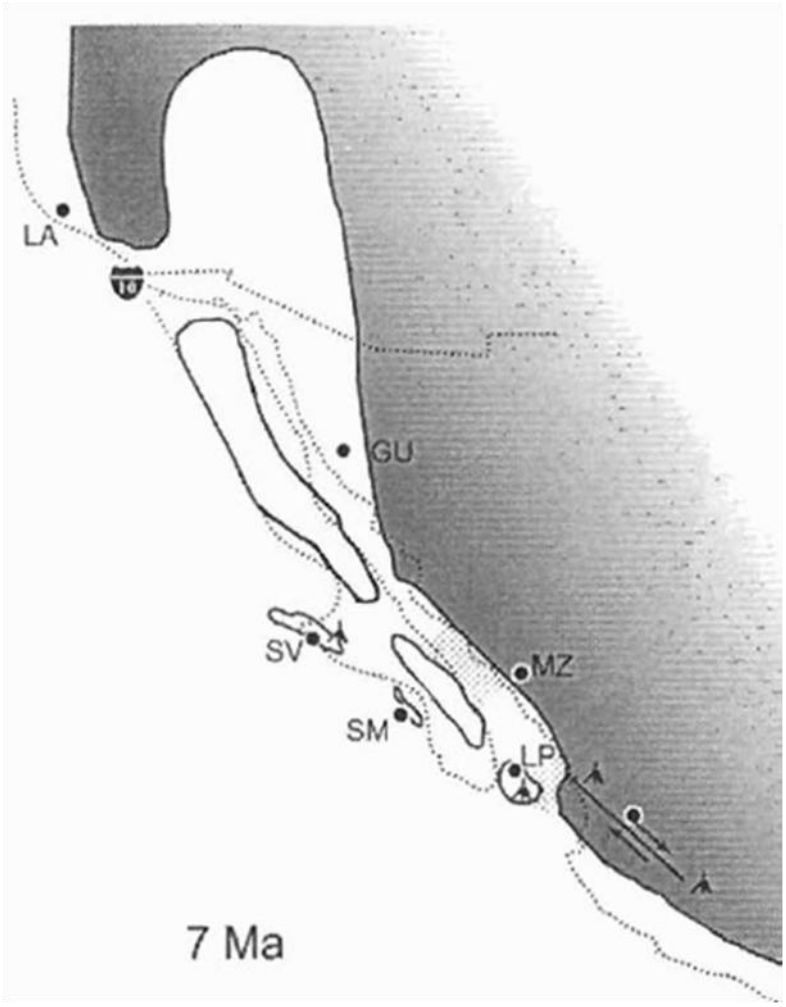

Fig. 1.2. Paleogeographic reconstruction of western North America for the Late-Miocene. The stripped areas symbolize the temporary gulf and the dashed line represents the contemporary coast shoreline (from Murphy & Aguirre-Leon 2002)

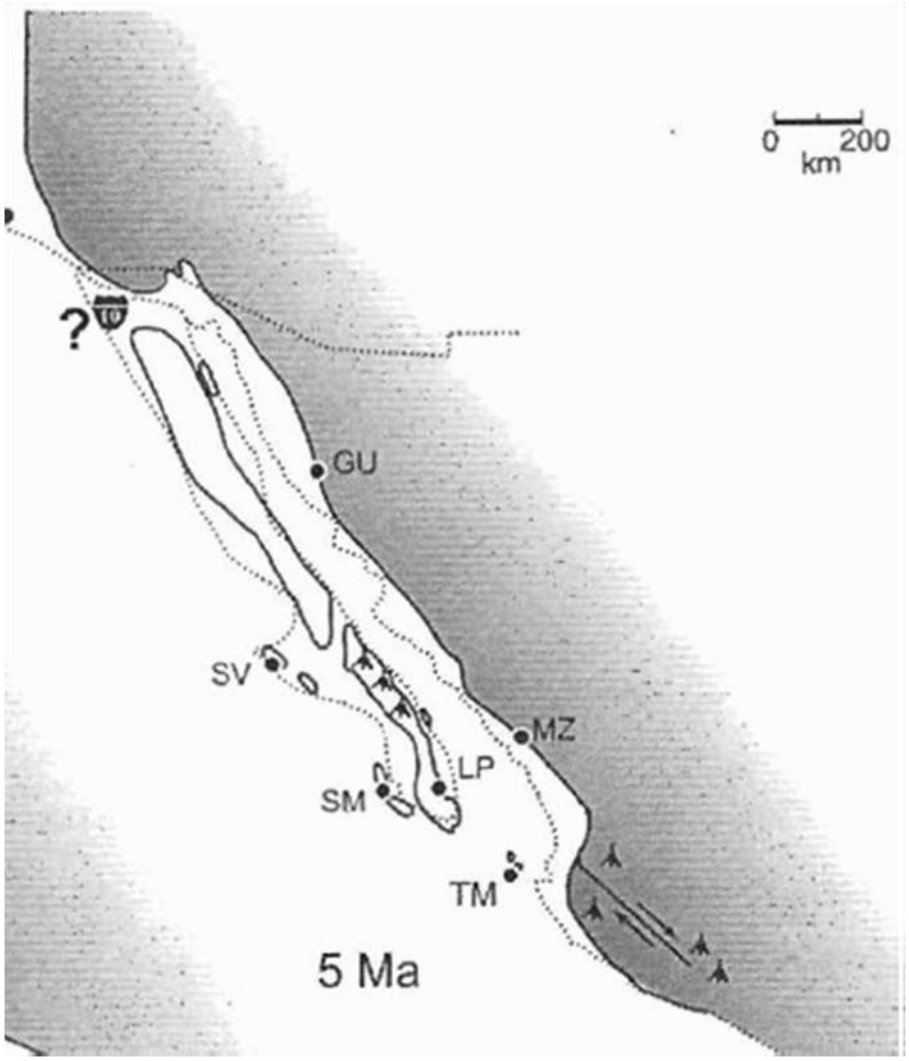

Fig. 1.3. Paleogeographic reconstruction of western North America for the Miocene-Plionece. The stripped areas symbolize the temporary gulf and the dashed line represents the contemporary coast shoreline (from Murphy & Aguirre-Leon 2002)

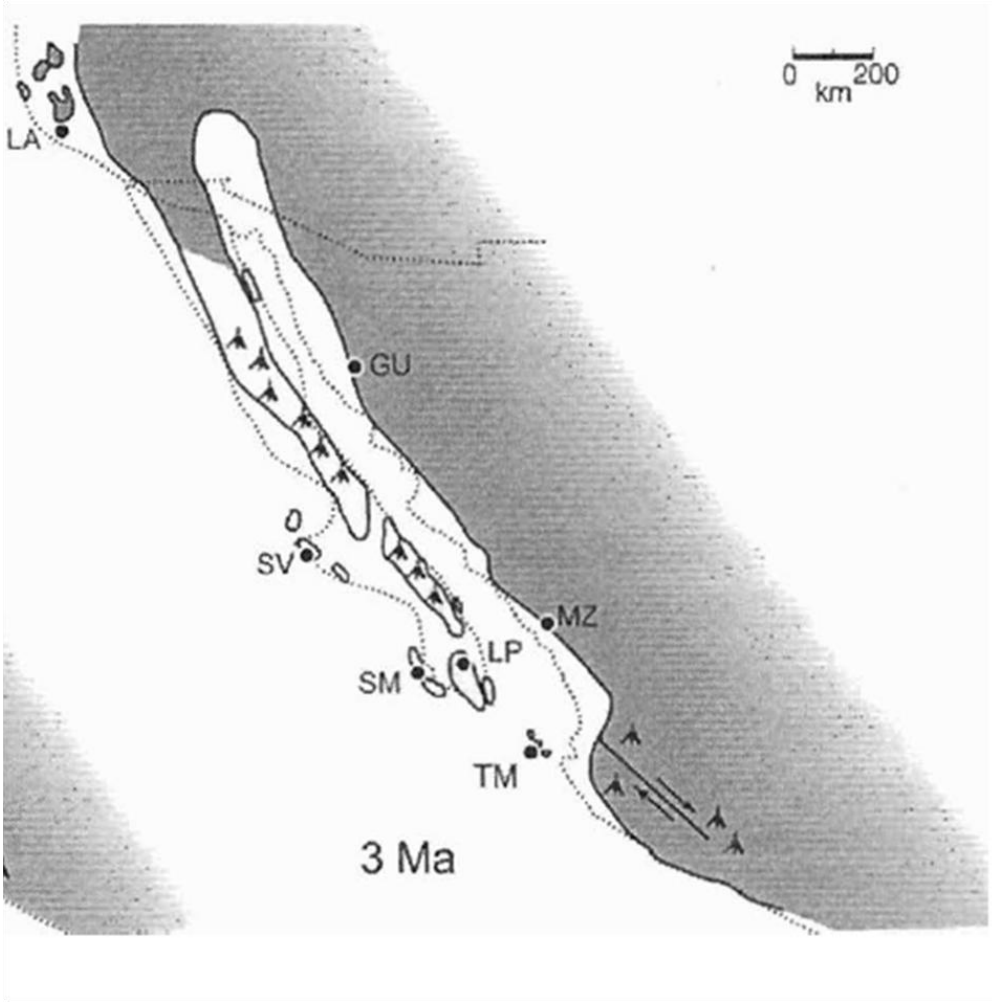

Fig. 1.4. Paleogeographic reconstruction of western North America for the Late-Pliocene. The dashed line represents the contemporary coast shoreline (from Murphy & Aguirre-Leon 2002).

1.3.2 Oceanography

The Gulf of California is a long narrow semi-enclosed sea. It is localized between semitropical and temperate zones, promoting a latitudinal gradient of ecological conditions. With a length of ~1500 km and a width between 80 and 300 km, the Gulf covers an area of more than 300,000 km^2 and various climatic zones and oceanographic regions characterizing four distinct bioregions (Fig. 1.5; Lavín & Marinone 20033; Ortega *et al.* 2010):

The Open Gulf – The main feature of this region is its transitional state. It has a great influence from the Pacific Ocean, an outgoing flux close to the peninsula and ingoing flux along the mainland coast, and a small cyclonic circulation in its centre (Mascarenhas *et al.* 2004). This region presents a wide continental shelf and most of the area is oceanic with depths up to 3,500m. The salinity and temperature are constant (35-36 ‰ and 22-28°C, respectively) and the tidal range is no greater than 1.5 m. With low concentration of nutrients and wind-driven upwelling only during winter, this area has the lowest productivity in the Gulf of California (Lavín & Marinone 2003; Ortega *et al.* 2010).

The Lower Gulf - This bioregion has very narrow continental shelf and depths of 1,500 m. As in the open gulf, the lower gulf shows low variation in temperature and salinity with reduced tidal range. However, the region has a very dynamic oceanography. A series of geostrophic gyres (i.e. eddies) about 50-150 km in diameter are often present in the area. These can be cyclonic or anti-cyclonic without an evident seasonal pattern, but with marked effects in the circulation and thermodynamic of the Gulf. Wind-driven upwellings are generated on the mainland during winter and on the peninsula during summer but the region has relatively low concentration of nutrients that limit primary productivity (Ortega *et al.* 2010; Lavín & Marinone 2003).

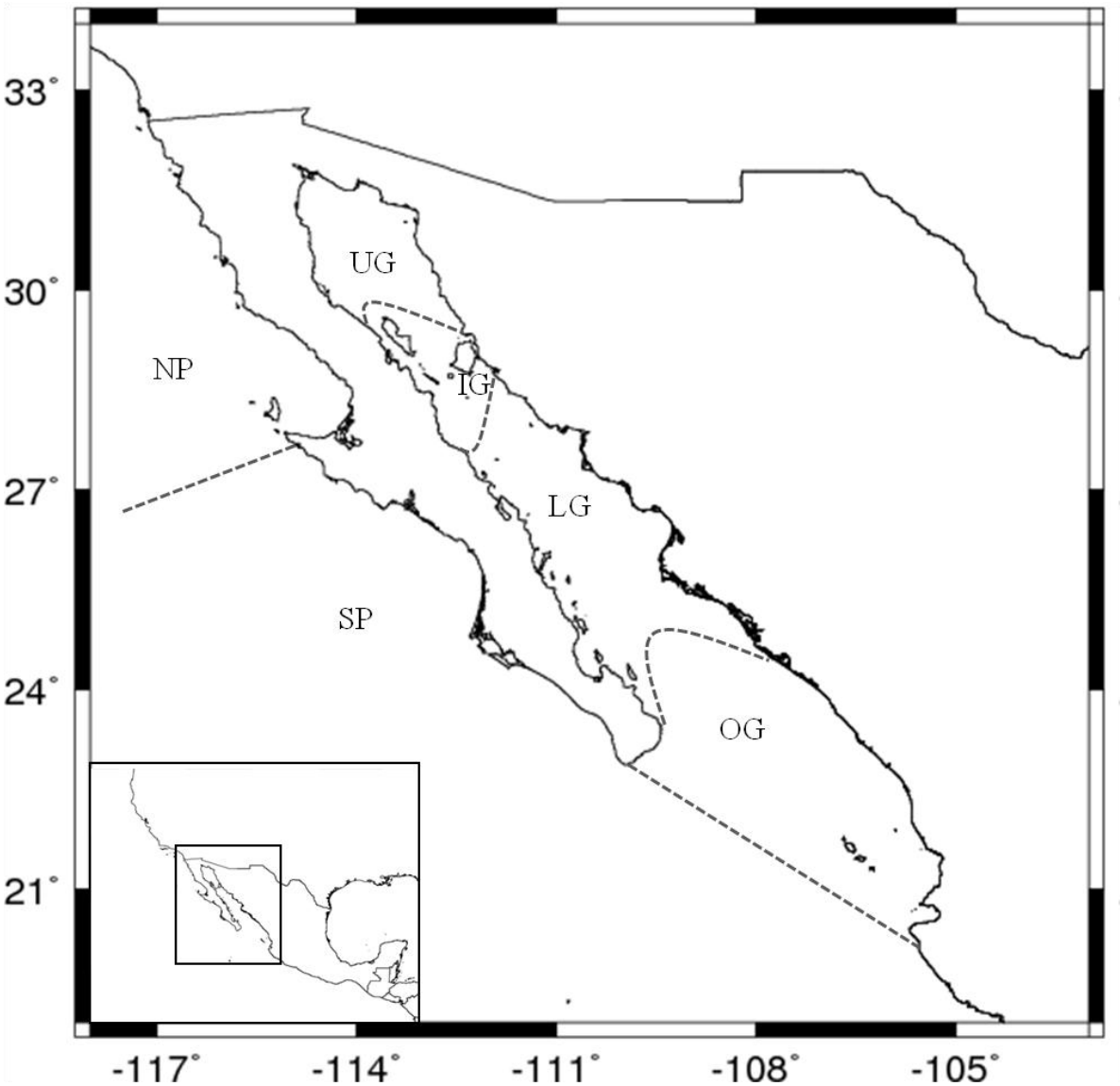

Fig1.5. The Gulf of California and the Baja California Peninsula. Bioregions are separated by dashed lines; Baja California Peninsula: North Coast (NP) and South Coast (SP); and for the Gulf of California: Lower Gulf (LG), Islands (IG), Upper Gulf (UG) and Open Gulf (OP).

The Islands Region - This is the smallest bioregion in the Gulf, characterized by the largest island and several narrow deep channels with up to 1,600 m depth. Buts it most distinctive feature is the presence of extensive and strong tidal mixing. As tide squeezes through the straits, tidal currents are intensified to such an extent that surface water boils and a strong upwelling is produced. The upwelled waters are very cold and have relatively low oxygen but high nutrients concentration, promoting primary productivity. In fact, this area has the lowest temperature and oxygen saturation of the Gulf throughout the year. Nevertheless, the Island Region it is the most productive bioregion in the gulf (Ortega *et al.* 2010; Lavín & Marinone 2003).

The Upper Gulf - With an average depth below 100 m, this is the shallowest region in the Gulf, being a tidal, evaporative basin (1m/year). This region comprised an estuarine environment but after the damming of the Colorado Riverit became an inverse estuary with high salinity (up to 40‰) throughout the year. This region also shows the wider range of temperature, from 9°C in winter to 38°C in summer and tidal ranges up to 10 m. This tidal range produces a strong mix and upwelling that allows high nutrient concentration and primary productivity (Ortega *et al.* 2010; Lavín & Marinone 2003).

1.3.3 Biodiversity and Economy

The high productivity and habitat complexity of the Gulf of California have promoted the evolution and diversification of a spectacular collection of biodiversity. The Gulf of California is among the five most diverse marine ecosystems of the world (Alvarez-Borreo 1983) and is recognized worldwide for its biological richness, productivity rates and scenic beauty. Since 2005, the islands and protected areas of the Gulf of California have been listed as World Heritage sites (UNESCO 2011)

The Gulf has more than 2500 species of micro and macroalgae, 62 are endemic and near to 5,000 species of macroinvertebrates. Approximately 700 species of macroinvertebrates are endemic, and hundreds are thought to be described (Brusca 1973a). Around 875 species of fishes inhabit this sea, 87 are endemic and others are yet to be described (Castro-Aguirre *et al.* 1995). Out of the seven extant marine turtles species worldwide, five are found in the Gulf and some use it as nesting area (Lluch-Cota *et al.* 2007). There are 887 species of terrestrial plants and animals living on the islands of the gulf, including 90 endemic species. Approximately 181 marine bird species are distributed in the GC, at least 26 of them use the islands as reproductive areas, four species are quasi endemic (95% of the global population are in the Gulf) and two are endemic (Lluch-Cota *et al.* 2007). There is a total of 38 species of marine mammals in the Gulf, the region is also recognised for the high diversity of cetaceans, with 40% of all known cetacean species found in the region (Vidal 1993).

Moreover, out of the ~1100 accepted species of elasmobranchs over 90 have been reported in the GC, and at least 3 are endemic to the area. Approximately 52 species of shark from 23 genera, 15 families and six orders could be found in this rich ecosystem along with 41 species of batoids (rays, skates and mantas), form 17 genera, 9 families and 4 orders (Castro-Aguirre & Pérez 1996; Epinosa-Pérez *et al.* 2004). Some of these species are abound and are target species in the important commercial fishery of the GC.

Unfortunately, there are approximately 10 million people living in the Gulf of California, which is the region with one of the highest rates of population growth in Mexico (1.9%) (Enríquez-Andrade *et al.* 2005). In addition to the habitat degradation and pollution caused by humans, economical activities linked to exploitation of natural resources (e.g. fishing) have resulted in a decline of natural populations and ecosystems productivity, with negative

economic impacts (Lluch-Cota *et al.* 2007). The ecological and economical value of the region has resulted in the establishment of natural reserves based on estimates of local species diversity. However, different stakeholders involved with management and conservation in the Gulf (i.e. Mexican Government, ONG's, fishermen) recognize that the current levels of biological knowledge about the region are not sufficient to provide sound advice for the design of a network of marine protected areas.

1.4 Molecular markers to study elasmobranchs from the Gulf of California

In this thesis, information from nuclear and mitochondrial DNA markers was combined to study genetic structure and speciation in elasmobranchs from the Gulf of California.

Mitochondrial DNA (mtDNA) - Because of its small size and conserved gene regions, animal mtDNA is a relatively easy to isolate and to amplify using the polymerase chain reaction (PCR) (Kocher *et al.* 1989). In addition, mtDNA is universally distributed in the animal kingdom, is usually homoplasmic, and shows haploid matrilineal inheritance. Thus, it is easily comparable among taxa, normally providing clearly defined maternal genealogies without the effect of the recombination (Moritz *et al.* 1987; Avise 1995; Avise 2004). Moreover, the rate of mutational substitution in mtDNA is comparatively faster than in nuclear DNA, increasing rates of lineages sorting and consequently the resolution of analyses (Moritz *et al.* 1987; Avise 2004). These factures have made this a widely used marker in investigations of phylogeny, demography, dispersal, speciation and systematics (Avise 2004). In this study mtDNA control region sequences were used to address questions about speciation, population structure and population history (Chapter 2, 3 and 4). The control region is a non-coding gene that participates in the control of the mitochondrial genome replication, and typically shows the fastest mutational rate of the mtDNA (Avise 2004).

However, the largely uniparental inheritance of this genome limits its power in disclosing fine-scale population structure and levels of differentiation in species with sex-bias dispersal tendencies (Hoelzer 1997), including elasmobranchs. Also, the organellar features of the mtDNA makes this essentially a one-locus marker (Moritz *et al.* 1987). Because gene histories and signals of demographic changes can vary substantially across loci, phylogenetic and genealogical inferences derived from a single locus may not reflect the true evolutionary history of the population or species under study (Rosenberg & Tao 2008). Therefore, information from multiple unlinked loci is necessary for robust conclusions about population structure and population history to be made.

A type of nuclear marker that has grown in popularity in recent years for population genetic studies of non-model species are the amplified fragment length polymorphisms (AFLPs) (Meudt & Clarke 2007). These markers are especially useful when species-specific markers such as microsatellites (see below) are not available for the organisms under study and the work involves multispecies comparative analysis, which is exactly the case in this thesis. The technique consists in consecutive enzymatic reactions. First, genomic DNA is cut by a restriction endonuclease digestion. Then a set of adapters are ligated to the restriction fragment. These adapters are used as primers for a selective PCR reaction, which results in a unique reproducible profile per individual (Vos *et al.* 1995). Usually, the profile consist of hundreds of loci that are widely distributed throughout the genome (Meudt & Clarke 2007). Since this method screens for genome-wide variation, some loci might deviate from neutrality but most independent markers would be neutral, showing unequal levels of genetic differentiation. This allows the identification of loci under selection (Beaumont & Balding 2004). Comparing patterns between loci under selection and neutrality allows analyses of the effect of natural selection in reproductive isolation. Perhaps the major disadvantage of the

AFLP markers is their dominance, reducing the informative power of each AFLP locus. However, the analysis allows the generation of numerous genome-wide markers with a high discriminatory power by the simultaneous assay of a large number of markers (Meudt & Clarke 2007).

For this study AFLP protocols were used to assess levels of gene flow and genetic structure within species and to detect possible effects of divergent selection in genetic differentiation. Unfortunately, after an initial successful period with the AFLP protocol commonly used in our laboratory, I experienced several complications that limited my progress for several months. At that time, several hundred restriction digestions were performed followed by the corresponding ligation, which apparently worked as expected. However, the subsequent selective PCRs did not work properly. After trying a range of modifications and optimizations, enough data were generated for only two (Chapters 2 and 3) out of the three species included in this thesis. For the third species it became necessary to explore alternative methods.

Microsatellite DNA have been one of the most popular genetic markers in population and individual level studies (Goldstein & Schlötterer 1999) because of their multi allelic nature, co-dominant mode of inheritance, high reproducibility and abundance (Schlotterer 2004). Microsatellites are short tandem repeats of a sequence that shows high mutational rates and polymorphism. Usually, in population genetic studies, di-, tri- or tetra-nucleotide repeat motifs are used (Sunnucks 2000). The rapid evolution of microsatellites allow them to detect fine-scale population structure over relatively short spatial and temporal scales (Sunnucks 2000). Until the end of 2010 there were no microsatellite markers available for any of the elasmobranch genera subject of this research. Fortunately, microsatellite primers that cross

amplify within the genus *Mustelus* (the group for which AFLP problems could not be resolved – as above) were recently reported in the literature (Boomer and Stow 2010). In this study microsatellite markers were used to infer patterns of genetic connectivity and population structure, and to assess sex-biases in dispersal (Chapter 4).

1.5 Thesis Outline

This work combines phylogenetic, population genetics and seascape genetic approaches to assess evolutionary divergence, reproductive isolation, and genetic structure in three co-distributed groups of elasmobranchs from the Gulf of California and the Pacific coast of the Baja California Peninsula, in Mexico.

The study groups are the guitarfish *Rhinobatos productus*, the angel shark *Squatina californica* and the brown smooth-hound *Mustelus henlei*. *R. productus* is a demersal guitarfish with a wide distribution from San Francisco, California to Guerrero Mexico (Fischer *et al.* 1995). *S. californica* is a temperate demersal shark that ranges from Alaska to the Gulf of California and from Peru to southern Chile (Compagno 2005). *M. henlei* is an epibenthic species found in temperate and sub-tropical waters from northern California to the Gulf of California and from Ecuador to Peru (Compagno 2005). Elasmobranchs have a wide range of mobility capacity, from the sedentary benthic coastal rays to the pelagic ocean sharks (Compagno 1990; Musick *et al.* 2004). The three species are co-distributed and are abundant in the Gulf of California. Although *S. californica* and *R. productus* have similarly low dispersal capacity, *M. henlei* is expected to show much higher vagility. It is expected vagility to be negatively correlated with genetic structure and speciation rate, a theory that has not been genetically tested in elasmobranchs.

The fascinating study region of this thesis provides a geologically and oceanographically complex system to assess the influence of geological and ecological processes on population divergence and speciation. Having this region as the geographic arena for population comparisons, the overarching aim of the thesis is to assess the influence of life history (in this case, potential species mobility) on genetic structure and speciation in elasmobranchs.

The thesis follows the format of three stand–alone data chapters (Chapters 2, 3 and 4) that contain the relevant information about each study system, study aims, results and discussion. In addition, the thesis contains a chapter that provides background information and introduce the study (Chapter 1) and a final concluding chapter (Chapter 5). Since the data chapters have been prepared as articles for submission to scientific journals and deal with elasmobranch populations collected along the same region, there will be information that have been inevitably repeated in these three chapters.

Chapter 2 investigates genetic divergence associated to bioregional divisions with the aim of assessing the relationship between ecological processes and speciation in the *R. productus* complex. Phylogenetic and genetic evidence about cryptic species is presented and seascape genetics is used to test for associations between genetic divergence and oceanographic factors. In Chapter 3, the importance of historical biogeography as barriers to gene flow was analysed in the context of low mobility in *S. californica*. This chapter presents evidence for allopatric speciation and ecological isolation in angel sharks. Finally, Chapter 4 presents an analysis of connectivity and sex-bias dispersal in a high mobility species, *M. henlei*, a heavily exploited coastal shark from the Gulf of California and the Pacific Coast.

Chapter II Ecological speciation in an elasmobranch from the Gulf of California: the guitarfish genus *Rhinobatos*

2.1 Introduction.

Speciation has persisted as one of the most hotly contested topics in evolutionary research, especially in regard to mechanisms of diversification (Schluter 2001; Sobel *et al.* 2010). Many evolutionary biologist agree with the idea that most species evolve by natural selection, and in particular, by the process of "ecological speciation" in which reproductive isolation evolve between populations adapted to different environments or ecological niches (Schluter 2009; Via 2009). During the last decade, theoretical and empirical evidence for ecological speciation accumulated, challenging the dominant paradigm of physical mechanisms of diversification that underpin allopatric speciation (Rundle & Nosil 2005;Schluter 2009; Via 2009). Nevertheless, the link between speciation and natural selection is not always evident, and the role of ecological adaptation and divergence in the speciation process remains controversial (Coyne & Orr 2004; Nosil 2008; Schluter 2009). Fortunately, emergent analytical phylogeographic approaches based on coalescent theory and Bayesian statistics can be used to track the history of lineage divergence and provide information to assess the contribution of particular factors in species formation (Kidd & Ritchie 2006; Avise 2009; Knowles 2009). Moreover, progress in genome scan methods for detecting genomic regions under selection (Beaumont & Balding 2004; Jensen *et al.* 2007; Storz 2005), and phylogeographic information systems protocols (Kidd & Ritchie 2006), the importance of

divergent selection in the speciation process can now be assessed in the context of the geographic distribution of organisms.

In marine ecosystems, vicariant events and oceanographic discontinuities have been recognized as the main causes of population disjunctions that resulted in speciation (Palumbi 1994; Gaither *et al.* 2010). Oceanographic interfaces are regions with contrasting environments or ecological gradients that are potentially strong candidates for ecologically divergent natural selection. Yet, the role of oceanographic processes as drivers of ecological speciation has not been satisfactorily addressed (Beheregaray & Sunnucks 2001; Pastene *et al.* 2007). Studies aimed to assess the relative role of geographic isolation and divergent natural selection in the sea could benefit from population genetic and phylogeographic surveys of regional biota exposed to active geological history and complex oceanography. In this context, the Gulf of California (GC) and its adjacent Baja California Peninsula (BCP) provides an ideal region to study marine speciation. The geomorphological history of the Gulf has been particularly dynamic (Helenes & Carreño 1999; Murphy & Aguirre-Leon 2002), and its oceanographic conditions show high temporal and spatial variability (Lavín & Marinone 2003; Ortega *et al.* 2010). The processes that culminated in the formation of the GC and the BCP started ~12 million year ago (Mya), with the detachment of a proto-peninsula from the mainland. Tectonic activity transported the proto-peninsula and a volcanic archipelago 300 km northwest forming the GC ~ 6 Mya (Fig2.1A). At that time, the GC was connected to the Pacific Ocean by seaways between islands and the proto peninsula. By the early Pleistocene (~3 Mya), emerging land attached the islands and the proto-peninsula, closing the seaways (Fig2.1B and C) and subsequently forming the present day BCP (Murphy & Aguirre-Leon 2002). The formation of the peninsula allowed the establishment of the existing oceanographic condition inside the GC. These conditions (current dynamic, temperature, salinity, depth and productivity) divided the gulf in four well delimited provinces (Fig. 2.1D)

(Lavín & Marinone 2003): The open gulf (OP) is greatly influenced by oceanic waters from the Pacific, with an average outgoing flux close to the peninsula and ingoing flux along the mainland coast. The lower gulf (LG) has a dynamic oceanography with a series of geostrophic gyres affecting the circulation and thermodynamic of the area during all seasons. The islands region (IG) has channels that are over 500 m deep and is characterized by strong tidal-mix upwelling that maintain high productivity and low temperatures (~11°C) throughout the year. The upper gulf (UG) has shallow waters (< 100 m on average), high salinity (up to 40 ‰), large temperature variation (9-38°C) and large tidal ranges (>6 m) (Lavín & Marinone 2003; Ortega *et al.* 2010). Undoubtedly, the distribution and connectivity of the local marine biota has been affected by the history of formation of the region and its ecological complexity. Some molecular studies have examined the influence of these factors on genetic differentiation in GC-BCP organisms (Bernardi *et al.* 2003; Huang & Bernardi 2001; Hurtado *et al.* 2010; Jacobs *et al.* 2004; Lin *et al.* 2009; Riginos 2005; Sandoval-Castillo *et al.* 2004; Stepien *et al.* 2001), but most of them have focused on vicariant biogeography especially between Pacific and Gulf of California (i.e. allopatric diversification).

Although allopatric speciation is important in the origin of marine diversity, it probably does not fully account for the diversity of many sharks and rays because several elasmobranchs show high potential for dispersal (Compagno 1990; Speed *et al.* 2010)that could translate into high gene flow among localities. Thus, an alternative possibility accounting for elasmobranch diversity is ecological speciation, in which divergent selection in alternative environments supersedes gene flow and drives populations along the speciation process (Coyne & Orr 2004). Ecological speciation has so far not been tested in any elasmobranch. With around 1,000 species described, elasmobranchs are the second most diverse group of vertebrates in the ocean (Compagno 1990). This high species diversity is most likely underestimated

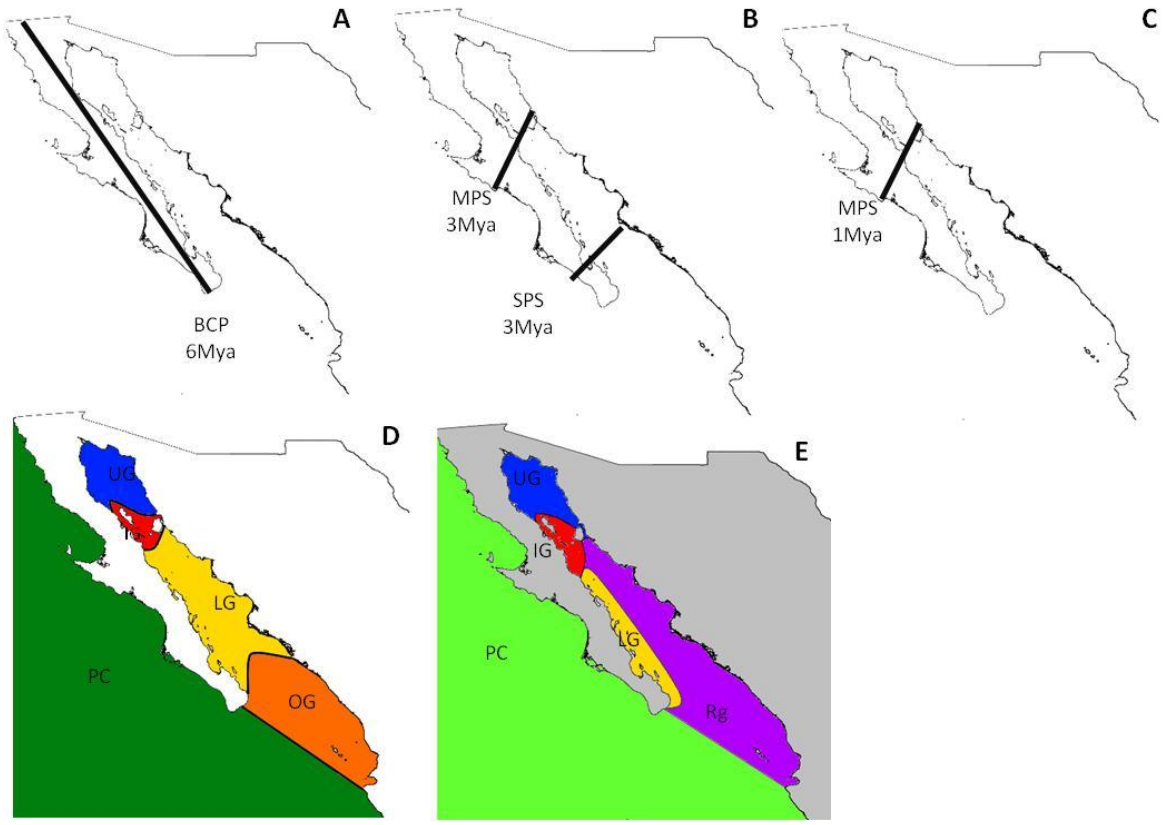

Fig 2.1 Maps of the Gulf of California and Baja California Penisnula , showing the putative vicariant events (A, B, C), the actual bioregions in the area (D), and the geographic distribution of the different *Rhinobatos* mitochondrial lineages (E). Baja California Peninsual (BCP), Southern Peninsula Seaway (SPS) , Middle Peninsula Seaway (MPS), Upper Gulf (UG), Islands (IG), Lower Gulf (LG), Open Gulf (OG), Pacific Coast (PC), *R. glaucostigma* (Rg), million years ago (Mya).

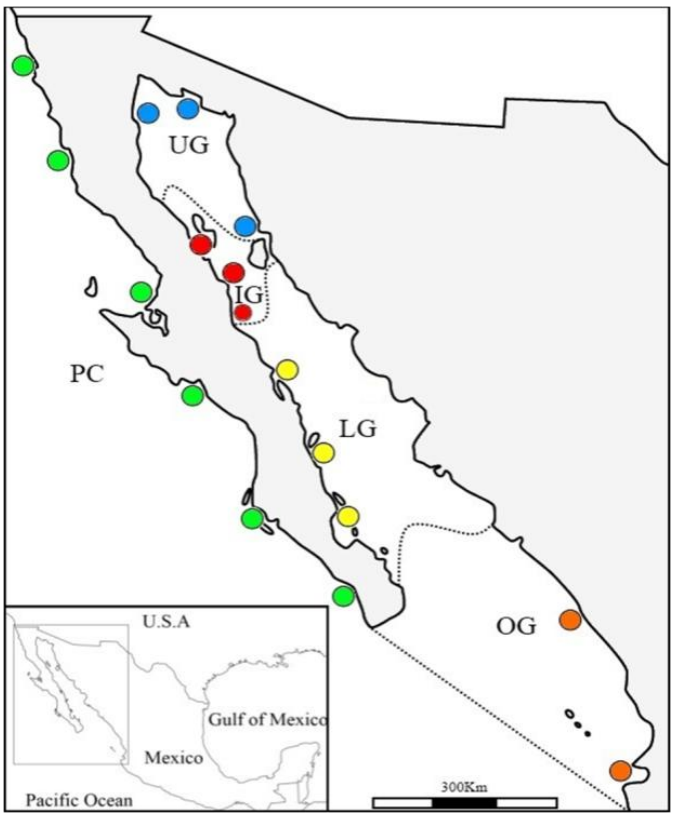

Fig. 2.2. The Gulf of California and the Baja California Peninsula in Mexico, showing the 17 sampling sites, the Pacific Coast (PC) and the four bioregions inside the Gulf: the Upper Gulf (UG), the Islands (IG), the Lower Gulf (LG) and the Open Gulf (OG).

because of the high conserved morphology and lack of extensive taxonomic studies in elasmobranchs (Ebert & Compagno 2007; Last 2007). One such example is the guitarfish genus *Rhinobatos*; one of the oldest genera of modern elasmobranchs with around 36 valid species (Compagno 2005). Guitarfish species generally show high levels of morphological stasis and, consequently, few diagnostic characters and limited interspecific morphological differentiation (Cappetta *et al.* 1987; Norman 1926; Randall & Compagno 1995). As a result, delineation of recently diverged lineages could be particularly difficult in this genus, leading to an underestimation of species diversity. Four guitarfish species have been reported in the GC-BCP region, but only three are currently accepted: *R. glaucostigma* (speckled guitarfish), *R. leucorhynchus* (whitenose guitarfish) and *R. productus* (shovelnose guitarfish) (Compagno 2005). Nevertheless, molecular studies have suggested cryptic lineages of *Rhinobatos* for the GC-BCP; Sandoval-Castillo *et al.* (2004) reported two allopatric mitochondrial DNA (mtDNA) lineages, one distributed along the GC, and one along the Pacific coast of the BCP. Since both the recognised and cryptic species are found in vast geographic areas with complex oceanographic conditions in the GC and BCP, they offer the opportunity to assess different stages of speciation and the relative roles of vicariant biogeography and natural selection on the formation of species.

Our main hypothesis was that the mobility capability of guitarfish is enough to overcome the historical vicariant events in the GC-BCP region, but its environmental variation promotes local adaptations and the subsequent genetic diversification. Here, a powerful analytical framework considering both mitochondrial and nuclear DNA data was used to assess the influence of geological history and ecological discontinuities on genetic divergence and speciation in *Rhinobatos* from the GC-BCP region. First, genetic divergence, migration rates, reproductive isolation and the delineation of cryptic lineages were assessed by a combination of phylogenetic and population genetic methods. Coalescent-based phylogeographic

simulations were then conducted to test the fit of a gene tree to particular population histories predicted by competing evolutionary hypotheses (Fig. 2.3). Finally, analyses based on seascape genetics, ecological niche modelling and outlier tests for loci under divergent selection were used to infer ecological factors underpinning genetic structure.

2.2 Methods.

2.2.1 Sampling.

Muscle tissue samples of 178 shovelnose (*R. productus*) and 21 speckled guitarfish (*R. glaucostigma*) were collected from commercial fisheries in 17 sites covering the four bioregions of the GC and the Pacific Coast (PC) of the peninsula (Fig. 2.2). Whitenose guitarfish (*R. leucorhynchus*) from the Gulf of Tehuantepec (Oaxaca, Mexico, ~1400 km south of the GC) and Brazilian guitarfish (*R. horkelii*) from southern Brazil were also sampled and included in phylogenetic analyses. All samples were preserved in 95% ethanol.

2.2.2 DNA analysis.

Genomic DNA was extracted from all samples and ~800 bp of the mitochondrial DNA (mtDNA) control region were sequenced using primers designed from conserved elasmobranch sequences (FPro200 5'-RYC YTT GGC TCC CAA AGC-3' and RCR900 5'-GGG MGG RCK RKA AAT CTT GA-3'). Nuclear data were generated for 189 samples (175 shovelnose and 14 speckled guitarfish) using a modified protocol of amplified fragment length polymorphism profiling (AFLPs) (Zenger *et al.* 2006).. Loci were determined with a threshold system that bins peak-height using AFLPSCORE (Whitlock *et al.* 2008; locus

threshold=25%, phenotype-calling relative threshold=10%). Methodological error rate was assessed by running 24 samples twice from the DNA extraction step, and using a mismatch error rate analysis in the software AFLPSCORE. Monomorphic loci for all samples (that score in less than 5% or more than 95% of all individuals) were excluded for subsequent analyses.

2.2.3 Oceanographic dataset.

The average annual oceanographic data of six variables (temperature, salinity, oxygen saturation, nutrients concentration, total chlorophyll and bathymetry) for the last 100 years were obtained from the NOAA World Ocean Data Base (www.nodc.noaa.gov/OC5/SELECT/dbsearch/dbsearch.html). Gridded maps at 0.09-degrees resolution of each oceanographic variable were generated using the DIVA algorithm in ODV.4 (Schlitzer 2010).

2.2.4 Statistical Analysis.

Phylogenetic analysis. MODELTEST 3.7 (Posada & Crandall 2001) was used to select the most appropriate substitution model for the mitochondrial dataset. Phylogenetic analyses were performed using Bayesian inferences in MRBAYES 3.11 (Huelsenbeck & Ronquist 2001; random seed, generation= 10,000, long chains = 8, burn-in= 1,000, iterations = 10,000). Samples of *R. leucorhynchus* and *R. horkelii* were used as outgroup. Genealogical relationships between mtDNA haplotypes were inferred with a haplotype network constructed in TCS (Clement *et al.* 2000).

Genetic structure and demographic history. Genetic differentiation among localities was assessed by the pairwise fixation indexes Φ_{ST} (mtDNA) in ARLEQUIN 3.5 (Excoffier &

Lischer 2010) and F_{ST} (AFLP) in AFLP-SURV v1.1 (Vekemans 2002). Hierarchical patterns of population structure were tested for both mtDNA and AFLP data using an analysis of molecular variance (AMOVA) implemented in ARLEQUIN 3.5 (Excoffier & Lischer 2010) The genetic variation was partitioned within sampled localities, among localities and among lineages (PC, UG, IG, LG and *R. glaucostogma*). Genetic structure and the most likely number of discrete populations was assessed for the AFLP data with a Bayesian approach for dominant markers in STRUCTURE 2.3 (Hubisz *et al.* 2009; admixture model with correlated alleles, burn-in=1,000, interactions =1,000000, with and without prior population information). In addition, historical demographic parameters (θ as population sizes, and M as gene flow between populations) were estimated with the mtDNA data based on a full migration model using MIGRATE-N 3.6 (Beerli 2006; slice proposal distribution, exponential with windows prior distribution, long chain=1, burn-in=10,000, run = 1,00,000, replicates=7, static heating with five chains= 1, 1.5, 3, 6 and 100). MIGRATE-N implements Markov Chain Monte Carlo (MCMC) and Bayesian inferences to estimate the probability of demographic parameters, in the context of isolation with migration model and coalescent theory. Parameters are escalated by mutational rate $\mu = 1.3x10^{-07}$, based in an average substitution rate of $8x10^{-3}$ substitutions per site per million years (Duncan *et al.* 2006) and generation time of 16 years (Villavicencio-Garayzar 1993). Finally, the time to the most recent common ancestor was inferred for all phylogroups using BEAST (Drummond & Rambaut 2007; standard priors, burn-in=1,000, generations=10,000,000). This Bayesian MCMC approach allows the use of relaxed molecular clock and different coalescent tree topologies to measure time in phylogenies. A molecular clock of 0.8% per million years was used (Duncan *et al.* 2006).

Detection of natural selection. The number of outlier AFLP loci under divergent selection was estimated using a Bayesian F_{ST}-outlier approach (Beaumont & Balding 2004) in the

application MCHEZA (Antao & Beaumont 2011), which provides an easy to use platform to implement the DFDIST package (http://www.rubic.rdg.ac.uk/~mab/stuff). Loci under directional selection were identified by pairwise comparisons between inferred phylogroups (i.e. main mitochondrial lineages). For each analysis the null distributions of F_{ST} values was generated using 50000 simulations, a θ of 0.01 and a confidence interval of 0.995. Loci identified as outliers in more than two comparisons (i.e. repeat outliers) were considered as loci under selection (Campbell & Bernatchez 2004).

Ecological isolation To evaluate the contribution of geographic and environmental factors to inferred genetic patterns, correlations between genetic structure (i.e. measured by mtDNA θ_{ST} and AFLP F_{ST}) and geographical and environmental distances were assessed using partial Mantel tests in IBSWS (Jensen *et al.* 2005; permutation= 10,000). Geographic distances between sampling localities were measured along the coastline using GOOGLEEARTH (2009). Environmental distances were estimated as the difference of each oceanographic variable between sampling sites. To determine the extent of ecological overlapping between lineages, predictive distribution models were generated using maximum entropy as implemented in MAXENT 3.3 (Phillips & Dudík 2008). Here, phylogeographic groups were considered as taxa. A total of two or three commercial fisheries sites per locality (9-15 per taxon) were used as the taxa presence dataset; and gridded maps of six oceanographic variables were used as environmental layers. Models performances were measured by the area under the ROC curves (AUC), in this case AUC was calculated using a pseudo-absence data (background) and represent the probability of random chosen present site is ranked over a random background site (Phillips & Dudík 2008). Niche overlap among phylogroups was quantified using niche similarity index (*I*) and tested using identity tests (Warren *et al.* 2008) implemented in ENMTOOLS (Warren *et al.* 2010). The *I* index ranges from 0 (no overlap in taxa distributions) to 1 (all areas are equally suitable for both taxa). For the identity test, a null

distribution of overlapping score between taxa was generated based on 1,000 occurrence point pseudoreplicates per taxon. Niche identity was rejected when the actual observed *I* index was significantly lower than that expected for the pseudo-replicated dataset.

Assessing alternative scenarios of evolutionary divergence. Finally, MESQUITE 3.7 (Maddison & Maddison 2009) was used to test competing phylogeographic hypotheses. Coalescent genealogies were simulated constrained within the population history predicted by each hypothesis (Fig. 2.3). Tree topologies were determined by two parameters, time since splitting (branch lengths) and population sizes (branch widths). Results from MIGRATE-N were used to set each extant population size, and ancestral population sizes were set as average descendant population sizes. Major historical geological events were used to set putative splitting times: (1) The Formation of the Gulf 6 million years ago (Mya, Oskin & Stock 2003), (2) the opening of a seaway crossing Baja California in the southern part of the Gulf 3 Mya (Helenes & Carreño 1999) and (3)the opening of a putative seaway at mid-peninsula 3 Mya (Ochoa-Landin *et al.* 2000) or 1 Mya (Upton & Murphy 1997). The hypotheses tested were as follow: H1a considers the isolation of the PC lineages 6 Mya with the formation of the GC. Following the closure of the seaways 3 Mya, an ecological radiation is proposed inside the gulf, with a final split between IG/PC after the closure of a seaway 1 Mya. H1b is the same as H1a but only considers the closure of a seaway 1Mya. H2a considers the geological history only, with the isolation of GC/PC 6 Mya. Here, the LG lineages split after closure of the seaways 3 Mya and the separation of PC/IG and Rg/UG occurs after the seaway 1 Mya. In H2a, LG lineage is isolated with the formation of the GC 6 Mya. Here the closure of the seaway 3 Mya produces another split between the lineages PC-IG and Rg-UG, and finally 1Mya all the lineages are isolated with the closure of the last seaway. H3 considers a single ecological radiation event, after the last seaway was closed and the contemporary

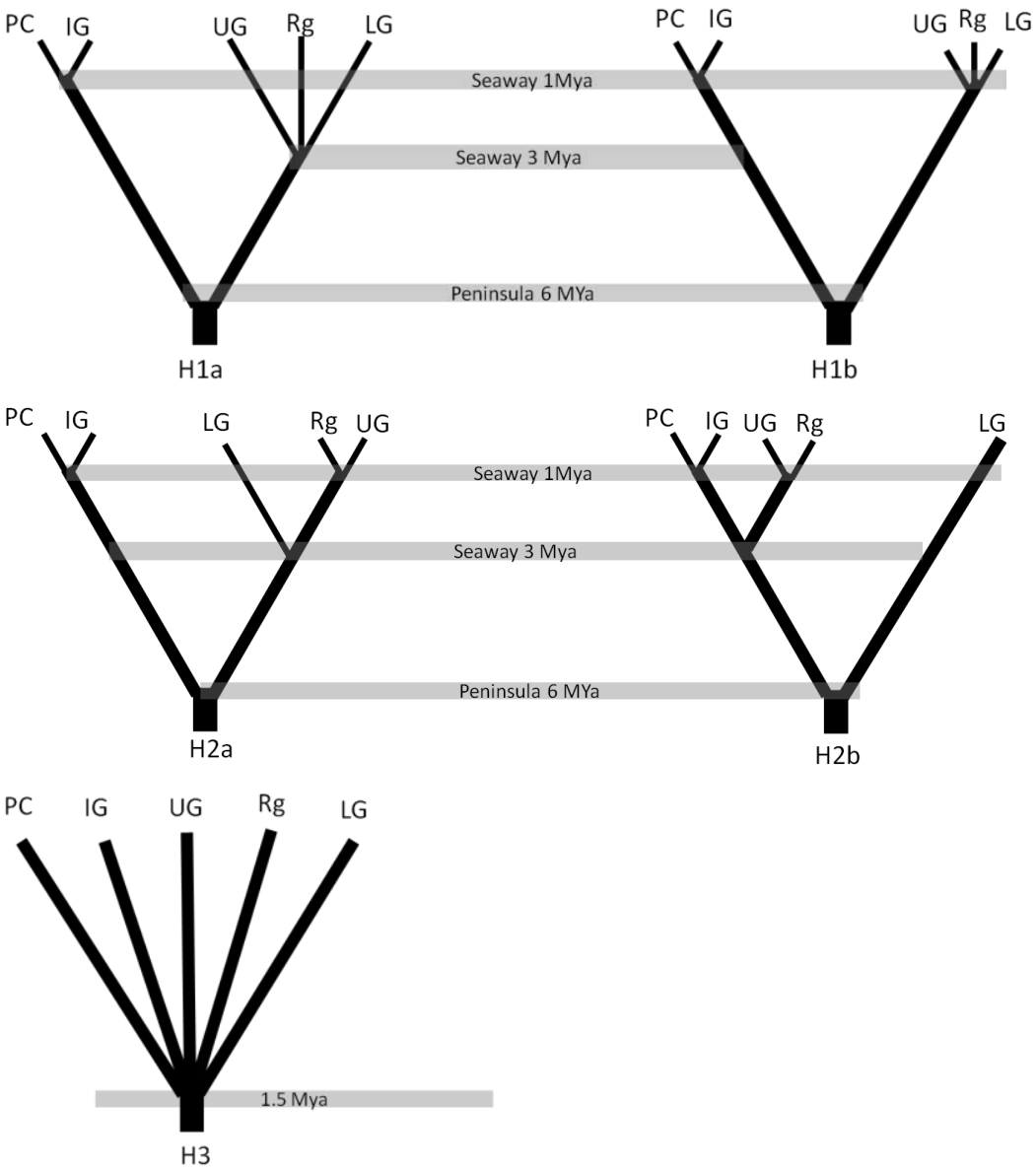

Fig 2.3. Phylogeographic hypotheses for diversification of *Rhinobatos*. H1: Vicariance and ecological radiation, three vicariance events at 6 Mya formation of the GC, 3Mya southern peninsula seaway closed, or 1 Mya middle peninsula seaway closed (see Fig 2,1A, B and C); and one ecological radiation 3 Mya (H1a) or 1 Mya (H1b). H2: Geological history, three vicariance events at 6, 3 and 1 Mya. Isolation PC vs GC 6 Mya with the formation of the GC (H2a), Isolation PC vs GC 1 Mya with the closure of the middle peninsula seaway (H2b). H3: Ecological radiation, one common ancestor for all lineage that diversify after the actual ecological condition were established 1 Mya.

oceanographic conditions were established. For each phylogeographic model, 1,000 coalescent genealogies were simulated using the substitution model determined by MODELTEST 3.7, and the scaling factor that generated levels of diversity similar to the empirical data. Subsequently, Maddison's deep coalescences (DC) were calculated for each tree. DC measures the discordance between the gene tree and the taxa subdivision (Maddison 1997). The tree originated by MRBAYES was used to calculate the empirical value, and the simulated genealogies were used to generate a null distribution of the DC statistic. Then for all scenarios a one-tailed test of significance was performed at the 5% level. MESQUITE 3.7 was used because it provides analytical methods with considerable flexibility to build taxon-specific null distributions in the context of models of historical population structure and coalescent theory.

2.3 Results and Discussion

2.3.1 Genetic diversity

A total of 52 mitochondrial haplotypes was identified from 199 guitarfish samples. Of these, 15 haplotypes were exclusive to the UG, nine to the IG, seven to the LG, 15 to the PC and eight to the Rg lineage (Table 2.1; Fig. 2.4). Nucleotide diversity was low in all populations ranging from 0.09 to 0.38%, while the haplotype diversity was moderate to high ranging from 0.551 to 0.952 (Table 2.1). There were 1116 AFLP fragment were scored, but just 541 loci were retained after apply the threshold system with a 2.7% error rate (Table 2.2). Out of the 541 retained loci 73 were considered under selection (13%).All loci were polymorphic, and the total expected heterozygosity ranged from 0.313 to 0.407.

2.3.2 Cryptic speciation

Nuclear and mitochondrial genetic data provide strong evidence for five species of *Rhinobatos* in our sample from the GC-BCP region. One lineage (Rg) corresponds to all organisms identified in the field as *R. glaucostigma*. However, samples originally identified as *R. productus* were divided into four lineages. These four lineages show strong phylogeographic concordance, each corresponding to a different biogeographic region comprising the Upper Gulf (UG), Islands (IG), Pacific Coast (PC), and Lower Gulf (LG). The *R. glaucostigma* lineage occurs in both the Open Gulf (OG) and in the LG, in sympatry with the LG cryptic lineage of *R. productus*. Phylogenetic analyses of mtDNA sequences show these species comprise five well-supported lineages (>78 Bayesian support), with four of them being reciprocally monophyletic (Fig. 2.4, 2.5). The marked phylogenetic subdivision is reflected in strong genetic structure among populations from the 17 sites (mtDNA Φ_{ST}=0.876, P<0.01; AFLP F_{ST}=0.139, P<0.01; Φ_{PT}=0.282, P<0.01). Results from AMOVA show that molecular variation is best explained by differences between lineages (mtDNA Φ_{CT}=0.869, P<0.01; AFLP Φ_{RT}=0.244, P<0.01) (Table 2.3). In addition, using all the 541 AFLP loci, the Bayesian clustering method identified K=5 and 6 (with and without prior population information respectively) as the most likely number of clusters. Here all individuals were allocated accurately to each mitochondrial lineage with very little to no signal of nuclear gene flow or introgression between lineages (Fig. 2.5b). These results agree with estimates of historical migration in which all pairwise migration parameters (m) in MIGRATE-N point to a higher probability distribution near to zero (Table 2.4).

Two of the four divergent lineages of *R. productus* (UG and LG) are reciprocally monophyletic and appear more closely related to the morphologically distinct *R. glaucostigma*, a conclusion supported by both genealogical and phylogenetic analyses (Fig.

Table 2.1. Sampling localities, lineages and total level of diversity. Sample size (n), number of haplotypes (Hp), haplotype (*h*) and nucleotide (π) diversity, heterozygosity observed (H_j), and proportion of polymorphic loci (PL%).

	Mitochondrial sequences				AFLP loci		
	n	Hp	h	π%	n	Hj	PL%
(PC) Pacific Coast	**56**	**12**	**0.741**	**0.15**	**59**	**0.368**	**96.8**
(1) Ensenada	5	3	0.8	0.13	4	0.313	76.5
(2) San Quintin	11	4	0.709	0.11	15	0.374	95.5
(3) Laguna Manuela	15	5	0.676	0.14	15	0.388	97
(4) San Ignacio	10	5	0.756	0.17	12	0.343	94.4
(5) Bahia Almejas	15	6	0.809	0.18	13	0.374	97.2
(LG) Lower Gulf	**38**	**7**	**0.606**	**0.1**	**25**	**0.357**	**94**
(6) La Paz	9	4	0.583	0.08	2	0.034	2.1
(7) Loreto	12	3	0.682	0.1	9	0.317	87.3
(8) Mulege	17	4	0.551	0.09	14	0.363	81.3
(IG) Islands	**39**	**11**	**0.645**	**0.22**	**44**	**0.384**	**95.9**
(9) San Bruno	14	5	0.659	0.38	15	0.404	96.6
(10) El Barril	10	4	0.644	0.09	10	0.407	100
(11) Bahia de los Angeles	15	5	0.629	0.12	19	0.374	93.8
(UG) Upper Gulf	**45**	**15**	**0.876**	**0.22**	**41**	**0.342**	**91.9**
(12) San Felipe	15	6	0.819	0.16	11	0.354	90
(13) Puerto Peñasco	15	11	0.952	0.26	15	0.378	91
(14) Bahia Kino	15	6	0.848	0.22	15	0.354	92.5
(Rg) R.galucostigma	**21**	**8**	**0.848**	**0.26**	**14**	**0.335**	**87.2**
(6) La Paz	5	4	0.900	0.20	5	0.353	79.3
(8) Mulege	6	3	0.601	0.13	9	0.351	85.7
(15) Barra de Piaxtla	5	2	0.717	0.33	0		
(16) Cruz de Huanacxtle	5	3	0.816	0.41	0		
Total	**199**				**183**		**100**

Table 2.2. Primer sets to generate AFLP genotype in *Rhinobatos* samples, number of scored fragments, removed fragments and retained loci per primer set.

Primer Set	Fragments Detected	Fragments Removed by Locus Threshold	Monomorphic Loci Removed	Loci Retained
MseICAGC/EcoRIAAC	268	147	6	115
MseICAGC/EcoRIACT	288	169	15	104
MseICAGC/EcoRIAGT	283	76	9	198
MseICAGC/EcoRIATC	277	139	14	124
Total	1116	531	44	541

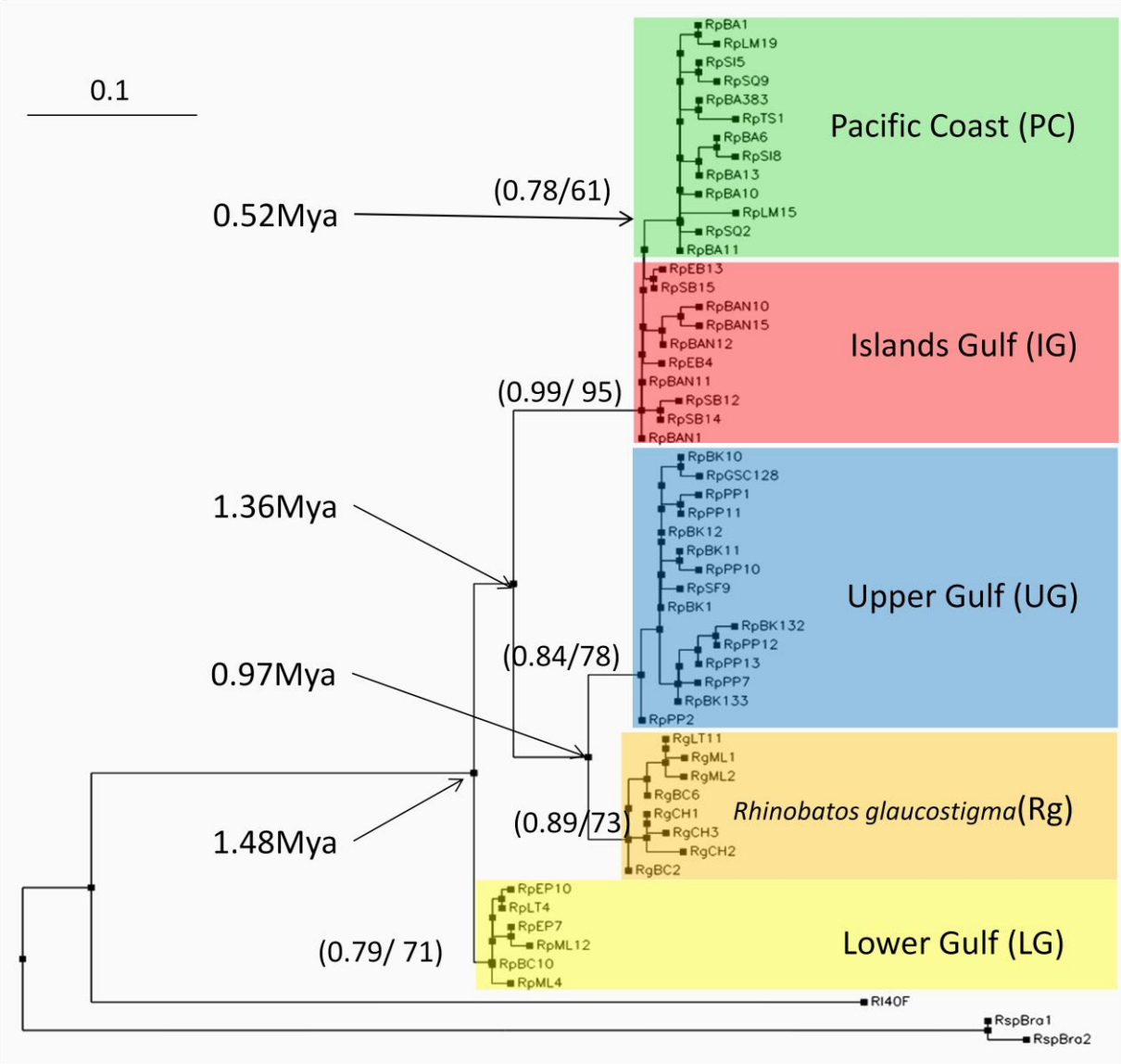

Fig. 2.4. mtDNA phylogeny for *Rhinobatos* complex from the GC-BCP region. It is based on Bayesian (By) and Maximum Parsimony (MP) analyses of 800 bp of the mtDNA control region, and shows the posterior probability (By) and bootstrapping value (MP) of each lineage in brackets, the estimate time of the most recent common ancestor in millions of years (Mya). Sequences of *R. leucorhynchus* from Mexico and *R. horkelii* form Brazil were used as outgroups.

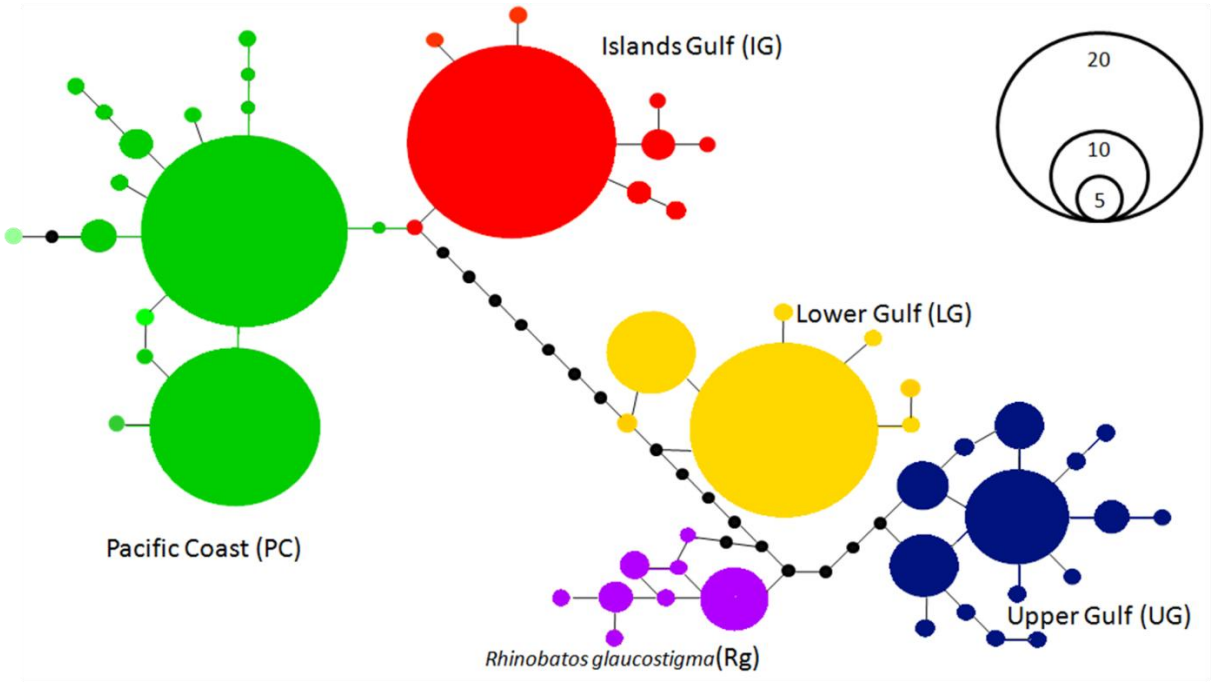

Fig. 2.5. mtDNA genealogical network for *Rhinobatos* complex from the GC-BCP region.. The haplotype network is based on statistical parsimony. The relative area of the circles reflects the frequency in the population and the colour represent the location, black dots represent hypothetical haplotypes, and separation indicates one mutation.

Table 2.3. *Rhinobatos* hierarchical analysis of molecular variance (AMOVA) based on 468 neutral AFLP loci and ~800 bp mitochondrial control region. For 17 localities and five bioregions. Significant results are in bold.

	Mitochondrial sequences			AFLP loci		
	% of variation	Fixation index	*P*	% of variation	Fixation index	*P*
Among Regions	86.8	**ΦCT=0.87**	**<0.001**	24	**ΦRT=0.24**	**<0.001**
Among Localities	0.72	ΦSC=0.055	0.002	4	**ΦPR=0.05**	**<0.001**
Within Localities	12.39			72		
Total		**ΦST=0.88**	**<0.001**		**ΦPT=0.28**	**<0.001**

2.4, 2.5). The other remaining *R. productus* (PC and IG) also comprise distinct lineages but have not yet achieved reciprocal monophyly. Nonetheless, just as the case of the UG and LG lineages, PC and IG are also highly divergent and show nil historical gene flow with other *Rhinobatos* species found in the GC-BCP region. Delimiting species on the basis of genetic data can be controversial, but the phylogenetic distinction and levels of reproductive isolation depicted here, in conjunction with results about niche partition and ecological divergence (below) satisfy a number of different properties used by biologists to delineate species. These include operational criteria used in several species concepts such as the biological species concept (Mayr, 1942; Dobzhansky, 1950) and some versions of the phylogenetic species concept (Nelson & Platnick, 1981). Thus, a total of four cryptic lineages with likely different stages of evolutionary separation are reported here for *R. productus*. In terms of taxonmy, it is conceivable that the LG lineage might represent the spiny guitarfish *R. spinosus*, a taxon first described on the basis of a single museum specimen (Günther 1870). Although morphological data based on three specimens suggested that *R. spinosus* occurs in one locality from the LG region (Castro-Aguirre & Pérez 1996), this taxon has been more recently synonymized with *R. productus* (Compagno 1999). There are no described taxa or synonyms that could be associated with other cryptic lineages, but reproductive data suggest the existence of a distinctive species in the UG region (Kena Romo-Curiel personal communication). Comparative morphological studies are necessary to determine the taxonomic status of GC and BCP lineages and identify phenotypic diagnostic characters that could be used to describe the cryptic species.

2.3.3 Phylogeographic hypotheses

The estimated population sizes were very similar for all lineages; ranging from 17308 individuals in LG to 53846 in PC (Table 2.4). The coalescent simulations rejected the three

Table 2.4. Historical gene flow and population sizes of *Rhinobatos* lineages, estimated with MIGRATE-N. Effective number of migrants per generation $N_em=\Theta M$, source on row and sink on columns. Effective female population sizes $Ne= \Theta/\mu$.

	PC	IG	UG	Rg	LG	*Ne*
PC		0	0	0	0	215385
IG	<0.001		0	0	<0.001	123077
UG	0	0		0	0	184615
Rg	<0.001	<0.001	0		0	130769
LG	0	0	0	0	0	69231

Table 2.5. Simulation tests based on deep coalescence of evolutionary hypotheses of diversification in *Rhinobatos*. Values highlighted in bold are scenarios that could not be rejected. H1: Vicariance and ecological radiation, three vicariance events at 6 Mya formation of the GC, 3Mya south seaway closed, or 1 Mya middle peninsula seaway closed; and one ecological radiation 3 Mya (H1a) or 1 Mya (H1b). H2: Geological history, three vicariance events at 6, 3 and 1 Mya. Isolation PC vs GC 6 Mya with the formation of the GC (H2a), Isolation PC vs GC 1 Mya with the closure of the middle peninsula seaway (H2b). H3: Ecological radiation, one common ancestor for all lineages that diversify after the actual ecological condition were established ~1 Mya.

Hypotesis	Scenario	Observed value	***P*** value
H1a	Vicariance and Ecology	36	0.045
H1b	Vicariance and Ecology	36	**0.055**
H2a	Geological History	36	0.024
H2b	Geological History	31	0.017
H3	Ecological Radiation	31	**0.230**

hypotheses dominated by historical vicariant biogeography (H1a *P*=0.045; H2a *P*=0.024; H2b *P*=0.017) (Table 2.5). On the other hand, the hypothesis of a single and more recent ecological radiation was strongly supported (H3 *P*=0.230) and that involving the historical formation of the BCP and ecological factors was marginally significant (H1b *P*=0.055). Thus, statistical phylogeographic methods based on coalescence suggest a possible combined influence of the BCP on initial isolation of *Rhinobatos* lineages followed by an ecological radiation at around 1 Mya. The primary cause of adaptive radiation is divergent selection for efficient utilization of alternative resources, which often drives incipient lineages to colonize new habitats (Schluter 2009). Historical environmental changes have been associated with opportunities for niche specialisation due to newly available habitat (Schluter 2000). The BCP was finally formed around 2.5 Mya, an event that is thought to have allowed the establishment of current environmental conditions of the GC by the early Pleistocene between ~2 and 1 Mya (Murphy & Aguirre-Leon 2002). These novel conditions could have produced alternative niches that promoted the adaptive radiation of *Rhinobatos* in the region, as further discussed below.

2.3.4 Ecological adaptation and speciation

The first requirement for ecological speciation is a source of divergent selection, and perhaps the main cause of divergent selection is habitat structure or contrasting niches (Schluter 2001). The analysis of environmental niche modelling shows high entropy with nil to moderated geographic overlap (Fig. 2.6). Niche identity was rejected for all pairs of lineages, with moderated *I* index values, except for LG vs Rg (Table 2.6). These lineages are sympatric, showing high niche overlap and therefore niche identity between them could not be rejected. The predicted niche models for *Rhinobatos* lineages are congruent with the habitat structure proposed for the GC with respect to productivity, salinity, temperature,

Table 2.6. Ecological niche identity test among lineages of *Rhinobatos*, based on ENMTool. Ecological niche overlap index (I) and *P* values of ecological niche identity test are presented above and below the diagonal, respectively.

	PC	IG	UG	Rg	LG
PC		0.33	0.32	0.53	0.49
IG	<0.01		0.48	0.42	0.47
UG	<0.01	<0.01		0.38	0.43
Rg	<0.01	<0.01	<0.01		0.76
LG	<0.01	<0.01	<0.01	**0.26**	

nutrients concentration and bathymetry (Ortega *et al.* 2010). These results provide convincing support for ecological partition among lineages in close association with the four described bioregions in the area.

In addition, the F_{ST} outlier analysis found that 73 out of 541 AFLP loci (13.5%) are either directly under directional selection or linked to selected genes. This is a high proportion of outliers considering that empirical genomic scans generally report 2-10% (Nosil *et al.* 2007). It is arguable that long term reproductive isolation facilitates the accumulation of neutral divergence that could be confused with genetic differentiation affecting selected traits (Schluter 2000; Rundle & Nosil 2005; Via 2009). However, our Bayesian analyses suggest a relatively recent time to the most recent common ancestor for *Rhinobatos* lineages (<1.5 Mya; Fig. 2.4). Moreover, clustering analyses with AFLP data show a heterogeneous genomic diversification, neutral loci still show high admixture and outlier loci present a strong isolation pattern (Fig. 2.7). In the early stages of ecological speciation or in comparisons between recently isolated ecological species, genetic differentiation is heterogeneous throughout the genome, and segregation associated with divergent selection can be identified with relative ease (Nosil *et al.* 2009; Schluter 2009; Via 2009). In addition, the contrasting results for analyses using the total and the putatively neutral AFLP datasets suggest that divergent selection has overcome the gene flow driving the lineages to genetic differentiation. It has been demonstrated that speciation with ongoing gene flow can occur under strong divergent selection, and several ecological mechanisms are now well described (Via 2002; Rundle & Nosil 2005; Nosil 2008). Therefore, the high proportion of outliers and the heterogeneous genomic diversification could be attributed to a strong selection component in the recent diversification of *Rhinobatos* lineages.

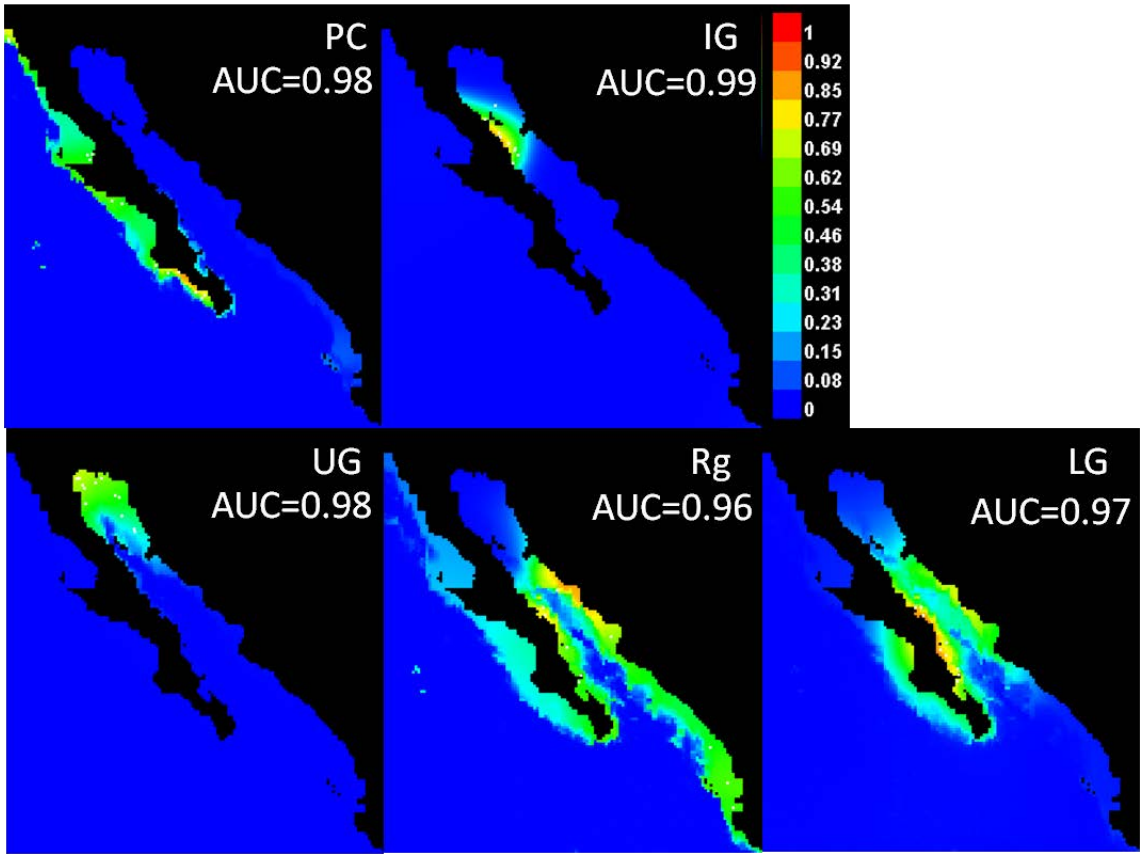

Fig. 2.6. Ecological niche models for lineages in *Rhinobatos* using 6 oceanographic data layers. Locality points are given in yellow dots, MaxEnt probability value levels are listed in a graded series from blue (0) to red (1). Pacific Coast (PC), Islands (IG), Upper Gulf (UG), *R. glaucostigma* (Rg), Lower Gulf (LG), area under the ROC curve (AUC).

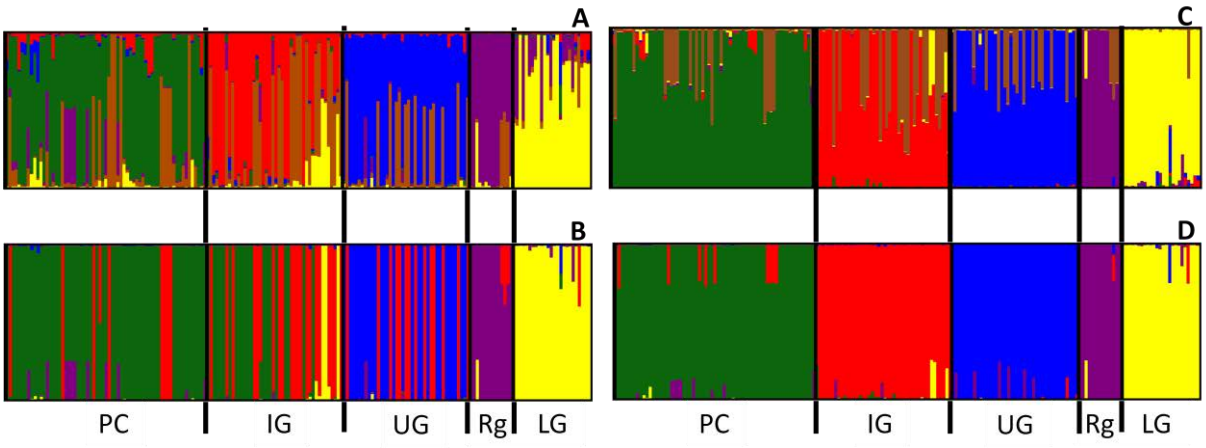

Fig 2.7. Genetic groups in the *Rhinobatos* species complex identified with a Bayesian clustering method based on 541 AFLP loci. Analyses were conducted with neutral loci only (A, B) or with all AFLP loci (C, D) and without prior population information (A, C) and with prior population information (B, D).

An alternative explanation for the high percentage of outliers involves the concomitant action of endogenous (not related with environmental variations) and exogenous selection (related with environmental variation) to promote the genetic differentiation of substantial proportion of neutral genome in a relatively short evolutionary time. Thus, the proportion of outliers under selection and the importance of ecology-driven selection could be overestimated (Bierne et al. 2011). Even though, this hypothesis also requires some degree of local adaptation, the challenge is distinguish the contribution of the different form of selection on the extreme differentiation. Unfortunately, more extensive work on gene expression and genomic adaptation are required to identify the driving force that promotes initial reproductive isolation and subsequent the accumulation of gene flow barriers (Wolf et al. 2010). Nevertheless, it is recognized that genetic-environment association provide evidences of exogenous selection action (Bierne et al. 2011). Therefore, the strong correlation ecological vs genetic distance and the ecological isolation between *Rhinobatos* lineages suggest an important role of exogenous selection in the diversification of this genus.

The final component of ecological speciation is the translation of the divergent selection to reproductive or genetic isolation (Kirkpatrick & Ravigné 2002). Both nuclear and mitochondrial genetic distances show a significant autocorrelation with geographic distance, temperature, salinity, nutrient concentration, oxygen saturation and bathymetry. For mitochondrial and outlier data, positive trends between genetic distance *vs* nutrients concentration, oxygen saturation and bathymetry remained significant after controlling for theeffect of geographic distance (Table 2.7). This result provides strong evidence of genetic isolation by ecological distances, and consequently reproductive isolation by natural selection. Oxygen consumption requirements are different between species of elasmobranchs, but in general most species have a critical oxygen concentration well above 50% of saturation – low oxygen saturation creates selection pressure for physiological adaptations that reduce oxygen

consumption via metabolic and ventilator depressions (Routley *et al.* 2002; Renshaw *et al.* 2010). Perhaps the relatively low oxygen saturation found in the IG region (Lavin and Marinone 2003) provides a "pseudo-hypoxic" niche to be exploited by specialized guitarfish species with reduced oxygen consumption. In addition, the distribution and abundance of several fish species have been linked to bathymetric factors (Maravelias 1999). Bathymetry has been considered an important barrier for the dispersal of elasmobranchs, especially for benthic coastal species such as *Rhinobatos* spp (Musick *et al.* 2004). Most species of guitarfish have a reduced bathymetric range, from 0 to 100m, being more abundant in the first 20 m where benthic habitats are more productive and complex (Compagno 1990; Musick *et al.* 2004). Therefore, the deep channels (>500m) in the IG region and the reduced continental shelf in the southern point of the BCP (Lavín & Marinone 2003) could function as physical or behavioural barriers, isolating the IG and the UG from other regions and restricting the migration between the PC and the GC. Although nutrient concentration may not directly affect predators such as elasmobranchs, this factor is strongly correlated with primary productivity, which in turn affects the abundance and diversity of herbivorous organisms (Zimmer et al. 2003; Korpinen *et la*. 2010). Differential concentration of nutrients between regions could be associated with difference in abundance, composition and diversity of *Rhinobatos* prey. Thus, it is proposed here that the development of feeding specializations in ecologically distinct areas has promoted the radiation of the genus. In fact, a key factor in the elasmobranchs evolutionary success has been their ecological and anatomical feeding specializations that allowed them to radiate into numerous niches (Wilga *et al.* 2007).

Table 2.7.- Correlations between genetic differentiation and geographical and ecological distances for lineages of *Rhinobatos*. *P* values are based on partial Mantel tests of

							Controlling by Geographical Distance					
	mtDNA		AFLP		AFLPS		mtDNA		AFLP		AFLPS	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Geographical Distance	0.46	**0.001**	0.50	**0.001**	0.41	**0.004**						
Temperature	0.33	**0.001**	0.46	**0.002**	0.21	**0.042**	0.11	0.15	0.16	0.13	0.16	0.93
Salinity	0.36	**0.002**	0.42	**0.006**	0.41	**0.006**	0.16	0.93	0.16	0.15	0.08	0.25
Oxygen Saturation	0.16	0.08	0.11	0.26	0.25	**0.023**	0.19	**0.046**	0.15	0.20	0.29	**0.012**
Nutrients	0.46	**0.001**	0.49	**0.001**	0.52	**0.001**	0.30	**0.009**	0.25	**0.046**	0.37	**0.006**
Chlorophyll a	0.04	0.66	0.04	0.25	0.11	0.16	0.03	0.40	0.04	0.37	0.06	0.24
Δ Temperature	0.27	**0.010**	0.44	**0.001**	0.28	**0.013**	0.06	0.69	0.16	0.14	0.01	0.44
Bathymetry	0.14	0.09	0.11	0.20	0.39	**0.007**	0.20	**0.033**	0.15	0.14	0.44	**0.003**

2.3.5 Sympatric speciation?

Sympatric speciation is one of the most controversial aspects of speciation theory. Even though empirical evidence and strong theoretical foundation support sympatric speciation, there is strong reluctance to accept its importance as a process generating biodiversity (Via 2001; Coyne & Orr 2004). This is mainly due to the difficuly proving cases in nature. Nonetheless, Coyne and Orr (2004) proposed four criteria to determine sympatric speciation in nature: (I) the species must be largely sympatric, (II) species must have substantial reproductive isolation, (III) sympatric taxa must be sister groups, and (IV) allopatric phase mitochondrial control region (mtDNA), neutral AFLP loci (AFLP) and AFLP loci including outliers (AFLP Sel). Values highlighted in bold are statistically significant. between species must be very unlikely. In this respect, LG and Rg lineages show extensive sympatric distributions, mitochondrial and nuclear data confirm significant reproductive isolation between them (Table 2.4, Fig. 2.6), and they are closely related genealogically (Fig. 2.4). Although the geological history of the GC makes it complicated to discard an allopatric period between lineages, vicariance events between them are unlikely, as discussed above. Hence, we suggest that the LG lineage evolved in sympatry with *R. glaucostigma*. Further research using more comprehensive sampling and multilocus sequence markers that accommodate for coalescent stochasticity are needed for comprehensively inferring speciation history and the suggestion of sympatric speciation in these lineages.

2.3.6 Isolation barriers

Three main causes of ecological speciation have traditionally been recognised: environmental differences, sexual selection and ecological interaction (Rundle & Nosil 2005; Schluter 2009).

Although distinguishing between them is difficult since they are not fully independent, it is useful to attempt to separate them because their consequences on speciation may vary (Rundle & Nosil 2005). Environmental differences are a relatively frequent source of divergent selection, and are surely an important mechanism for allopatric and sympatric speciation for a wide range of organisms (Schluter 2000; Coyne & Orr 2004). The genetic and ecological differentiation among lineages, the isolation by ecological distances and the high number of outlier loci detected support the idea of an ecological radiation by habitat differentiation in *Rhinobatos* from the GC. Environmental differences could produce reproductive isolation by spatial segregation. Divergent habitat preferences could generate reproductive isolation if mating occurs in a determined preferred habitat (Rundle & Nosil 2005). Reproductive philopatry is the fidelity of some individuals to mating locations, and it is the manifestation of habitat preferences. This behaviour has been reported in several elasmobranchs, and has been considered as a driver of genetic structure in some species (Hueter *et al.* 2005). In fact, habitat preferences for reproduction have been reported in several species of *Rhinobatos*, including *R. productus* (Villavicencio-Garayzar 1993; Márquez-Farías 2007). Consequently, the diversification of *Rhinobatos* in the GC could be produced by divergent preference in habitats for reproduction potentially related to oxygen saturation, nutrients concentration and bathymetry.

2.4 Conclusions

This is the first study showing evidence for ecological speciation in elasmobranchs. We identified five lineages of *Rhinobatos* in the GC-BCP region that are affected by strong divergent selection, show ecological niche partitioning, and genetic isolation correlated with environmental conditions. Furthermore, we discarded a fully vicariant speciation model for all

lineages within the Gulf and propose that its oceanographic conformation created the habitat diversity necessary for the ecological radiation of *Rhinobatos*. We suggest that philopatry, a behaviour reported for several elasmobranchs, could be a driver of reproductive isolation in *Rhinobatos* populations along the ecological speciation process. In addition, we also propose that two linages (LG and Rg) likely diverged in sympatry. These outcomes have implications for the conservation and management of elasmobranchs in general and are important to better understand speciation in the oceans.

Chapter III Phylogeography, seascape genetics and speciation of angel shark *Squatina californica*

3.1 Introduction

One of the main goals in speciation research is to clarify mechanisms influencing reproductive isolation (Coyne & Orr 2004). Several barriers to gene flow, and models of how these barriers can isolate populations, have been proposed (Howard & Berlocher 1998b; Coyne & Orr 2004). Yet, an enduring challenge is distinguishing between historical and contemporary barriers by which species become genetically distinct. Most such studies have been conducted on species after the speciation process is completed. In such cases, they are more likely to offer information about the characteristics of species than about processes that give rise to species (McPhail 1994; Beheregaray & Sunnucks 2001). By studying partially reproductively isolated populations, invaluable insight into historical and modern connectivity among populations can be gained. Furthermore, this could reveal important aspects about temporal and spatial components of the barriers to gene flow and the genetics of reproductive isolation (Schluter 2009; Via 2009).

Marine populations often display complex genetic patterns dominated by substantial spatial and temporal variation (e.g. Johnson & Black 1984; Banks *et al.* 2010). This complexity tends to hamper researchers' ability to understand the process of population divergence and connectivity, especially when conventional analytical methods are used (Selkoe *et al.* 2008). The development of coalescent-based phylogeographic and seascape genetics approaches has

facilitated the identification of barriers to historical and contemporary gene flow, and even helped to distinguish the effects of multiple evolutionary forces on reproductive isolation (Selkoe *et al.* 2008; Knowles 2009; Storfer *et al.* 2010; Chan *et al.* 2011), broadening the ecological, evolutionary and conservation implications of such studies in the sea.

A number of barriers affecting connectivity and genetic structure have been reported for marine organisms, including oceanographic currents (Banks *et al.* 2007; White *et al.* 2010, Möller *et al.* 2011) environmental discontinuities (Beheregaray & Sunnucks 2001; Piggott *et al.* 2008; Mendez *et al.* 2010), geographic distances (Polato *et al.* 2010), and historical vicariance (Waters 2008; Pfenninger *et al.* 2010). It is generally expected that life history is a strong determinant about how species will respond to physical and environmental barriers. Putting it simply, the population structure of highly mobile marine species often reflects contemporary oceanographic patterns, while low dispersal species are expected to better reflect the influence of historical geography (Kelly & Palumbi 2010). However, gene flow does not depend entirely on dispersal potential. Organisms with specialised habitat requirements to complete their life cycles tend to exhibit genetic structure concordant with current environmental conditions, despite dispersal capacity (Pelc *et al.* 2009). Thus, species with low mobility and highly specialised environmental requirements, particularly those distributed over a geologically and environmentally complex region, offer interesting models to assess the nature and chronology of barriers influencing population connectivity and speciation in the sea.

The geological history and oceanographic conditions of the Gulf of California (GC) and the Pacific Coast (PC) of Baja California Peninsula BCP provide an appropriate system to test the relative importance of vicariance events, environmental discontinuities and spatial distances on genetic structure of marine organisms. The oceanographic complexity of the GC-BCP

region has been proposed as a determinant of contemporary barriers that delimit species and population distributions (Walker 1960; Aceves-Medina *et al.* 2004; Riginos 2005). Due to differences in oceanographic variables, the GC is divided into four well differentiated bioregions, the open gulf (OG), the lower gulf (LG), the island (IG) and the upper gulf (UG) (Fig. 3.1D). The UG is characterized by shallow waters (<100m on average), large temperature variation (9-38°C), high concentration of nutrients and elevated primary productivity. The IG has channels that are over 500m deep, and an extensive strong upwelling with very low water temperature and oxygen saturation, but high concentration of nutrients that allow the highest primary productivity in the GC. The LG presents geostrophic gyres that affect the circulation and thermodynamics of the whole GC. In the OG the conditions are similar to the LG but with strong influences of ocean waters from the Pacific Ocean (Lavín & Marinone 2003; Fig. 3.1). Along the west coast of the BCP, the confluence of cold and warm currents, and the presence of geostrophic gyres in the same point (Punta Eugenia), divides the area into south (SP) and north (NP) coasts, almost exactly on the middle of the BCP (Bernardi *et al.* 2003). Most of the differences between some regions are abrupt and could represent a strong physiological or physical barrier for the dispersal of marine organisms. On the other hand, geological activity produced historical vicariant events that created genetic discontinuities in several organisms (Bernardi *et al.* 2003; Jacobs *et al.* 2004; Riginos 2005). The formation of the GC and the BCP has been a very active geological process starting ~12 million years ago (Mya), with a proto peninsula and several small islands that delimited a proto-gulf. Through volcanic and tectonic activity, the proto-peninsula and the archipelago migrated ~300km northwest around 6 Mya forming the permanent GC. This gulf was in contact with the PC by sea channels that separated the islands in the middle and the cape regions of the actual peninsula. By the late Pliocene/early Pleistocene, geological activity and sea level fluctuations closed the seaways forming the actual BCP and GC (Fig 3.1A, B and C) (Murphy & Aguirre-Leon 2002).

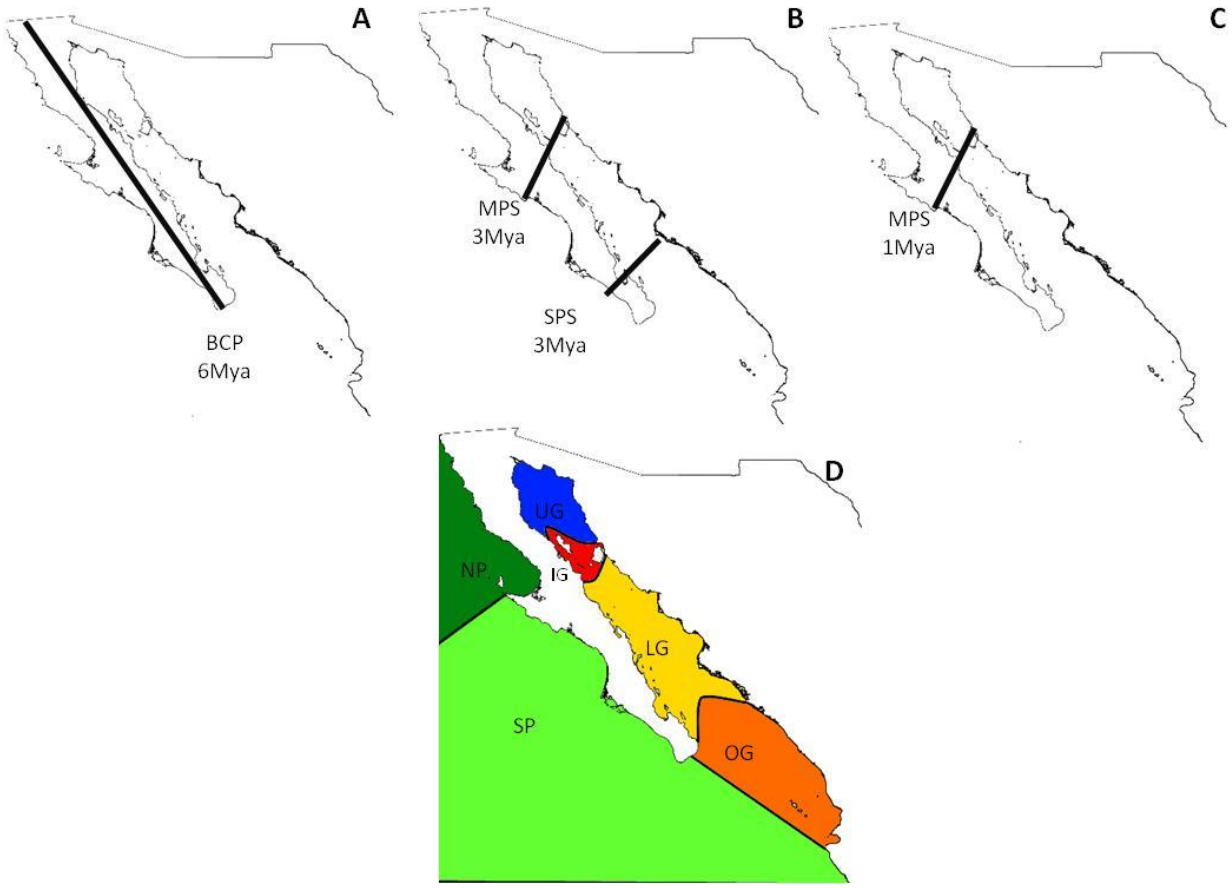

Fig. 3.1 Maps of the Gulf of California and the Baja California Peninsula, showing the vicariant events (A, B, C) and the bioregions (D). Baja California Peninsula (BCP), Middle Peninsula Seaway (MPS), Southern Peninsula Seaway (SPS), North Pacific (NP), South Pacific (SP), Open Gulf (OG), Lower Gulf (LG), Island (IG) and Upper Gulf (UG), Million year ago (Mya)

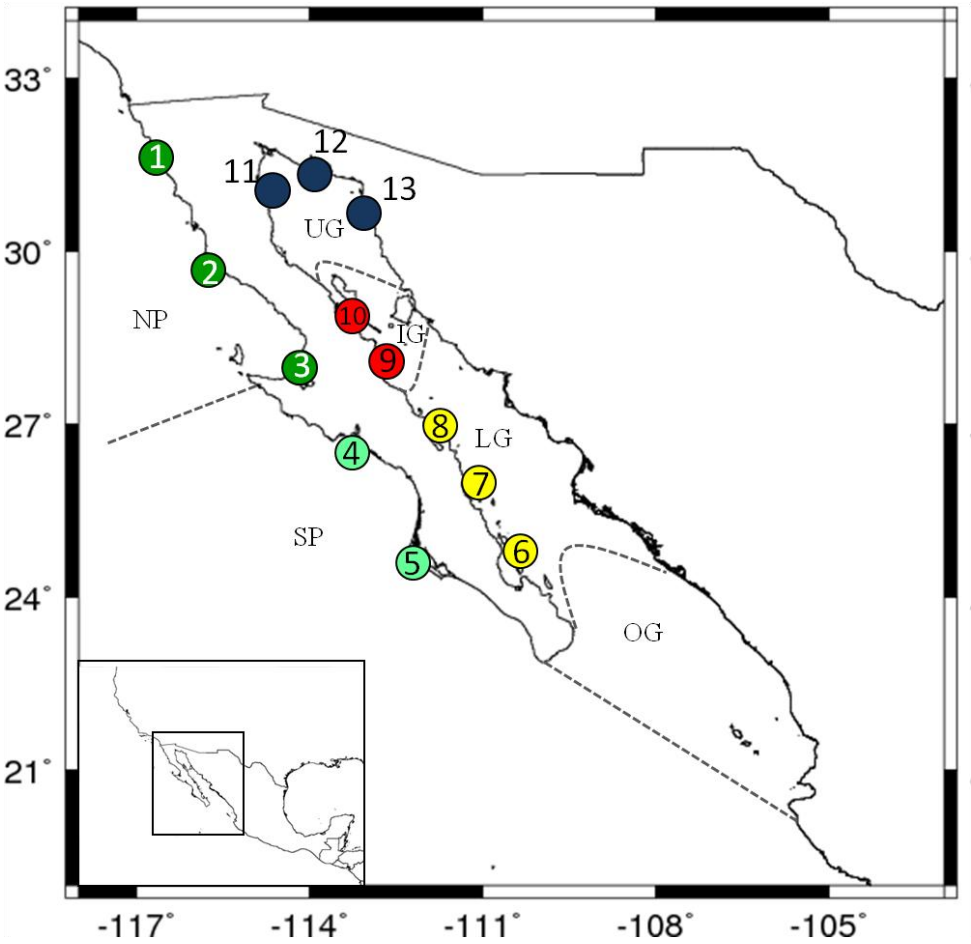

Fig. 3.2. The Gulf of California and the Baja California Peninsula. Sampling sites are: (1) Popotla, (2) El Faro, (3) Laguna Manuela, (4) San Ignacio, (5) Bahia Almejas, (6) La Paz, (7) Loreto, (8) Bahia Concepcion, (9) El Barril, (10) Bahia de los Angeles, (11) San Felipe, (12) Gulfo de Santa Clara, (13) Puerto Peñasco. The bioregions are separated by dashed lines; Baja California Peninsula: (NP; dark green) North Coast and (SP; light green) South Coast; and for the Gulf of California: (LG; yellow) Lower Gulf, (IG; red) Islands, (UG; blue) Upper Gulf and (OP) Open Gulf.

The study system of this work is the Californian angel skark, *Squatina californica*. This is a bottom-dwelling shark that ranges from Alaska, USA to Baja California Sur, Mexico, including the Gulf of California, and from Costa Rica to Chile (Compagno 1984). Despite its relatively wide distribution, the Californian angel shark does not show long migratory movements (Standora & Nelson 1977; Pittenger 1984), and strong population structure over relatively short geographic distances (< 300km) has been reported in this species for the Californian coast (Gaida 1997). In addition, preliminary reproductive and genetic data suggest a different lineage in the Gulf of California compared to the Pacific Coast population (Compagno in preparation; Stelbrink *et al.* 2010). However, using PCR-RFLP analysis of the mitochondrial control region, Grijalva-Chong *et al*. (Grijalva-Chon *et al.* 2002) reported genetic homogeneity in samples of California angel shark, from the UG and LG regions in the GC. This result was found in spite of the low dispersal potential in the species and potential barriers represented by the oceanographic complexity and geological history of the GC (Murphy & Aguirre-Leon 2002; Lavín & Marinone 2003).

In this work mitochondrial control region sequences and amplified fragment length polymorphism (AFLP) markers were used to investigate phylogeographic history, spatial genetic structure and reproductive isolation in angel sharks *S. californica* from the GC-BCP regions. Coalescent-based analyses and seascape genetic approaches were used to assess the relative impact of vicariant events, oceanographic complexity and geographic distances on genetic structure in this low mobility shark. If vicariant events represent the primary mechanisms driving population divergence, any main inferred genetic break is expected to match the hypothetical position of historical biogeographic barriers, and divergence times between population groups should coincide with the known chronology of these barriers. On the other hand, if environmental conditions are the main drivers of isolation between

populations, phylogeographic breaks should be associated to oceanographic discontinuities and genetic differentiation is expected to correlate with ecological parameters.

3.2 Materials and Methods

3.2.1 Sampling

Between May and September of 2008, muscle tissue samples of 192 Californian angel sharks were collected from commercial fisheries at 13 localities around the BCP and the GC. All sharks were captured within ~75 km from their landing sites. These localities cover five bioregions: two regions in the PC, the north (NP) and the south (SP) coast of the BCP; and three regions inside the GC, Upper Gulf (UG), Island (IG) and Lower Gulf (LG) (Fig. 3.1D, Table 3.1). Samples of *Squatina guggenheim* were obtained from Brazil for outgroup comparison. All tissue samples were stored in ~95% ethanol.

3.2.2 DNA analyses

Genomic DNA was extracted using a modified salting out protocol Sunnucks & Hale 1996. Mitochondrial DNA (mtDNA) sequence data were collected for all samples and a subset of 156 samples was genotyped using AFLPs (Table1). A fragment of the mitochondrial control region of ~550 bp was amplified by PCR using primers designed from conserved elasmobranch sequences (FPro200 5'-RYC YTT GGC TCC CAA AGC-3' and RCR900 5'-GGG MGG RCK RKA AAT CTT GA-3'). Each reaction (10µL) contained 0.5µM of each primer, 5mM $MgCl_2$, 100µM each dNTP, 1X colourless GoTaq® flexi buffer, 1U Taq

Table 3.1 Sampling localities for *Squatina californica*, bioregions and genetic diversity. Sample size (n), number of haplotypes, haplotype (h) and nucleotide (π) diversity, heterozygosity observed (H_o), and proportion of polymorphic loci (PL%).

	mtDNA				AFLP		
	n	Haplotypes	h	π%	n	Ho	PL%
(NP) North Pacific	**35**	**5**	**0.719**	**0.22**	**25**	**0.231**	**66.8**
(1) Popotla	5	2	0.6	0.44	4	0.295	63.8
(2) El Faro	10	3	0.622	0.16	10	0.220	65.3
(3) Laguna Manuela	20	3	0.695	0.17	11	0.236	69
(SP) South Pacific	**25**	**4**	**0.697**	**0.27**	**12**	**0.269**	**73.1**
(4) San Ignacio	10	3	0.711	0.23	5	0.279	64.6
(5) Bahia Almejas	15	4	0.695	0.31	7	0.263	68.7
(LG) Lower Gulf	**42**	**13**	**0.808**	**0.29**	**35**	**0.307**	**83.2**
(6) La Paz	20	8	0.742	0.24	15	0.314	91
(7) Loreto	20	9	0.847	0.33	15	0.317	87.3
(8) Bahia Concepcion	2	1	0	0	5	0.363	81.3
(IG) Islands	**40**	**6**	**0.579**	**0.17**	**40**	**0.252**	**73.9**
(8) El Barril	20	5	0.663	0.18	20	0.200	70.5
(9) Bahia de los Angeles	20	5	0.505	0.15	20	0.256	75.4
(UG) Upper Gulf	**50**	**12**	**0.772**	**0.29**	**45**	**0.283**	**79.1**
(10) San Felipe	20	8	0.758	0.22	20	0.286	77.6
(11) Golfo de Santa Clara	10	7	0.911	0.46	5	0.320	72
(12) Puerto Peñasco	20	7	0.768	0.27	20	0.287	76.2
Total	**192**	**30**	**0.883**	**0.33**	**157**	**0.329**	**100**

Table 3.2. Primer sets to generate AFLP genotype in *S.californica* samples, number of scored fragments, removed fragments and retained loci per primer set.

Primer Set	Fragments Detected	Fragments Removed by Locus Threshold	Monomorphic Loci Removed	Loci Retained
MseICAC/EcoRIAGC	61	19	9	33
MseICAA/EcoRIACG	294	83	57	154
MseICA/EcoRIAAC	246	102	44	100
Total	601	531	110	287

(Promega, WI, USA) and 30-100ng template DNA. The thermocycle profile consisted of one step at 94°C for 2 min followed by 30 cycles of 94° C for 45 s, 58°C for 45 s, 72°C for 60 s, and a final step of 72°C for 10 min. PCR products were sequenced using primer FPhe200 and Big Dye Terminator chemistry, in an ABI 3730 automated sequencer (Applied Biosystems, CA, USA). All chromatograms were checked by eye and aligned using SEQUENCHER 4.7 (Gene Codes Corporation, MI, USA). Sequences with ambiguous sites were resequenced in the reverse direction. An adapted protocol from Zenger et al. (2006) was used to generate AFLP typing for all samples. Selective reactions were completed in 10 μL of reaction containing 2 μL of diluted (1:10) preamplification product, 1.5 mM $MgCl_2$, 200μM each dNTP, 0.2μM fluorescently labelled *Eco*RI+AGC, +ACG, or +AAC primer, 0.5 μM *Mse*I+CAC, +CAA or +CA primer (Marshall personal communication), 1X colourless GoTaq® flexi buffer, 1U Taq (Promega, WI, USA). Selective PCR profiles consisted of an initial step at 94°C for 2 min followed by 30 cycles at 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s, and a final step of 72°C for 10 min. GeneScan™ 500LIZ® (Applied Biosystems, CA, USA) size standard was added to the PCR products, and the subsequent fragments were scanned using an ABI 377 automated sequencer (Applied Biosystems, CA, USA). The data was analysed and binned using GENEMAPPER 4.0 (Applied Biosystems, CA, USA). To estimate genotyping error rate, the whole processes from DNA extraction to peak detection was repeated in 24 samples. Loci were determined by applying a threshold system to bin peak-height data. Optimal thresholds that both minimize genotyping error and maximize the numbers of retained loci, was estimated for each set of primers using a mismatch error rate analysis in the software AFLPSCORE (Whitlock et al. 2008). Locus and phenotype-calling relative thresholds were set to 30% and 15% respectively. In addition, loci score in less than 5% or more than 95% of the individuals were considered monomorphic and excluded for subsequent analyses. The workbench MCHEZA (Antao & Beaumont 2011) was used to identify AFLP loci under directional selection. MCHEZA implement a Bayesian F_{ST}-outlier

approach (Beaumont & Balding 2004) to detect dominant marks loci likely subject to divergent natural selection. AFLP data were divided in four populations (Pacific Coast, Upper Gulf, Islas of the Gulf and Lower Guld) and loci under positive selection were identified by pairwise comparisons between populations. Outliers detected in multiple comparisons were considered as loci under selection and removed from the total AFLP data set for subsequent analyses

3.2.3 Ecological variables

Gridded maps at 0.09-degrees resolution for six oceanographic variables were generated based on oceanographic data using the DIVA algorithm in ODV.4 (Schlitzer 2010), based on annual averages of oceanographic data for the last 100 years, obtained from the NOAA World Ocean Data Base Website (www.nodc.noaa.gov/OC5/SELECT/dbsearch/dbsearch.html).

3.2.4 Phylogenetic and genealogical history

For the analysis of mitochondrial sequences, a model of base substitution was selected on the basis of maximum likelihood and Bayesian information criterion using MODELTEST 3.7 (Posada & Crandall 2001). Phylogenetic analyses were performed using Bayesian inferences in MRBAYES 3.11 (Huelsenbeck & Ronquist 2001). The analysis consisted of four runs of eight chains with 10,000,000 generations, tree sampling every 1,000 generations, and a burn-in period of 10,000 generations. Convergence between runs, convergence of parameters and appropriate levels of chain swapping were assessed using Tracer 1.4 (Rambaut & Drummond 2007). Sequences for *S.guggenheim* were used to root the tree. In addition, to visualise the

genealogical relationship of the Pacific angel shark, a statistical parsimony network at 95% confidence level was constructed using the program TCS 1.41 (Clement *et al.* 2000).

3.2.5 Summary Statistics

For mitochondrial data ARLEQUIN 3.5 (Excoffier & Lischer 2010) was used to calculate haplotype frequency, haplotypic diversity (h), nucleotide diversity (π) and fixation index (Φ_{SF}). For AFLP data, the percentage of polymorphic loci, expected heterozygosity and fixation index (F_{ST}) were calculated using AFLP-SURV v11 (Vekemans 2002). In addition Θ^B, an analogue of F_{ST} that accounts for uncertainty regarding the inbreeding coefficient (f) in dominant markers, was estimated using HICKORY 1.1 (Holsinger & Lewis 2007). The software was run with the default parameters (burn-in=50,000, samples=250,000, thin=5), using full, f=0, Θ^B=0 and f-free models.

3.2.6 Population structure

Analysis of molecular variance (AMOVA) was used to evaluate hierarchical spatial structure for the mtDNA data using ARLEQUIN 3.5 (Excoffier & Lischer 2010) and for AFLP data using GENALEX 6.4 (Peakall & Smouse 2006). Variance was assessed within localities, among localities within bioregions and among bioregions (Fig. 3.1D, Table 3.1). Genetic differentiation among localities was measured using pairwise Φ_{ST} for mitochondrial sequences and F_{ST} for nuclear data.

Population structure was further evaluated with AFLP loci using a Bayesian clustering approach for dominant markers implemented in STRUCTURE 2.3 (Hubisz *et al.* 2009). For

each K (maximum number of clusters) from 1 to 13, three independent runs were used with a burn-in phase of 10,000 iterations followed by a run phase of 100,000 iterations. The admixture model with correlated alleles was used, with default priors for average and standard deviation of *F* (0.01 and 0.05 respectively), and α (0, 10).). The statistic ΔK was used to determine the most likely number of clusters as suggest by Evanno *et al.* (2005). To visualise the STRUCTURE results spatially, geographical interpolation of admixture levels were created a code provided by O. Francois and E. Durant, ran using R 2.12.2 (2011).

3.2.7 Isolation by geographical and ecological distances

Isolation by geographic and ecological distances was assessed with a partial Mantel test in ISBWS 3.16 Jensen *et al.* 2005 using 1,000 randomizations. This method estimates the correlation between more than two distance matrices, allowing the comparison between two variables while controlling the effect for a third one. The fixation index values (Φ_{ST} for mitochondrial and F_{ST} for nuclear data) were used as genetic distances. Geographic distances were calculated as the distances between sampling localities measured along the coastline using GOOGLEEARTH (2009). Environmental distances were estimated as the difference in of each of the seven oceanographic variables (temperature, salinity, oxygen saturation, nutrients, chlorophyll and bathymetry) between sampling sites.

Historical demography and divergence times

Historical demographic parameters (migration and population size) were estimated using mtDNA sequences in MIGRATE-N 3.2.7 (Beerli & Palczewski 2010). This software implements an extension of coalescence theory considering migration, and uses a Bayesian algorithm to sample coalescent genealogies that better fit the given genetic data under a specific mutational model. The mutation model of Felsenstein (1984) was used, in three runs

consisting of one long chain, five independent replicates, 1,000,000 sampled genealogies, 100 sampling increment, burn-in of 10,000 trees per chain, and a static heating scheme of 4 temperatures (1, 1.5, 3 and 10). Full and stepping-stone migration models were tested using Bayesian factor (*BF*) calculated from Bézier-corrected log marginal likelihood of each model. The full model considered bidirectional gene flow between all bioregions, while under the stepping-stone model only migration between neighbouring bioregions was estimated. Population size dynamics and time since divergence between bioregions were inferred using BEAST 1.5 (Drummond & Rambaut 2007). Since there is not a specific angel shark molecular clock for the mitochondrial control region, dating analysis was based on the mtDNA sequences, assuming an average mutational rate of 0.8% per million years Duncan *et al.* 2006. This clock was calibrated used divergences between populations of the scalloped hammerhead shark across the Isthmus of Panama, and is commonly used in genetic studies of sharks.

Time to most recent common ancestor (T_{mrca}) for each population of angel shark was estimated using a relaxed clock model in BEAST 1.5. This program, as in MIGRATE-N, uses a Bayesian approach to average over coalescence genealogy space assuming a determinate mutational model. The HKY+I was used with standard priors, to run the analysis for 10,000,000 generations, sampling every 100^{th} generation with the first 1000 samples discarded as burn-in. Stationarty and sample size for all parameters were evaluated using TRACER 1.4 (Rambaut & Drummond 2007).

3.2.8 Biogeographic hypotheses

Coalescent-based tests were conducted on five phylogeographic hypotheses (Fig. 3.3) using MESQUITE 2.6 Maddison & Maddison 2009. This approach simulates coalescent genealogies constrained within the population history predicted by each hypothesis. Two

parameters determine the tree topology: time since splitting (branch lengths) and population sizes (branch widths). Major historical geological events were used to set possible split times: (1).- Formation of the Gulf around 6 Mya; (2).- Closing of a seaway crossing the Baja California Peninsula at the southern part of the Gulf around 3 Mya; (3).- Closing of a putative seaway at mid-peninsula at 3 Mya or 1 Mya (Murphy & Aguirre-Leon 2002). Results from MIGRATE-N were used to set each extant population size, while ancestral population sizes were set as the average descendant population sizes. The hypotheses tested were as follows:

H1 Geological History: considers only the geological history, with the isolation of GC/PC at 6 Mya, then the split of LG/UG after the closure of seaways 3 Mya and the separation of PC/IG by the closure of a seaway 1 Mya.

H2 Vicariance and ecological isolation: isolation of GC/PC 3Mya when seaways were closed, and then two sequential splits IG/(UG/LG) by the gradual establishment of the oceanographic conditions in the GC.

H3 Two vicariant events: Closure of seaway 3 Mya produced isolation of PC/GC and then the closure of a second seaway produced the splits PC/IG and UG/LG.

H4a Pacific origin: Isolation of PC/GC 6 Mya, and then UG isolated when north seaway was closed 3 Mya, and a final split of LG/IG 1 Mya.

H4b Gulf origin: The formation of the GC originated the isolation of LG/UG 6Mya, then 3Mya the closure of the seaways produced the split of UG/IG, and a final split IG/PC occurred when the last seaway was closed 1Mya.

For each phylogeographic model, 1,000 coalescent genealogies were simulated using the best fit substitution model determined by MODELTEST 3.7. Subsequently, Maddison's deep coalescences (DC) were calculated for each tree. The tree reconstructed by MRBAYES was used to calculate the empirical value, and the simulated genealogies were used to generate a null distribution of the DC statistic. Then for all scenarios, a one-tailed test of significance was performed at the 5% level.

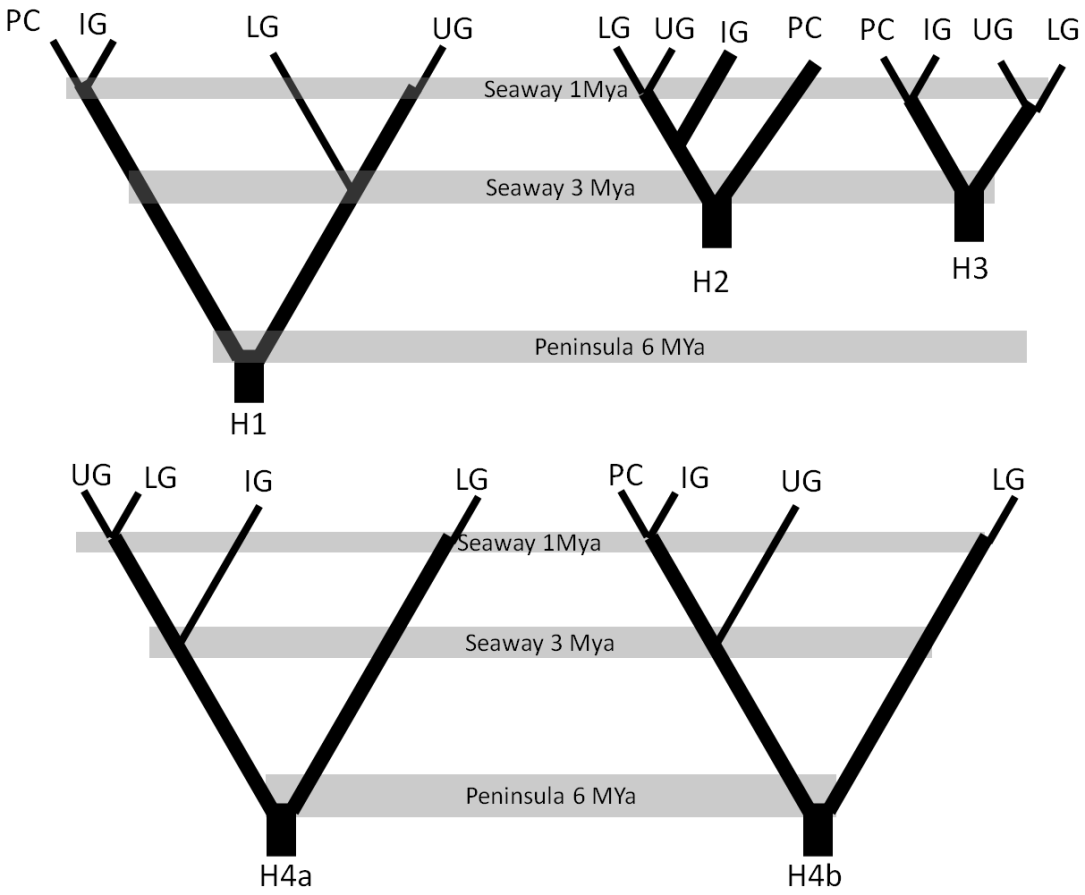

Fig. 3.3 Phylogeographic hypotheses for *Squatina californica* in the GC.

H1 Geological History.- consider only the geological history, with the isolation GC/PC 6Mya by the formation of the Baja California Peninsula, then the split LG/UG after closure of seaways 3Mya and the separation of PC/IG by the closure of a seaway1 Mya. H2 Vicariance and ecological isolation.- isolation of GC/PC 3Mya when seaways were closed 3Mya, and a then two sequential splits IG/(UG/LG) by the establishment of oceanographic conditions in the GC,. H3 Two vicariant events. Closure of seaway 3Mya produce isolation PC/GC and then the closer of a second seaway produce two splits PC/IG and UG/LG. H4a Pacific Origen. Isolation PC/GC 6Mya with the formation of the GC, and then UG isolated when north seaway was closed 3Mya, and a final split LG/IG 1Mya. H4b Gulf Origen.- The formation of the GC originated de isolation of LG/UG 6Mya, then 3Mya the split of UG/IG by the closure of seaways in the north and a final split IG/PC when the last seaway was closed 1Mya in the mid gulf.

Finally, main barriers for gene flow were highlighted geographically, by plotting the genetic distances between populations on a map. The edges with the largest associated distance in the triangulation network were identified as barriers, applying a Delaunay triangulation, a Voronoi tessellation and a Monmonier's maximum distance algorithm with the software BARRIER 2.2 (Manni *et al.* 2004). To test for the significance of barriers, four matrices of genetic differentiation were used. The AFLP data were divided into three matrices, according to the set of primer used (Table3.2), then the populations pairwise F_{ST} were calculated for each set and used with the mitochondrial Φ_{ST} as genetic distances matrixes. The first five barriers for each independent set of data were displayed, and divisions identified in all sets were considered as significant barriers.

3.3 Results

3.3.1 Genetic diversity

A total of 30 mtDNA haplotypes was resolved for the sample of 192 Californian angel sharks. Six haplotypes were exclusive to the PC and another 23 were found in the GC, with only one haplotype shared between these two regions. In the PC the two most common haplotypes are found in 70% of the samples, while in the GC 47% of the samples showed the most common haplotype (Fig. 3.4). Nucleotide diversity was low in all populations, ranging between 0.17 and 0.29%, whereas haplotype diversity was moderate to high, ranging between 0.602 and 0.790 (Table 3.1). A total of 287 polymorphic AFLP loci was resolved with a error rate of 2.2%, and expected heterozygosity ranging between 0.238 and 0.363 (Table 3.1 and 3.2).

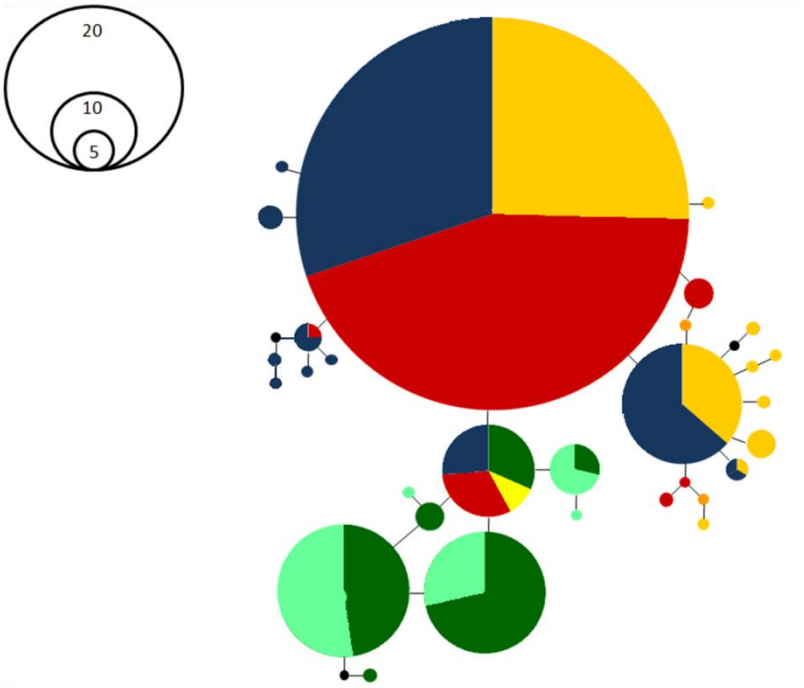

Fig. 3.4. mtDNA genealogical network for *Squatina californica* from the GC-Baja California Peninsula region.. The haplotype network is based on statistical parsimony. The relative area of circles reflects haplotype frequency in the population and the colour represents the location, black dots represent hypothetical haplotypes, and lines indicate one mutation. Colours correspond to bioregions, Upper Gulf (UG) blue, Islands (IG) red, Lower Gulf (LG) yellow, North Pacific (NP) light green and South Pacific (SP) dark green.

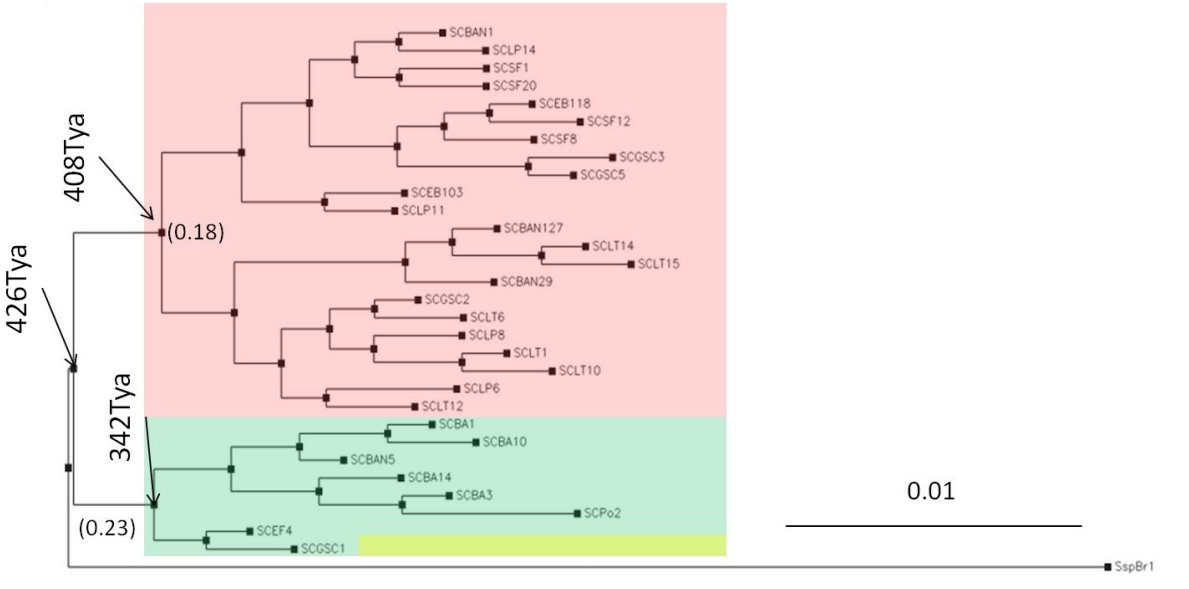

Fig. 3.5. mtDNA phylogeny for *Squatina californica* from the GC-Baja California Peninsula region. Based on a Bayesian analysis of 550 bp of the mtDNA control region. The posterior probability of each clade is shown in brackets and the estimate time of the most recent common ancestor in thousands of years. *Squatina guggenheim* from Brazil was used as outgroup.

3.3.2 Phylogenetic and genealogical patterns

The best substitution model for *S. californica* mitochondrial control region was F81+I, with I=0.8585. The phylogenetic analysis provides support for two major clades, one composed by sharks sampled exclusively in the GC, the other containing all samples collected in the PC plus one haplotype found in sharks sampled in both the PC and the GC (Fig. 3.4 and 3.5). However, these two clades are not statistically well supported (<0.2 Bayesian Inference). The haplotype network shows a cohesive group of maternal lineages that also supports the phylogeographic break between the GC and the PC. No phylogeographic groupings are evident in populations of the GC (Fig. 3.4). Several low frequency haplotypes are found in only one bioregion, but the most frequent haplotypes are shared between bioregions. These haplotypes appeared to have recently given rise to most of the matrilineal diversity observed, suggesting a pattern of historical connectivity between bioregions.

3.3.3 Population structure

Substantial genetic structure was detected with both mtDNA (Φ_{ST}=0.403, P<0.0001) and nuclear data (F_{ST}=0.1510, P<0.0001 and Φ_{PT}=0.258, P<0.0001). AMOVA results show that the variation can be well supported by differences among regions, a result observed for mtDNA (Φ_{CT}=0.378, P<0.0001) and AFLP (Φ_{RT}=0.216, P<0.0001) (Table 3.3). Genetic differentiation among localities ranged from zero to high structure based on both mtDNA (Φ_{ST}=0.000-0.6599) and AFLP (F_{ST}=0.000-0.299; Φ_{PT}=0.000-0.510) (Table 3.4). Overall, fixation indexes were higher between the PC and GC localities than between localities found along the coast.

According to the ΔK statistic, STRUCTURE determined 4 to be the most probable number of genetic clusters. The spatial distribution of such clusters matches well the geographical division of bioregions. Low to moderate levels of admixture and migration are suggested by this analysis, especially between bioregions in the GC (Fig. 3.6 and 3.7). All PC samples show strong membership to a single population, whereas in the gulf the IG population appears to be the most distinct.

3.3.4 Isolation by distance

A significant correlation was detected between geographic and genetic distances for both marker datasets (mtDNA, $P<0.0001$; AFLP, $P<0.0001$), indicating limited dispersal in angel sharks. Also for both markers genetic differences were correlated with temperature, salinity and nutrients concentration. After controlling for geographical distance, only the correlation for temperature (AFLP, $P<0.0001$) and bathymetry (ALFP, $P=0.009$) remained significant across the entire sample (Table 3.5). When only GC samples are used, there was a significant effect of temperature, nutrient concentration, oxygen saturation and bathymetry on AFLP genetic distance, even after controlling for geography (Table 3.6). These results suggest that isolation by ecological distances is greater within the Gulf, but also evident between GC and PC angel sharks.

Table 3.3. Hierarchical analysis of molecular variance (AMOVA) for *Squatina californica* based on 268 AFLP loci and ~550 bp of mtDNA control region.

	Mitochondrial sequences			AFLP loci		
	% of variation	Fixation index	P	% of variation	Fixation index	P
Among Regions	37.8	**Φ_{CT}=0.378**	**<0.001**	21.7	**Φ_{RT}=0.216**	**<0.001**
Among Localities within Regions	2.48	Φ_{SC}=0.04	0.0557	4.2	**Φ_{PR}=0.053**	**<0.001**
Within Localities	59.7			74.1		
Total		**Φ_{ST}=0.403**	**<0.001**		**Φ_{PT}=0.258**	**<0.001**

Table 3.4. Results of population pairwise fixation index for *Squatina californica*. Below the diagonal are Φ_{ST} based on mtDNA. Above diagonal are Φ_{RT} based on AFLP. Significant values after sequential Bonferroni correction are depicted in bold. Localites are: Popotla (Po), El Faro (EF), Laguna Manueal (LM), San Ignacio (SI), Bahia Almejas (BA), La Paz (LP), Loreto (LT) Bahia Concepcion (BC), El Barril (EB), Bahia de los Angeles (BAN), San Felipe (SF), Golfo de Santa Clara (GSC) and Pueto Peñasco (PP). The four bioregions are the Pacific Coast (PC), Lower Gulf (LG), Islands (IG) and Upper Gulf (UG).

		Po	EF	LM	SI	BA	LP	LT	BC	EB	BAN	SF	GSC	PP
PC	Po		0.089	0.046	0.111	0.119	**0.237**	0.232	0.184	**0.431**	**0.345**	**0.215**	0.236	**0.242**
	EF	0.190		0.152	0.167	0.276	**0.365**	**0.352**	**0.351**	**0.510**	**0.454**	**0.348**	**0.409**	**0.352**
	LM	0.260	0.000		0.148	0.105	**0.331**	**0.324**	**0.342**	**0.491**	**0.434**	**0.312**	0.372	**0.326**
	SI	0.168	0.000	0.000		0.150	0.279	**0.277**	0.252	**0.469**	**0.405**	**0.279**	0.311	**0.298**
	BA	0.234	0.063	0.076	0.000		**0.283**	**0.280**	0.254	**0.467**	**0.392**	**0.260**	0.314	**0.297**
LG	LP	**0.612**	**0.577**	**0.567**	**0.559**	**0.502**		0.001	0.063	**0.231**	**0.182**	**0.135**	0.121	**0.176**
	LT	**0.559**	**0.527**	**0.534**	**0.514**	**0.468**	0.029		0.057	**0.198**	**0.150**	0.084	0.086	**0.125**
IG	BC	0.336	0.411	0.309	0.310	0.156	0.408	0.377		0.195	0.082	0.086	0.074	0.114
	EB	**0.632**	**0.586**	**0.560**	**0.564**	**0.500**	0.112	**0.225**	0.300		0.044	**0.189**	0.242	**0.184**
	BAN	**0.662**	**0.621**	**0.588**	**0.597**	**0.524**	0.081	**0.193**	0.395	0.000		**0.151**	0.176	**0.146**
UG	SF	**0.628**	**0.594**	**0.581**	**0.575**	**0.518**	0.004	0.098	0.416	0.077	0.052		0.052	0.022
	GSC	**0.474**	**0.480**	**0.506**	**0.459**	**0.423**	0.101	0.140	0.178	0.129	0.139	0.039		0.058
	PP	**0.577**	**0.537**	**0.534**	**0.522**	**0.472**	0.011	0.082	0.326	0.087	0.070	0.000	0.000	

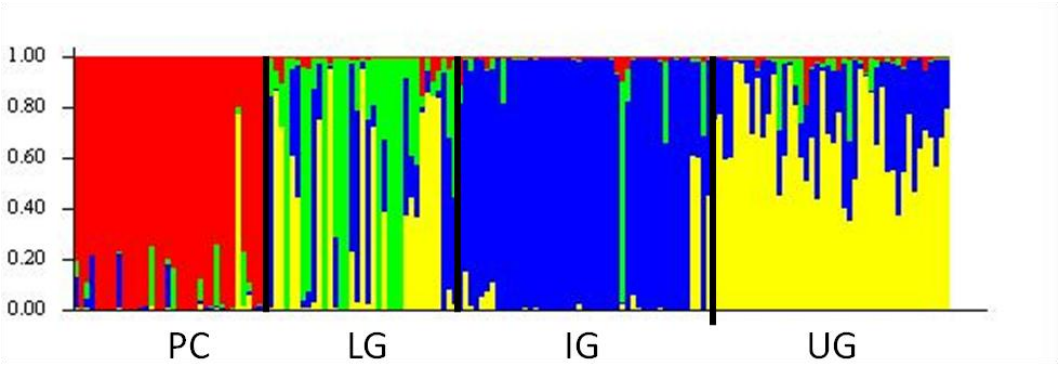

Fig. 3.6. Genetic clusters in *Squatina californica* identified with STRUCTURE 2.3 based on 268 AFLP loci. The graphic shows four clusters: Pacific Coast (PC), Lower Gulf (LG), Islands (IG), Upper Gulf (UG). The LG and UG have high levels of admixture

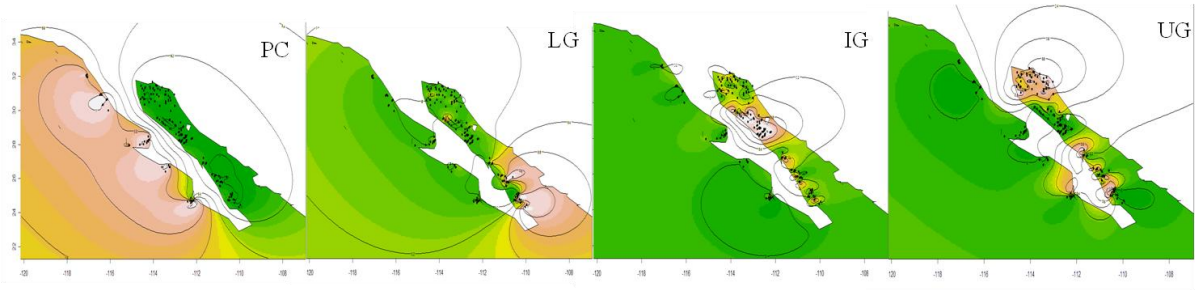

Fig. 3.7. Interpolation maps of the admixture level between regions: Pacific Coast (PC), Lower Gulf (LG), Islands (IG), Upper Gulf (UG). These maps were created using the statistical software R, and they show geographically the admixture coefficient estimated in STRUCTURE 2.3.2. Warm colours represent high admixture and cold colours represent low admixture

Table 3.5. Correlations between genetic differentiation and geographical and ecological distances for *Squatina californica*. *P* and *r* values are based on partial Mantel tests of mtDNA, and AFLP. Values highlighted in bold are statistically significant.

					Controlling by Geographical Distance			
	mtDNA		AFLP		mtDNA		AFLP	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Geographical Distance	0.766	**< 0.001**	0.767	**0.001**				
Temperature	0.535	**0.001**	0.632	**0.008**	0.021	0.398	0.563	**0.001**
Salinity	0.735	**<0.001**	0.722	**0.001**	0.029	0.416	0.001	0.832
Oxygen Saturation	0.009	0.805	0.005	0.630	0.018	0.882	0.081	0.284
Nutrients	0.341	**0.019**	0.386	**0.013**	0.002	0.994	0.001	0.782
Chlorophyll a	0.018	0.768	0.003	0.535	0.003	0.999	0.001	0.958
Bathymetry	0.015	0.392	0.124	0.067	0.085	0.193	0.472	**0.009**

Table 3.6. Correlations between genetic differentiation and geographical and ecological distances for *Squatina californica*. *P* and *r* values are based on partial Mantel tests of mtDNA, and AFLP. All analyses were conducted using only the GC localities. Values highlighted in bold are statistically significant

					Controlling by Geographical Distance			
	mtDNA		AFLP		mtDNA		AFLP	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Geographical Distance	0.015	0.545	0.748	**<0.001**				
Temperature	0.020	0.434	0.604	**0.010**	0.037	0.432	0.563	**0.012**
Salinity	0.001	0.937	0.250	0.211	0.005	0.961	0.123	0.374
Oxygen Saturation	0.027	0.586	0.593	**0.010**	0.047	0.589	0.473	**0.011**
Nutrients	0.034	0.496	0.3856	**0.009**	0.002	0.422	0.575	**0.010**
Chlorophyll a	0.017	0.545	0.017	0.308	0.001	0.547	0.146	0.537
Bathymetry	0.020	0.608	0.527	**< 0.001**	0.028	0.615	0.428	**< 0.001**

Table 3.7. Historical gene flow in *Squatina californica* between bioregions: Pacific Coast (PC), Lower Gulf (LG), Island (IG) and Upper Gulf (UG). The effective number of migrants per generation $N_em=\Theta M$ was estimated based on mtDNA data. Origen in rows and destination in columns

	PC	LG	IG	UG
PC		0.187	0.170	0.732
LG	0.0413		0.162	0.900
IG	0.0413	0.811		1.025
UG	0.0938	0.978	0.187	

3.3.5 Historical Demography

The full rather than the stepping stone migration model (*BF*=12.07) was best supported by the MIGRATE-N analysis. The population sizes range between 6938 and 34688. The levels of gene flow were low to moderate (Table 3.7). Migration between the PC and GC was low and asymmetrical, with higher migration coming from the PC to the GC. Inside the GC, migration between the UG and LG was symmetrical and moderately high. However, gene flow to the IG was reduced, but this bioregion provided a relatively high number of migrants to other populations.

3.3.6 Biogeographic hypothesis and barriers

All but one biogeographic hypothesis could be statistically rejected by the Mesquite analysis. The hypothesis considering vicariant isolation between the PC and GC around 3 Mya, and a sequential diversification inside of the GC was not rejected (Fig. 3.3; Table 3.8). Hypotheses that include the formation of the BCP 6Mya and/or a single ecological radiation inside the GC were rejected. This suggests a recent diversification (≤3Mya), influenced by the closure of the seaways. On the other hand, the software Barrier identified three main genetic breaks. The most important was the BCP, which divide the PC from the other populations. The other two are located at the border between the UG and IG regions, and the division between the LG and IG regions. (Fig. 3.8).

Table 3.8 Simulation test based on deep coalescence biogeographic hypothesis of population isolation in *Squatina californic*. H1 Geological History.- consider only the geological history, with the isolation GC/PC 6Mya by the formation of the Baja California Peninsula, then the split LG/UG after closure of seaways 3Mya and the separation of PC/IG by the closure of a seaway1 Mya. H2 Vicariance and ecological isolation.- isolation of GC/PC 3Mya when seaways were closed 3Mya, and a then two sequential splits IG/(UG/LG) by the establishment of oceanographic conditions in the GC,. H3 Two vicariant events. Closure of seaway 3Mya produce isolation PC/GC and then the closer of a second seaway produce two splits PC/IG and UG/LG. H4a Pacific Origen. Isolation PC/GC 6Mya with the formation of the GC, and then UG isolated when north seaway was closed 3Mya, and a final split LG/IG 1Mya. H4b Gulf Origen.- The formation of the GC originated de isolation of LG/UG 6Mya, then 3Mya the split of UG/IG by the closure of seaways in the north and a final split IG/PC when the last seaway was closed 1Mya in the mid gulf

Hypotesis	Scenario	Observed value	***P*** value
H1	Geological History	41	0.002
H2	Vicariance Ecology	48	0.055
H3	Vicariance Ecology	41	0.015
H4a	Gulf Origen	34	0.001
H4b	Pacific Origen	38	0.006

Fig. 3.8 Barriers to gene flow for *Squatina californica*. Genetic breaks as identified by BARRIER, using four matrixes of pair-wise F_{ST} comparisons. Gray lines represent the barriers and confidence is indicated in the weight of the line, with heavy lines indicating the break supported by all matrixes.

3.4 Discussion

3.4.1 Baja California Peninsula (BCP) as a phylogeographic barrier for a coastal shark

Results from a range of analytical tools based on both mitochondrial and nuclear markers provide strong support for a marked phylogeographic break and nil or low contemporary gene flow between PC and GC angel sharks, suggesting a cryptic incipient species of *Squatina* in the Gulf. These findings emphasise the role of the BCP as a biogeographic barrier for a coastal shark. With more than one thousand kilometres of coast/shoreline, the BCP is one of the longest peninsulas in the world. This region has been very active from a geologic perspective during the last six million years and has affected the evolution, distribution and genetic structure of both terrestrial (Upton & Murphy 1997; Grismer 2000; Riddle *et al.* 2000) and marine organisms (Bernardi *et al.* 2003; Jacobs *et al.* 2004). Several sister species have been identified between the GC and PC, suggesting a vicariant role of the BCP and allopatric speciation (Walker 1960; Castro-Aguirre *et al.* 1995). Nonetheless, since the Peninsula has existed for several million years (Oskin & Stock 2003), the number of species produced by the geographic isolation of the GC is relatively modest compared with more recent vicariant events, such as the emergence of the Panama Isthmus (Briggs 1974b). Nonetheless, a considerable number of organisms shows disjunct populations in both coasts of the BCP (Bernardi *et al.* 2003; Jacobs *et al.* 2004), suggesting that this barrier has exhibited historical permeability. This permeability could explain the levels of historical migration found between the PC and GC in *S. californica.* Two hypotheses have been proposed for historical gene flow between GC and PC biota and for the origin of disjunct populations and sister species in the region, one related to Pleistocene glaciation cycles (Brusca 1973b; Bernardi *et al.* 2003;

Jacobs *et al.* 2004) and the other to seaways across Baja California (Grismer 2000; Bernardi *et al.* 2003).

It has been suggest that during cold periods of the Pleistocene, habitat was lost in the Pacific coasts of California and Baja California. Aquatic populations used GC as a refuge, since of the impact of glaciations was apparently weaker in this region (Hall 2002; Jacobs *et al.* 2004). The end of the last glaciation allowed the expansion of GC populations to the PC, but subsequent increase of temperature in the southern GC prevented dispersal between coasts (Brusca 1973b; Jacobs *et al.* 2004). The effectivity of this warm water barrier has been questioned since organisms inside the GC are exposed to higher temperatures during summer (Bernardi *et al.* 2003). However, other oceanographic conditions could simultaneously interact and act as barriers. Currents and gyres, for instance, have been proposed as important barriers to gene flow, especially in larval dispersers (Banks *et al.* 2007; White *et al.* 2010), but also for mobile adult species (Polovina *et al.* 2004; Mendez *et al.* 2011; Möller *et al.* 2011) elasmobranchs included (Speed *et al.* 2010; Würtz 2010). Geostrophic gyres have been described in the southern part of the GC (Figueroa *et al.* 2003). These gyres, in combination with a narrow continental shelf, and the physical isolation created by the BCP and prevent gene flow between GC and PC angel sharks.

The other hypothesis is that after formation of the BCP and GC ~ 6 Mya, the peninsula was actually an archipelago of at least three islands. The north island was the longest, separated from the mainland by a seaway in the northern part of the Gulf, and from the second island by a seaway in the middle of the GC. In the southern part, two islands separated by a small seaway formed the cape region. Geological evidence supports the existence of these seaways until 3 Mya, when apparently they were closed due to final emergence of the BCP and sea level changes (Lavín & Marinone 2003). Also, a seaway opening ~1 Mya has been proposed

on the basis of phylogeographic data for marine and terrestrial organisms (Upton & Murphy 1997; Riddle *et al.* 2000; Riginos 2005). These seaways could function as corridors for gene flow between the GC and PC populations for several fish species (Bernardi *et al.* 2003) and could account for historical gene flow between angel sharks from the GC and PC.

Different genetic signals are expected from these two hypotheses. The postglacial colonization of the PC would have produced signal for both demographic and spatial population expansion (i.e. range expansion). Older lineages should be found in the GC, as well as evidence for historical gene flow from the GC to the PC, possibly following a stepping-stone model. On the other hand, the presence of seaways in the BCP would result in similar levels of gene flow between PC and GC populations. Estimates of the time from most recent common ancestor suggest a GC origin. Also, levels of historical migration between PC and the Gulf do not differ among GC populations and the stepping-stone model was not statistically supported. In fact, it appears that migration is actually asymmetric from the PC to the GC, instead of the opposite direction. Finally, the biogeographic hypothesis analysis supports, although only marginally, the isolation between PC and GC species by the time when the seaways closed (Fig. 3.1). Therefore, the available evidence suggests that the seaways theory provides a better explanation for the historical connectivity between PC and GC angel sharks than glaciation cycling.

3.4.2 Ecologically distinct populations in the Gulf of California

Three populations of angel sharks could be identified inside the GC. The distribution of these populations match with bioregions proposed based on oceanographic variables, and the distribution of phytoplankton and fish species (Round 1967; Walker 1960; Aceves-Medina *et al.* 2004; see Fig. 3.1D). Moreover, the limits of these populations correspond with genetic

break reported for teleosts (Riginos 2005) and marine mammals (Gonzalez-Suarez *et al.* 2009; Segura-Garcia 2011).This concordance points to common barriers for gene flow likely acting at a biotic level within the Gulf (discussed below).

Seaway across the mid-Baja California – While some authors propose the seaways as a facilitator for gene flow between the PC and GC (Bernardi *et al.* 2003), others suggest that a mid-peninsula seaway worked as barrier for gene flow between populations inside the GC (Riginos 2005). The author based this hypothesis on a phylogeographic congruence; reptiles and mammals from the BCP show a genetic break in the mid part of the peninsula Upton & Murphy 1997; Riddle *et al.* 2000), as well as some fishes in the PC of the BCP show a genetic subdivision at a similar latitude (Bernardi *et al.* 2003). However these separations could also be explained by strong ecological and climatic gradients, in both marine and terrestrial habitat (Grismer 2002; Burton 1998). In addition, divergence times between disjunct populations vary considerably among species. Riginos (2005) suggests this result could be related to problems associated with molecular clock calibrations for so many different species; nevertheless it could be evidence for different waves of diversification (Leaché *et al.* 2007). In the latter case, the seaway theory would provide an insufficient explanation. Also, the two genetic breaks (UG *vs* IG and IG *vs* LG) are ~ 200 and 100 km away, respectively, from the hypothesized position of the seaway. Even though a genetic break could change location in evolutionary time (Pringle & Wares 2007), the congruent position of the break for several species with different dispersal capabilities (Riginos 2005; Gonzalez-Suarez *et al.* 2009; Segura-Garcia 2011) suggests that other factors should be considered.

Oceanographic variables – The GC shows a very complex oceanography, with large seasonal and geographic variation that promote the formation of well-differentiated regions (Lavín & Marinone 2003; Fig 1). Extreme salinity (40‰), and a wide range of temperatures (9-38°C) in

the UG; the deep channels (>500m), low temperature (~11°C) and oxygen saturation in the IG; as well as the dynamic LG in the south, could have acted as barriers for gene flow between bioregions. Some of these characteristics have been discussed as possible factors delimitating connectivity in the GC (Riginos 2005; Segura-Garcia 2011), but their correlation with the genetic structure of the marine organism has not been tested.

The distinction of three angel shark populations congruent with bioregional division and the statistical support for genetic isolation by ecological distances are consistent with the oceanographic barriers theory. Abrupt bathymetric differences can function as strong barriers for dispersal in several marine organisms (Schultz *et al.* 2008; Hoffman et al. 2011). The division between coastal and oceanic habitat at around 200 m could restrict the migrations of costal elasmobranchs, especially benthic species such as angel sharks (Musick *et al.* 2004; Schultz *et al.* 2008; Speed *et al.* 2010). The contrast between the shallow waters in the UG with the deep channels (>500m deep) in the IG perhaps act as a semipermeable barrier that divides the GC into northern and southern regions. Moreover, oxygen saturation influences metabolism and swimming activity of elasmobranchs (Carlson, 2003). Thus, low oxygen concentration could represent a physiological barrier affecting the distribution and dispersal of sharks (Heithaus *et al.* 2009; Froeschke *et al.* 2010). It is proposed here that the relative low oxygen saturation found in the IG region provides a physiological barrier for migration of angel sharks.

In addition, temperature differences provide perhaps the most important physiological barrier in animals; affecting the distribution and dispersal capacity of several organisms (Somero 2010). During most of the year, UG and LG regions are warmer than IG. The temperature also is one of the most important factors controlling elasmobranch mating, gestation and birth seasons (Pratt *et al.* 1990; Wourms & Demski 1993). In the GC, the reproduction of

Californian angel sharks takes place during winter and spring (Salomón-Aguilar *et al.* 2009), when the strongest upwelling and lowest temperature and oxygen saturation are found in the IG region (Lavín & Marinone 2003). These putative oxy, thermo and bathymetric barriers could be semi-permeable, allowing some migrants to pass through. However, the low temperature and oxygen concentration in the IG during reproductive season provides a strong selection pressure again immigrants with high oxygen consumption and warmer temperature preferences. This could perhaps explain the lower levels of gene flow to the IG population and the higher observed admixture (Fig.3.6 and 3.7) between the more ecologically similar UG and LG.

3.4.3 Implications for conservation management and taxonomy

The high genetic differentiation and the low or absent historical and contemporary gene flow between the PC and GC angel sharks supports the idea of two species of *Squatina* in the region. Despite being genealogically distinct, one haplotype was found in sharks from both regions (Fig. 3.4 and 3.5). The lack of reciprocal monophyly could be interpreted as a lack of isolation (Sites & Marshall 2003), however, two divergent species will require a considerable amount of time after initial isolation before they are reciprocal monophyletic (Hudson & Turelli 2003; Knowles & Carstens 2007). Our estimates indicate that angel sharks from the PC and the GC started to diverge only around <1 Mya. In addition, reduced gene flow appears to have accompanied this recent process of lineage separation, which together with the very low mutational rates in elasmobranchs (Martin 1999) might account for the lack of reciprocal monophyly. Overall, the evidence presented here strongly indicates that a recently isolated and incipient cryptic species of *Squatina* is found in the Gulf. Given that the type locality of *S. californica* is from San Francisco, USA (Ayres 1859), the Gulf lineage likely represents an undescribed species. No morphological, physiological or demographic comparative studies

are available for *Squatina* in the region. Such studies are therefore needed to assess morphological distinctiveness, identify diagnostic characters, and revisit the taxonomy of the genetically and ecologically distinct lineage found in the GC.

The genetic architecture of angel shark populations inside the GC reflects the existence of three populations that should be considered as distinct management units. Unfortunately, there are no species-specific data of historical catch of *S. californica* in Mexico. Empirical knowledge based on fishermen and researcher observations suggest a strong reduction in the species' stocks over the last few years. The genetic diversity found in GC populations is within the range of other species of elasmobranchs (Sandoval-Castillo *et al.* 2004; Heist 2004a; Corrigan *et al.* 2008). Nevertheless, the K-strategy life history of angel sharks, coupled with the very limited geographic range of each population and, possibly that of the species (endemic to the GC), make them extremely vulnerable to heavily localized fishing pressure, such as that currently occurring in the region (Bizzarro *et al.* 2009; Pérez-Jiménez & Sosa-Nishizaki 2008). Given the existence of three genetically distinct populations of angel shark, the management police of elasmobranchs in the GC must consider possible demographic distinction (e.g. fecundity, size at first maturity) that could differentially influence the vulnerability of each population to overexploitation (Hutchings 2000; Cortés 2002). In addition, genetic diversity has significant effects in ecological and evolutionary process, such as population evolutionary potential and species ecological resilience (Hughes *et al.* 2008; Becks *et al.* 2010). Thus, the conservation of the three genetic differentiate angel shark clusters is primordial to maintain the potential of each population to become a new species, as well as the resilience of species to exploitation, habitat degradation of climatic change effects.

Seascape genetic analysis points to the importance of temperature and oxygen saturation in the delineation of GC populations and their subsequent vulnerability to climatic oscillations. Theoretical and empirical studies suggest that many marine species respond to ocean warming shifting their distribution. This response could produce local extirpation and subsequent ecological effects (Parmesan 2006; Cheung *et al.* 2009; Banks et al. 2010). Also, species with narrow ranges restricted by oceanographic barriers are more vulnerable to climatic change due to the possible reduction or loss of essential habitat (Klausmeyer & Shaw 2009). Knowledge about thermal tolerance and functional genetic variation (e.g. based on next generation sequencing approaches) in *Squatina* from the GC would allow an understanding of the potential effects of ongoing climate and environmental change on shark populations with narrow distributions.

Chapter IV "Trans-peninsular" connectivity in a heavily exploited coastal shark: the brown smooth-hound *Mustelus henlei* from the Gulf of California and the Mexican Pacific Coast

4.1 Introduction

A central goal in marine ecology is to understand ecological and evolutionary processes that maintain the functioning and persistence of marine ecosystems. A critical component of this goal is to quantify the rate of connectivity among populations (Almany *et al.* 2009; Cowen & Sponaugle 2009). The large-scale, long-term gene flow or interchange of organisms in a geographical sense is essential for the evolution and persistence of populations and species. On a local scale, the stability of a population could be compromised by the absence of migrants that maintain population fitness (Hellberg 2009). The movement of organisms amongst ecosystems can also influence the numbers of ecological interactions, as well as the flow of nutrients and energy, affecting long-term ecosystem resilience (Folke *et al.* 2004; Hughes *et al.* 2005). Furthermore, individual dispersal over long distances allows the expansion of the species range. In this way, the potential for species diversification can be enhanced along with its ability to adapt to changing environmental conditions (Avise 2000). Therefore, understanding and quantifying population connectivity is important not only for the conservation of marine ecosystems but also for elucidating general ecological and evolutionary processes.

The study of connectivity is challenging, since it depends on several attributes of species as well as on interactions between species and the landscape (Hodgson *et al.* 2009). Fortunately, successful migrants leave a trail of their movements in population genomes, offering means to estimate both historical and contemporary connectivity (Palumbi 2003; Hellberg 2009). In recent years, marine population biologists have examined the genetic makeup of several species in order to answer a range of questions about dispersal patterns in the sea (Palumbi 2003). These studies have largely focused on fish and invertebrate species with larval dispersal (e.g. Cowen and Sponaugle 2009; Weersing and Toonen 2009). On the other hand, the ecologically important group of elasmobranchs has been largely neglected in studies of both historical (Beheregaray 2008) and contemporary connectivity (Heist 2004b).

It was only in the last two decades that the conservation and management of shark populations has received some attention due to a growing understanding of their susceptibility to overexploitation and expansion of their fisheries (Bonfil 1994; Camhi *et al.* 1998; Stevens *et al.* 2000; Dulvy *et al.* 2008). Added to this is the fact that the implementation of shark management polices has been extremely difficult given the general absence of relevant biological information for most elasmobranch species (Camhi *et al.* 1998; Musick *et al.* 2000; Camhi *et al.* 2008; Camhi *et al.* 2009; Herndon *et al.* 2010). In the absence of traditional life-history studies, inferences from genetic data are a valuable source of information for a range of aspects regarding an organism's natural history; including population connectivity (Schwartz *et al.* 2007).

The brown smooth-hound, *Mustelus henlei*, is an epibenthic shark with a distribution from Oregon, USA to the Gulf of California, Mexico and from Ecuador to the north of Peru (Compagno 1984). This is one of the most significant components of the large elasmobranch fishery along the Baja California Peninsula (BCP) and the Gulf of California (GC). *Mustelus*

henlei represents ~30% of the annual coastal shark catch in the region, corresponding to ~5 thousand tonnes and over 300,000 individuals of this species (Smith *et al.* 2009; Bizzarro *et al.* 2009; Pérez-Jiménez & Sosa-Nishizaki 2008; INAPESCA 2010; Sandoval-Castillo personal observations). Unfortunately, while the life history of *M. henlei* is relatively well known in California (Ebert & Squillante 2003; Campos *et al.* 2009), biological studies of this species are essentially absent in Mexico, with a few exceptions in the upper area of the Gulf of California (e.g. Pérez-Jiménez & Sosa-Nishizaki 2008; Perez- Jimenez et al. unpublished data). In that region, reproductive data suggest a GC population distinct from that found along the Californian coast (Pérez-Jiménez & Sosa-Nishizaki 2008).

The GC has very complex oceanography and topography such that four well-delimited regions are recognised. The upper gulf (UG) has shallow waters (<100m on average), high salinity (up to 40‰), large temperature variation (9-38°C) and large tidal ranges (>6m). The islands region (IG) has channels that are over 500m deep and is characterized by strong tidal-mix upwelling that maintains high productivity and low temperatures (~11°C) throughout the year. The lower gulf (LG) has more stable temperature and salinity, but is more dynamic oceanographically, with a series of geostrophic gyres affecting the circulation and thermodynamic of the area across all seasons. The open gulf (OP) is similar to the LG, but with less gyres, and a greater influence of oceanic waters from the Pacific (Lavín & Marinone 2003). On the other hand, the Pacific Coast (PC) of BCP is divided into north (NP) and south Pacific (SP) regions by an important biogeographic break around the mid BCP. This break is related with the convergence of currents with a dramatic temperature difference, and the presence of semipermanent oceanographic eddies (Brigge 1974, Hewitt 1981; Bernardi et al. 2003; Pelc et al. 2009). These oceanographic conditions create environmental discontinuities and physiological barriers that could limit dispersal between bioregions, both in the GC and the Pacific Coast of the BCP.

The GC-BCP area has a very active geological history. A transitory proto-gulf was formed ~12 million years ago (Mya) with the emergence of small islands and the detachment of a proto-peninsula from the mainland. Tectonic movements transported the archipelago and the proto-peninsula northwest, forming the permanent GC ~5.5 Mya. At that time the GC was connected to the Pacific Ocean by channels between islands and the proto peninsula. By the early Pleistocene (~3 Mya) emerging land and fluctuations in sea level closed the channels, attaching the islands and the proto-peninsula to subsequently form the present-day BCP (Murphy & Aguirre-Leon 2002). The complex geological history of the GC-BCP could have produced several vicariant opportunities affecting connectivity between GC and PC populations.

In this study, mitochondrial DNA (mtDNA) control region sequences and nuclear microsatellite markers were used to assess levels of connectivity and genetic structure in populations of *M. henlei* along the Baja California Peninsula and the Gulf of California, in Mexico. We combined and statistically explored genetic and environmental information to assess the relative efficiency of oceanographic and geological factors as barriers to connectivity in a heavily exploited coastal shark with presumably high dispersal. Low genetic differentiation in both marker datasets would suggest that known biogeographic barriers in the region are not effective for a high disperser. On the other hand, patterns of genetic structure consistent with bioregional divisions would support the hypothesis that coastal oceanographic processes influence species mobility. Finally, marked divergences between GC and PC populations would highlight the importance of the BCP as a vicariant biogeographic barrier. Our study has implications for understanding processes underpinning genetic structure in a

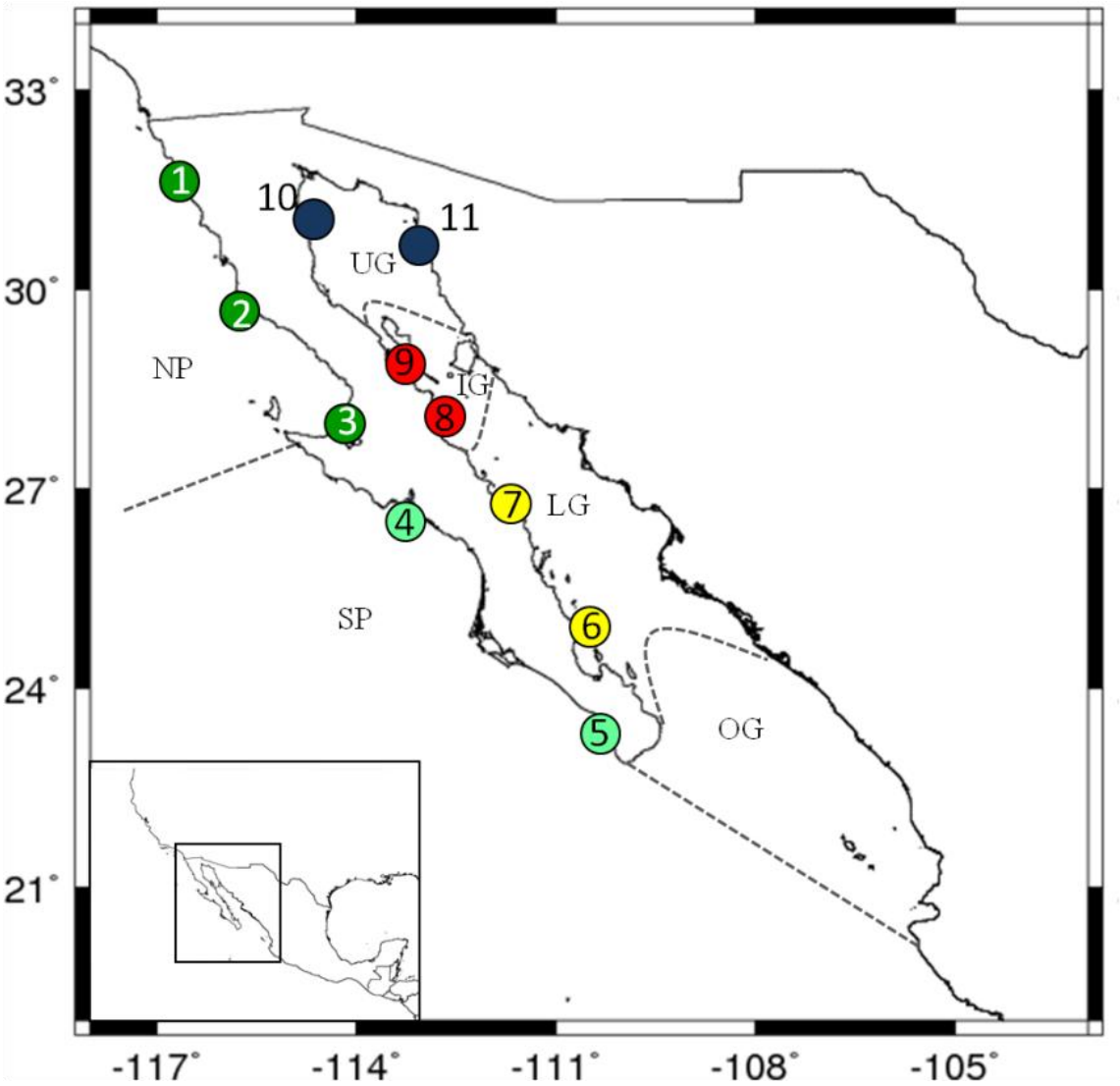

Fig. 4.1 Map of the Gulf of California and the Baja California Peninsula. Sampling sites are indicated by the circles (1) Popotla, (2) San Quintin, (3) Laguna Manuela, (4) San Ignacio, (5) Todos Santos, (6) La Paz, (7) Mulege, (8) El Barril, (9) Bahia de los Angeles, (10) San Felipe, (11) Puerto Peñasco. Bioregions are separated by dashed lines, for the Baja California Penisula North Coast (NP, dark green dots)and South Coast (SP, light green dots); and for the Gulf of California Lower Gulf (LG, yellow dots), Islands(IG, red dots), Upper Gulf (UG, blue dots) and Open Gulf (OP).

Table 4.1 Sampling localities, bioregions and total level of diversity. Sample size (n), number of haplotypes (H), haplotype (h) and nucleotide (π) diversity, heterozygosity observed (H_O), and expected (H_E), and mean number of alleles per locus (A).

		Mitochondrial sequences			Microsatellites		
	n	H	h	π%	H_O	H_E	A
(NP) North Pacific	**25**	**10**	**0.647**	**0.22**	**0.68**	**0.691**	**6**
(1) Popotla	5	1	0	0	0.73	0.7	3.7
(2) San Quintin	11	6	0.8	0.27	0.682	0.672	4.7
(3) Laguna Manuela	9	5	0.722	0.28	0.648	0.699	4.7
(SP) South Pacific	**23**	**7**	**0.708**	**0.2**	**0.754**	**0.713**	**5.7**
(4) San Ignacio	10	6	0.778	0.27	0.7	0.657	4
(5) Todos Santos	13	5	0.692	0.15	0.795	0.741	5.5
(LG) Lower Gulf	**24**	**12**	**0.888**	**0.36**	**0.696**	**0.683**	**5.1**
(6) La Paz	19	12	0.918	0.4	0.694	0.69	5
(7) Mulege	5	4	0.9	0.23	0.7	0.663	3.5
(IG) Islands	**30**	**11**	**0.881**	**0.39**	**0.683**	**0.643**	**4.8**
(8) El Barril	15	9	0.914	0.41	0.678	0.679	4.7
(9) Bahia de los Angeles	15	9	0.886	0.37	0.689	0.594	4
(UG) Upper Gulf	**39**	**8**	**0.823**	**0.31**	**0.738**	**0.679**	**5.7**
(10) San Felipe	20	8	0.853	0.33	0.758	0.693	5.5
(11) Puerto Peñasco	19	8	0.8421	0.31	0.717	0.662	4.8
Total	**141**	**24**	**0.84**	**0.33**	**0.712**	**0.684**	**7**

highly mobile and economically important coastal shark distributed along a complex and iconic marine ecosystem. It should also contribute substantially to conservation and management of the large elasmobranch fishery of the Gulf of California, not only for the brown smooth-hound, but also for other exploited coastal sharks with high dispersal potential.

4.2 Methods.

4.2.1 Tissue sampling

Sampling was conducted at eleven localities around the Baja California Peninsula and the Gulf of California where commercial fishermen land elasmobranchs. All samples were obtained from fishing trips conducted within ~75 km of landing sites. The localities were selected to cover three bioregions in the Gulf, UG, IG, LG; and two bioregions in the Pacific Coast, NP and SP (Fig. 4.1; Table 4.1). Muscle tissue samples of 141 brown smooth-hounds (82 females, 38 males and 19 unsexed) were collected and stored in ~95% ethanol. Samples of *Mustelus californicus*, *M. lunulatus* and *M. albipinnus* were also collected from the UG and used to confirm the identification of *M. henlei* samples.

4.2.2 Genetic markers

Genomic DNA was extracted using a salting out protocol (Sunnucks & Hale 1996), and genetic data were collected from all samples using mtDNA and microsatellite markers. A ~600 bp fragment of the mitochondrial control region was amplified using primers designed from conserved elasmobranch sequences (FPre200 5'-RYC YTT GGC TCC CAA AGC-3' and RCR900 5'-GGG MGG RCK RKA AAT CTT GA-3'). Each 10μL PCR contained 0.5μM of each primer, 3mM $MgCl_2$, 100μM each dNTP, 1X colourless GoTaq® flexi buffer,

1U Taq polymerase (Promega, WI, USA) and 30-200ng template DNA. The thermocycling profile consisted of 94°C for 2 min followed by 30 cycles at 94°C for 45 s, 58°C for 45 s, 72°C for 60 s, and 72°C for 10 min. PCR products were sequenced using primer FPhe200 using Big Dye Terminator chemistry in an ABI 3730 automated sequencer (Applied Biosystems, CA, USA). All chromatograms were checked by eye and aligned using the SEQUENCHER 4.7 program (Gene Codes Corporation, MI, USA). Ambiguous sites were confirmed by sequencing in the reverse direction. Microsatellites were PCR-amplified using six *Mustelus* microsatellite markers (MaND5, MaWGT, Ma07B, MaWD7, Ma6B5 and MaD2X) based on conditions described in Boomer and Stow (2010). Scanned data were analysed to detect bins using GENEMAPPER 4.0 (Applied Biosystems, CA, USA). MICROCHECKER 2.2.3 (Van Oosterhout *et al.* 2004) was used to assess scoring errors and null alleles in the amplified genotypes, with a Bonferroni adjusted at 95% confident interval and 1000 randomisations for the Monte Carlo simulation.

4.2.3 Data analysis

Genetic diversity. For the mitochondrial data, haplotype frequency, haplotype diversity (h) and nucleotide diversity (π) were estimated for each sampled locality and bioregion using ARLEQUIN 3.5.1.2 (Excoffier & Lischer 2010). The same program was used to calculate allele frequencies, and observed (H_O) and expected (H_E) heterozygosity per locality for the microsatellite data. Additionally, tests of locus independence and Hardy-Weinberg equilibrium were conducted using GENEPOP 4.0 (Rousset 2008).

Genealogical analysis of mtDNA sequences. Genealogical relationships were estimated with a statistical parsimony network (Templeton et al 1992) using the program TCS 1.41 (Clement et

al. 2000). This method estimates the number of mutations necessary to link all haplotypes parsimoniously using a 95% confidence level.

Historical demographic analysis. Migration among regions was estimated in MIGRATE-N 3.2.6 (Beerli & Palczewski 2010). This software implements an expansion of coalescence theory which considers migration, and uses a Bayesian algorithm to sample coalescent genealogies that best fit the given genetic data under a specific mutation model. For mtDNA sequences, the mutation model of Felsenstein (1984) was used with one long chain, two replicates, 200,000 sampled genealogies, 100 sampling increment, burn-in of 1,000 trees per chain; and a static heating scheme of four temperatures (1, 1.5, 3 and 100). For the microsatellite data, the Brownian motion model was selected for faster parameter estimation. The analysis was run as with mtDNA, but with three replicates, and 150,000 sampled genealogies. The full and stepping-stone migration models were compared using an un-nested model analysis with Bayes factor (Beerli 2010). The full model considers migration between each pair of populations in both directions, while the stepping stone model only considers migration between nearby populations. Since a calibrated molecular clock is not available for *Mustelus*, the demographic parameters were estimated considering mutation rates μ=4x10^{-8} and 1x10^{-3} for mitochondrial and microsatellite data, respectively, which are rates commonly used in elasmobranchs (Duncan *et al.* 2006; Corrigan *et al.* 2008; Karl *et al.* 2011). To provide other estimates of change in population size, the statistics Tajima's *D* (Tajima 1989) and Fu's *Fs* (Fu 1997) were calculated in ARLEQUIN, and tested for significance using 1000 simulations. If population sizes have been stable, both statistics are expected to return values close to zero. Significantly negative or positive values of *D* and *Fs* are expected under recent population expansion or bottleneck scenarios, respectively. The distribution of pairwise differences among haplotypes (mismatch distribution) was also assessed in ARLEQUIN. A unimodal mismatch distribution suggests recent population expansion, while multimodality

indicates the influence of migration, population subdivision or historical contraction. To test the fit of the observed data to a sudden expansion model, Harpending's Raggedness indices were calculated using 10,000 replicates. Small raggedness values are typical of expanding populations while larger values suggest stationarity (Harpending 1994).

Analysis of spatial population structure, migration and sex-biased dispersal. Levels of genetic differentiation among populations were estimated by calculating the pairwise fixation indices Φ_{ST} for mtDNA and F_{ST} for microsatellite loci. Hierarchical patterns of population structure were tested for both marker types using an analysis of molecular variance (AMOVA), implemented in ARLEQUIN. AMOVA divides the total variance into covariance components, in this case variance within sampling localities, among localities and among bioregions (NP, SP, LG, IG, UG). Localities were assigned to biogeographic regions according to the description in Lavin and Marinoni (2002; see Fig. 4.1, Table 4.1) and hierarchical fixation indexes were tested for significance using 1,000 permutations.

Nuclear genetic structure among sampled localities was also evaluated using the Bayesian clustering algorithm implemented in STRUCTURE 2.3.3 (Hubisz *et al.* 2009). Three independent runs for each K (maximum number of clusters) from 1 to 11 were run using the admixture model with correlated alleles, with a burn-in phase of 10,000 iterations followed by a run phase of 100,000 iterations. Default priors were used for average and standard deviation of F (0.01 and 0.05 respectively), and α (0, 10) were used. The statistic ΔK (Evanno *et al.* 2005) was used to determine the most likely number of clusters.

To estimate the spatial scale over which gene flow is occurring, an allele-based method for detecting geographic genetic structure was used in SASHA 1 (Kelly *et al.* 2010). This approach tests for a random distribution of the geographical distances between co-occurrence

of alleles (occurrences of same allele in different localities). Non-random distributions indicate departures from panmixia. The program also calculates the average geographic distance between occurrences of alleles that could be interpreted as the spatial scale of gene flow. In addition, to determine the geographic scale of genetic similarity across different distance classes, a spatial autocorrelation test was performed using microsatellite data in GENALEX 6.4 (Peakall & Smouse 2006). This method estimates an empirical outocorrelation coefficient (*r*) between al pair of individuals whose geographical separation are in a specified distance class. Then, by random permutation of genotypes a null distribution of *r* values is generated under the assumption of no geographical structure. Finally, using the 95% confidence interval of null distribution the statistical significance of each empirical *r* value is tested (Smouse & Peakall 1999). In this case, deviation from randomly distributed genotypes was tested using 10,000 permutations of individual genotypes and 1,000 bootstraps. Runs were conducted for all samples, as well as for females and males separately. Finally, using the same program, an assignment-based test was performed to assess differences in dispersal between sexes. This method estimates an assignment index correction (*AIc*) for the probability of an individual being born locally. Individuals with higher dispersal should show negative values, while those that are less dispersing will show positive values. Subsequently, mean values of the index were compared between sexes and statistically assessed with a Mann Whitney U-test.

Ecological isolation. Isolation by geographic and/or ecological distances was tested using Partial Mantel tests in IBWS 3.16 (Jensen *et al.* 2005). This is a nonparametric test for correlation among three distance matrices. Fixation indices (Φ_{ST} for mtDNA and F_{ST} for microsatellites) were the genetic distances between localities. Using GOOGLEEARTH (2009), geographic distances were estimated as the linear distances along the coastline between sampling localities. Environmental distances were calculated as the differences in

mean of seven oceanographic variables between sampling sites. Data of annual average for the last 100 years of key oceanographic variables (temperature, salinity, oxygen saturation, nutrients concentration, total chlorophyll concentration and bathymetry) were obtained from the NOAA World Ocean Data Base (www.nodc.noaa.gov/OC5/SELECT/dbsearch/dbsearch.html). These are variables that could potentially affect the distribution and dispersal of coastal sharks, either directly, or by influencing the distribution of their prey.

4.3 Results

4.3.1 High genetic variability

A total of 24 mitochondrial haplotypes was resolved in *M. henlei*. The most common haplotype (32.6% of the sample) was found at all localities, whereas the three most common haplotypes (63.1%) were found in all bioregions (Fig. 4.2). Overall, there was high haplotype diversity (0.8401) but low nucleotide diversity (0.33%). Haplotype diversity varied widely across localities (0-0.918). Variability in the nuclear genome was also moderate to high, both in number of alleles per locus (5 to 10, mean=7) and observed heterozygosity (0.594 to 0.704, mean=0.677). Nuclear and mtDNA diversities were generally higher in the GC than along the Pacific Coast (Table 4.1). For microsatellites, genotyping error or null alleles were not detected. In addition, tests of, Hardy-Weinberg and linkage disequilibrium were non-significant after Bonferroni sequential correction (Table 4.2 and 4.3). Thus, the null hypothesis of random mating in the populations was not rejected and alleles at different loci can be considered as independently assorting.

Table 4.2 Hardy-Weinberg equilibrium test significant values (*P*) for *Mustelus henlei* samples, by region and locus. Number the alleles (A) per locus. Pacific Coast (PC), Islands (IG), Lower Gulf (LG) and Upper Gulf (UG)

.

Region	*P*	
PC	0.844	
IG	0.507	
LG	0.399	
UG	0.503	
Locus	*P*	A
MaND5	0.899	8
MaWGT	0.145	7
Ma07B	0.542	5
MaWD7	0.939	6
Ma6B5	0.725	10
MaDX2	0.058	6

Table 4.3 Significant values for linkage disequilibrium between pair of *Mustelus henlei* microsatellite loci

Locus Pair	*P*
MaND5/MaWGT	0.319
MaND5/Ma07B	0.383
MaWGT/Ma07B	0.396
MaND5/MaWD7	0.828
MaWGT/MaWD7	0.977
Ma07B/MaWD7	0.683
MaND5/Ma6B5	0.252
MaWGT/Ma6B5	0.199
Ma07B/Ma6B5	0.755
MaWD7/Ma6B5	0.466
MaND5/MaDX2	0.165
MaWGT/MaDX2	0.785
Ma07B/MaDX2	0.855
MaWD7/MaDX2	0.468
Ma6B5/MaDX2	0.348

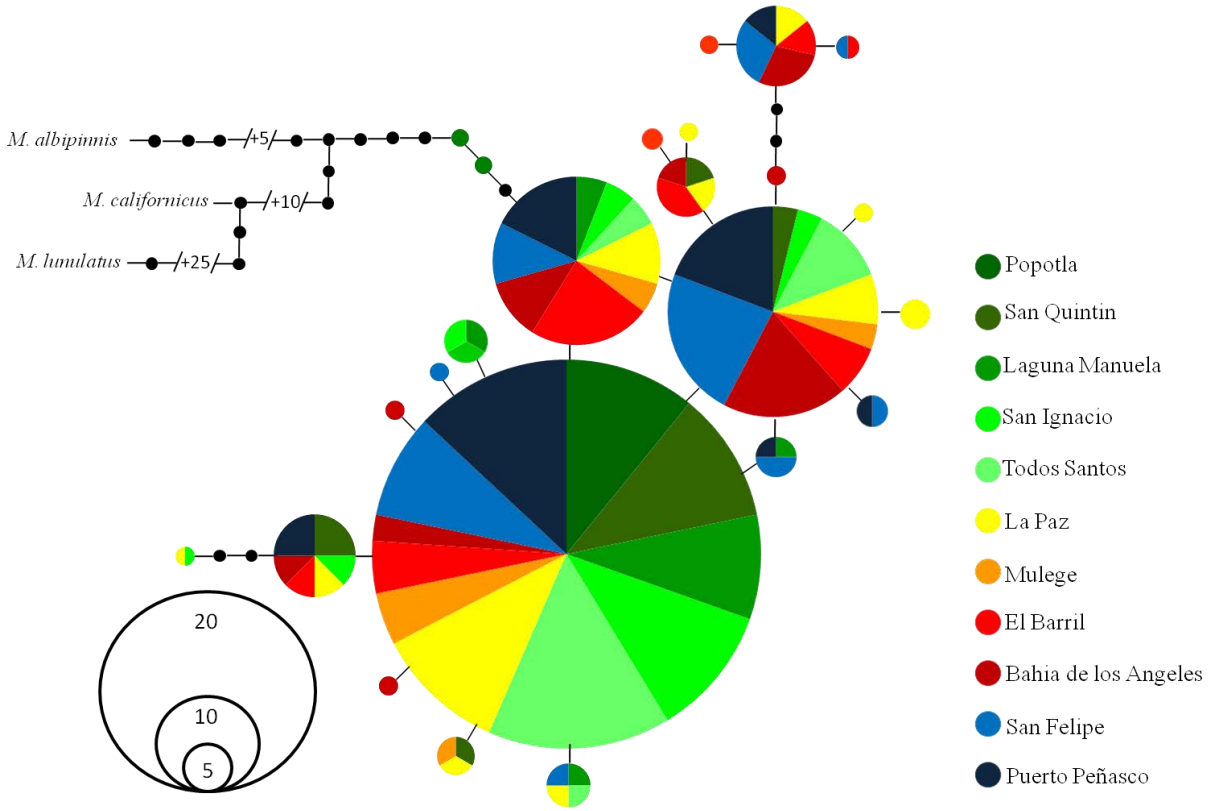

Fig. 4.2. Genealogical network of *Mustelus henlei* mtDNA control region haplotypes. Relative area of circles reflects haplotype frequency, and the colour represents sampling location. Black dots represent hypothetical haplotypes, and lines separating haplotypyes indicate one mutation.

4.3.2 Genealogical analysis

The *M. henlei* lineage can be clearly separated from other *Mustelus* species found in the region. The mtDNA haplotype network shows a closely related group of haplotypes, with lineages mostly arranged as small star phylogenies. The network is dominated by three haplotypes that are present across all regions (both inside and outside the Gulf), and are very common at most localities (Fig. 4.2).

4.3.2 Historical demography

Estimates of the number of migrants per generation differed for mtDNA and microsatellite data. While microsatellites generally showed high levels of gene flow, mtDNA shows limited migration between bioregions (Table 4.4); supporting sex-biased dispersal mediated by males (see below). The microsatellite results using the full migration model did not reveal stronger gene flow between neighbouring sites. Considerable (Nm>>1) migration occurred between all pair of populations, including the NP-UG bioregions that are separated by at least 1500km. Moreover, migration model analysis did not support the stepping-stone model (Table 4.4), suggesting stronger historical connectivity possibly related with the geological history of the area and/or recent population expansion.

Two tests of neutrality, Tajima's *D* and Fu's *Fs* statistics, were negative for all bioregions, and for both coasts (GC and Pacific). However, only Fu's *Fs* statistics were significantly different from zero (Table 4.5). Since, Fu's *Fs* are more sensitive to demographic expansion; the differences between indexes could be due to a relatively weak signal of a recent fluctuation in population sizes. Mismatch distributions were deemed unimodal for all bioregions and were not statistically different than a sudden expansion model (Fig. 4.3). An

Table 4.4 Gene flow between regions North Pacific (NP), South Pacific (SP), Lower Gulf (LG), Island (IG) and Upper Gulf (UG). Effective number of migrants per generation $N_em=\Theta M$ and $N_em=\Theta M/4$ from mitochondrial and microsatellite data, respectively (where Θ and M are estimator for population size and gene flow correspondingly). Source and sink populations are on rows and columns respectively.

	Microsatellites					Mitochondrial sequences				
	NP	SP	LG	IG	UG	NP	SP	LG	IG	UG
NP		3.75	3.34	2.07	2.28		0.14	4.52	0.16	0.14
SP	3.21		1.97	1.86	2.01	0.16		5.16	0.16	0.28
LG	3.48	2.88		2.57	1.21	0.12	0.12		0.14	0.13
IG	1.79	2.95	2.50		2.81	0.13	0.13	0.43		0.13
UG	4.55	3.48	2.88	2.71		0.18	0.14	2.98	0.78	

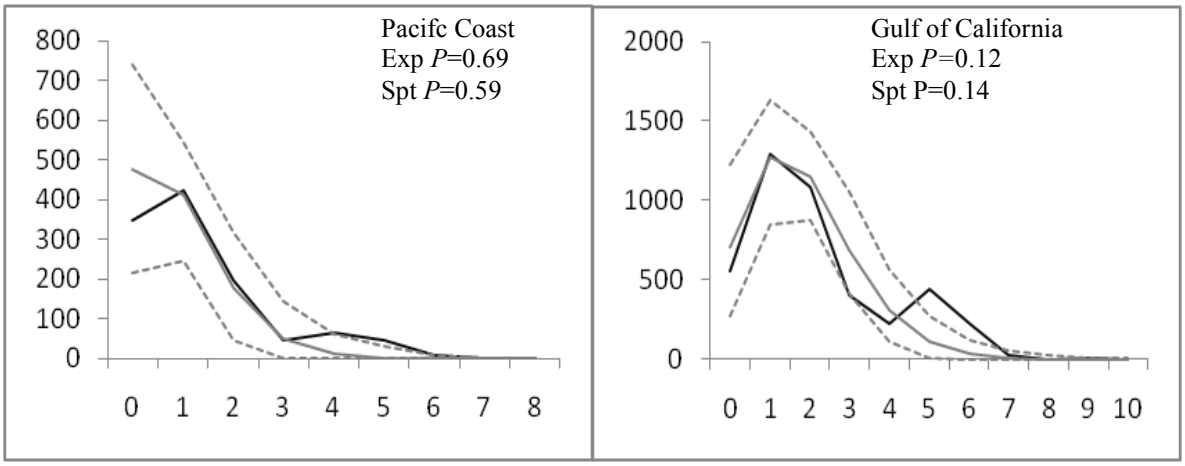

Fig. 4.3. Mismatch distribution of pairwise sequence differences in *Mustelus henlei* from the Pacific Coast and the Gulf of California. Black solid lines are observed frequencies of nucleotide differences between pair of samples, grey solid lines are the expected distribution under a model of demographic expansion, and the grey dashed lines represent the 99% confidence interval of 1,000 simulations. Harpending's Raggedness indices under significant demographic (Exp *P*) and spatial expansion (Spt *P*) models are also provided.

Table 4.5 Population expansion analyses results. Tajima's D, Fu's Fs, τ, corresponding 95% confidence interval, time since expansion ($t=\tau/2\mu$) in million years ago (Mya), P values for model of demographic expansion (Exp P) and spatial expansion (Spt P), for (NP) North Coast, (SP) South Coast, (LG) Lower Gulf, (IG) Islands, (UG) Upper Gulf and (OP) Open Gulf, (PC)Pacific Coast, and (GC) Gulf of California.

	Tajima's D	P	Fu'sFs	P	τ	5%	95%	Time Mya	Exp P	Spt P
NP	-1.01	0.18	-6.96	0.00	0.56	0.00	3.23	695800	0.81	0.79
SP	-1.12	0.13	-2.51	0.01	1.12	0.25	2.18	1401363	0.38	0.37
LG	-1.66	0.03	-5.75	0.00	1.90	0.84	3.37	2377925	0.55	0.58
IG	-0.47	0.37	-3.37	0.02	1.09	0.54	1.96	1367188	0.79	0.49
UG	-0.46	0.38	-1.62	0.23	1.45	0.88	2.20	1806638	0.03	0.06
PC	-1.37	0.06	-6.44	0.00	0.86	0.38	1.39	1074225	0.69	0.59
GC	-1.27	0.08	-8.29	0.01	1.80	1.37	2.37	2255863	0.12	0.14
Mh	-1.33	0.069	-11.26	0.001	1.63	1.23	2.09	2041013	0.097	0.144

exception was the UG that showed a negative but not significant Fu's *Fs* value, and a bimodal mismatch distribution significantly different from expansion expectations. These results suggest a different demographic history in that region. Time since population expansion estimates were similar for all bioregions, ranging from 0.7 to 2.4 Mya, with a slightly more recent estimate of expansion for the Pacific coast (Table 4.5). This suggests a common event that affected population demography in all regions.

4.3.3 High connectivity and low population structure

Significant differences in frequencies were detected among localities in both mitochondrial (Φ_{ST}=0.042, P=0.04) and microsatellite data (F_{ST}=0.017, P<0.0001), but overall levels of structure were low. The divisions of samples in biogeographic regions, localities within bioregions and within localities, revealed significant mtDNA variation among bioregions (Φ_{CT}=0.066, P=0.003), and nuclear variation among localities within bioregions (F_{SC}=0.011, P=0.025) (Table 4.6). Pairwise localities comparisons resulted in low to moderate values of differentiation in mtDNA (Φ_{ST}=0.0000-0.2399) and nuclear data (F_{ST}=0.0000-0.0105), with only two (for mtDNA data) and four (for microsatellite data) comparisons significant out of 55 possible comparisons (Table 4.7). Only the comparison between Popotla (Po) and El Barril (EB) was consistently significant for both marker datasets. In general, values of fixation indices were higher between GC and Pacific Coast localities than between localities within each coast (Table 4.7). On the other hand, results from STRUCTURE suggest a single genetic population in the region, with the most likely maximum number of cluster being one (K=1, posterior probability > 0.99). However, the latter result might have been influenced by the presence of a pattern of isolation by distance in the species (see below).

Table 4.6 *Mustelus henlei* hierarchical analysis of molecular variance (AMOVA), for 13 localities and five bioregions, based on six microsatellites and 60 bp of mitochondrial control region. Significant results are in bold.

	Microsatellites			Mitochondrial Control Region		
	Percentage of variation	Fixation index	P	Percentage of variation	Fixation index	P
Among Bioregions	0	R_{CT}=0.006	0.1916	7.53	**Φ_{CT}=0.066**	**0.004**
Among Localities	1.7	**R_{SC}=0.011**	**0.0254**	0	Φ_{SC}=0.000	0.824
Within Localities	98.3			95.86		
Total		**R_{ST}=0.017**	**<0.0001**		**Φ_{ST}=0.0420**	**0.040**

Table 4.7 Localities pairwise fixation indices. Below diagonal are Φ_{ST} based on mitochondrial DNA. Above diagonal are F_{ST} based on microsatellites. Significant values at 95% confidence level after sequential Bonferroni correction are in bold. Localites Popotla (Po), San Quintin (SQ), Laguna Manueal (LM), San Ignacio (SI), Todos Santos (TS), La Paz (LP), Mulege (ML), El Barril (EB), Bahia de los Angeles (BAN), San Felipe (SF) and Pueto Peñasco (PP).

	Po	SQ	LM	SI	TS	LP	ML	EB	BAN	SF	PP
Po		0.003	0.017	0.066	0.000	0.067	0.060	**0.105**	0.041	0.034	0.037
SQ	0.000		0.003	0.010	0.005	0.023	0.000	0.023	0.000	0.000	0.011
LM	0.033	0.000		0.000	0.000	0.000	0.000	**0.054**	0.000	0.021	0.023
SI	0.000	0.000	0.000		0.024	0.026	0.000	**0.084**	0.011	0.043	0.049
TS	0.023	0.000	0.000	0.000		0.011	0.018	**0.049**	0.000	0.020	0.010
LP	0.046	0.000	0.000	0.000	0.000		0.000	0.029	0.003	0.017	0.017
ML	0.125	0.000	0.000	0.059	0.000	0.000		0.001	0.000	0.000	0.001
EB	**0.239**	**0.158**	0.084	0.095	0.089	0.020	0.020		0.020	0.003	0.019
BAN	0.194	0.127	0.077	0.079	0.097	0.000	0.000	0.000		0.011	0.000
SF	0.130	0.047	0.015	0.005	0.003	0.002	0.000	0.001	0.000		0.006
PP	0.180	0.100	0.086	0.078	0.108	0.007	0.009	0.000	0.000	0.000	

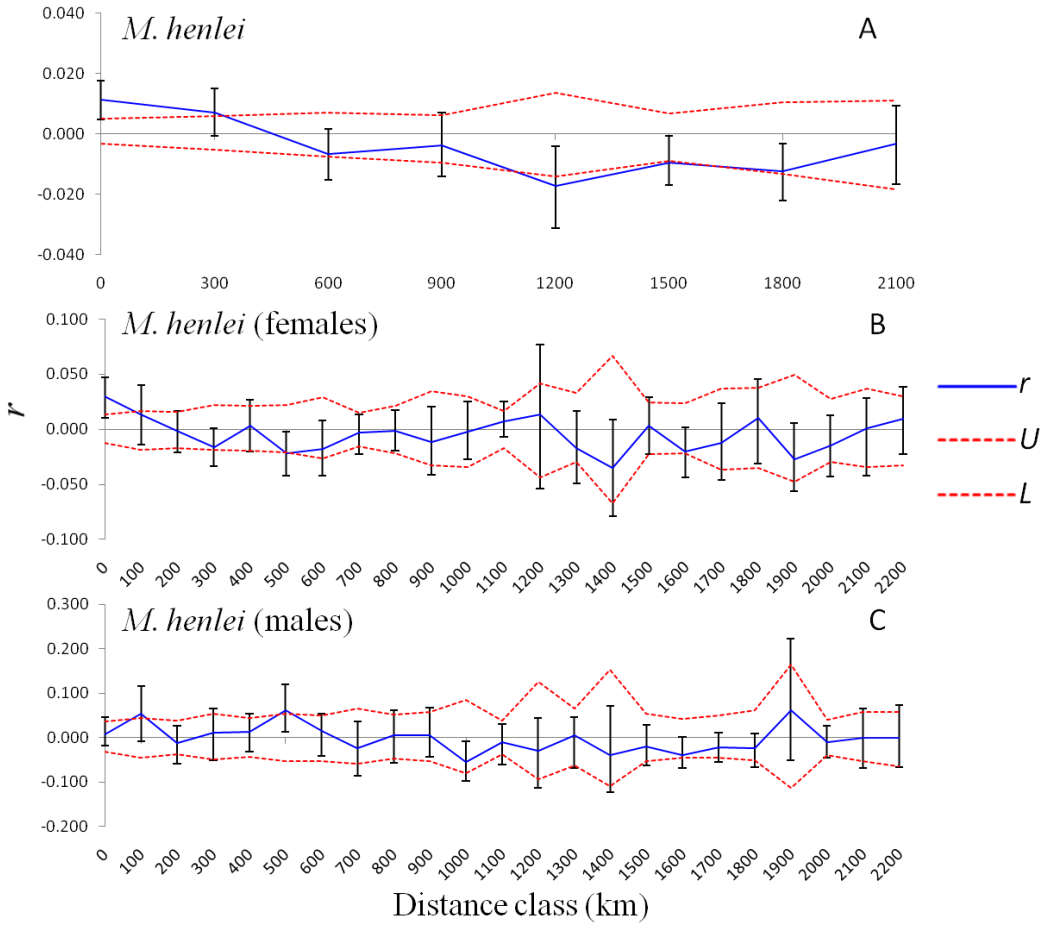

Fig. 4.4. Correlogram plots of the spatial autocorrelation coefficient r (using microsatellite data). Upper (U) and lower (L) bounds at 95% confidence. (A) All samples together. (B) Females. (C) Males.

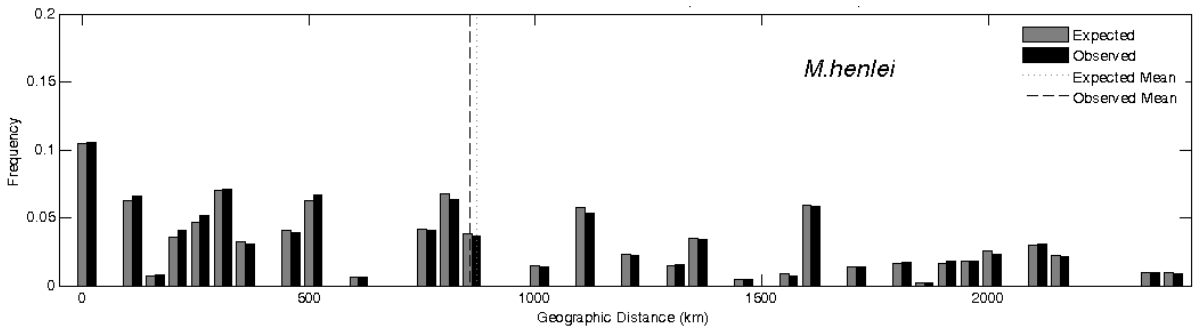

Fig. 4.5 Results for the spatial analysis of shared alleles. Frequency distribution of observed and expected distance between co-occurrences of alleles, with mean observed (856.32km) and expected (870.93km) distance indicated by dashed lines. This indicates the distance in km between copies of the same allele that occurred in different locations, and the average dispersal distance per allele. The *P* values (0.059) are the significance of the difference between values of our data and the expected under panmixia.

Table 4.8. Isolation by ecological and geographic distances. The significance value of the correlation between genetic (mitochondrial DNA mtDNA and microsatellites Mst), geographical and environmental distance for all localities, the Pacific coast (PC) and the Gulf of California (GC). The last four columns are the correlation of genetic and environmental distances controlling by geographical distances. Bold values are for significant correlations.

					Controlling/Geographical Distance			
	mtDNA		Mst		mtDNA		Mst	
	r	*P*	*r*	*P*	*r*	*P*	*r*	*P*
Geographical Distance	0.669	**<0.001**	0.486	**0.003**				
Temperature	0.282	0.138	0.165	0.199	-0.328	0.997	-0.328	0.747
Salinity	0.641	**0.002**	0.423	**0.009**	-0.465	0.606	0.016	0.573
Oxygen Saturation	0.091	0.312	0.145	0.437	0.097	0.217	0.097	0.404
Nutrients Concentration	0.575	**0.007**	0.032	0.116	0.185	0.072	0.018	0.654
Chlorophyll-*a*	0.022	0.799	0.023	0.854	-0.316	0.953	-0.316	0.923
Bathymetry	0.453	**0.017**	0.236	0.063	-0.298	0.064	-0.298	0.078

4.3.4 Isolation by geographic and environmental distances

Mantel tests showed significant correlations between genetic and geographic distances for both the mtDNA (P=0.0001) and microsatellite (P=0.003) datasets. Accordingly, for microsatellites spatial autocorrelation analysis revealed significant positive autocorrelation (i.e. greater-than-random genetic similarity) among individuals sampled at the same locality (r=0.011, P= 0.001) and in different localities up to 300 Km (r=0.006, P=0.029) (Fig. 4.4A). These results differ slightly from those of the SASHA analysis, which suggests overall reduced geographic structure (Dg=14.61, P=0.059; $Dcdf$=0.016, P=0.029) and significant gene flow occurring at scales of ~800km (Fig. 4.5). The analysis of ecological isolation showed that geographic distance and salinity have significant effects in the genetic structure of populations, a result observed for both markers. Nutrient concentration and bathymetry were significant only for the variation observed in the less resolving mtDNA dataset. However, none of these environmental variables were correlated with genetic structure after correcting for geographical distances (Table 4.8).

4.3.5 Sex-biased dispersal

Spatial autocorrelation correlograms were different for the two sexes. While females show positive significant correlation among individuals from the same locality (r=0.03, P=0.001), and an x-interception at 190km (Fig. 4.4B), males' correlation did not depart from the random distribution of genotypes (Fig. 4.4C), a result supporting more restricted dispersal of females. Moreover, mean AIc were negative for males (-0.092) and positive for females (0.043) (Fig. 4.6 and 4.7), also suggesting male-biased dispersal; nevertheless, mean AIc values were not significantly different between sexes (Z=-0.722, P=0.470).

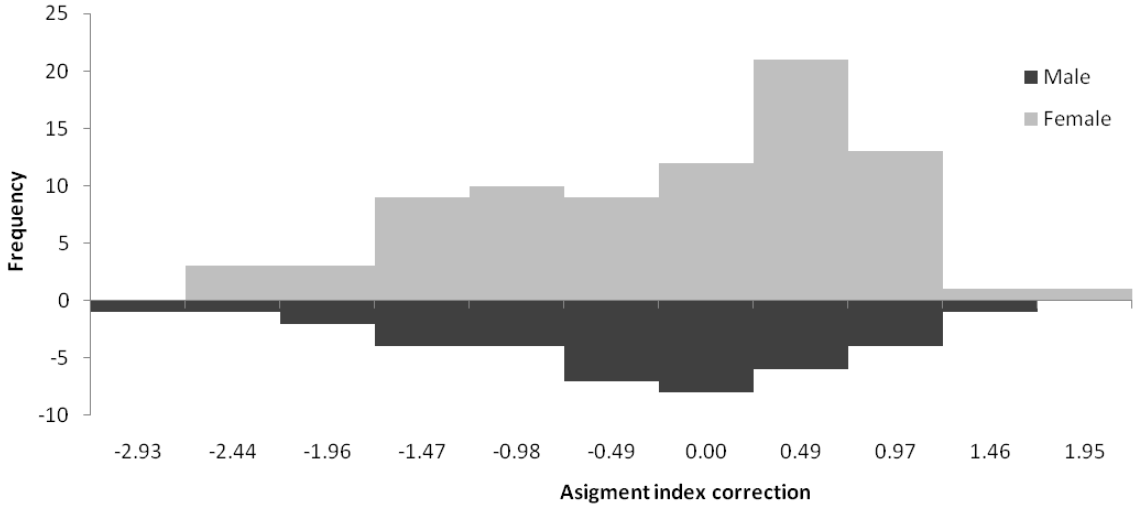

Fig. 4.6 Frequency distribution of assignment index correction values (*Alc*). This index measures the deviation of individuals from the mean population assignment probability. High dispersal individuals will show negative values, whereas those with positive values would be more likely to being born locally.

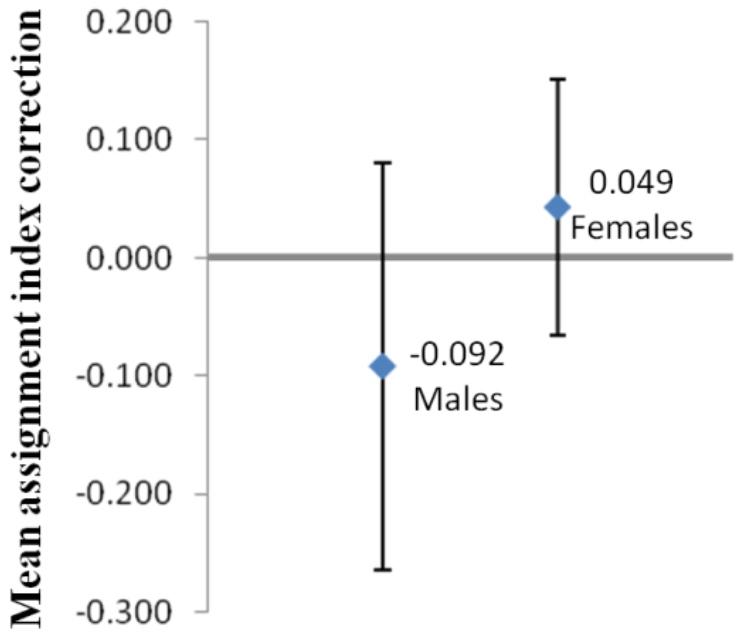

Fig. 4.7. Mean and standard deviation of assignment index correction, measuring the deviation of individuals from the mean population assignment probability. High dispersal individuals will show negative values.

4.4 Discussion

Our population genetics and phylogeographic analyses support the hypothesis of a relatively well-connected metapopulation of the brown smooth-hound, *Mustelus henlei,* in the Gulf of California (GC) and the Pacific Coast of Baja California Peninsula (BCP), in Mexico. Despite being composed of subpopulations spatially segregated in different bioregions, we found no evidence for marked genetic structure associated with historical biogeographic barriers, or for strong ecological isolation associated with the complex environmental discontinuities of the region, supporting the suggestion of high dispersal capability in *M. henlei*. Nonetheless, our results also strongly support a pattern of isolation by geographic distance among localities (especially between the GC and the BCP) and the hypothesis that, despite high dispersal potential, some individuals tend to remain and/or reproduce in their natal area, especially females. Our results have important implications for conservation management programs of the heavily exploited *M. henlei* fishery of the Gulf of California, and also for other coastal sharks with high dispersal potential.

4.4.1 Species identification and population history

The identification of *Mustelus* species found in the GC and BCP region is hampered by their very conserved morphology and small number of diagnostic characters (Pérez Jiménez *et al.* 2005). Although this could result in problems for genetic studies of connectivity, all of our study samples were grouped in a closely related lineage that includes a specimen with known identity (Perez-Jimenez et al. 2005), confirming our field identification. In agreement with the low morphological divergence among *Mustelus* species from the region, the sequence divergence between congenerics was also very low (ranging between 2.6 and 5.6%).

A shallow phylogeographic pattern is evident in the *M. henlei* mtDNA genealogy, which shows a group of closely related haplotypes with no geographic sorting (Fig. 4.2). A strong signal of population expansion of Pleistocene age was recovered for the species based on both mismatch analysis (Fig. 4.3 and Table 4.5). We propose that historical demographic growth in this species was associated with the last stages of formation of the GC during the early Pleistocene (Murphy & Aguirre-Leon 2002), which would have provided new habitat and opportunities for both demographic and range expansions. It is thought that population demography of several species of elasmobranchs were influenced by Pleistocene glacial cycles (Duncan *et al.* 2006; Chevolot *et al.* 2006; Schultz *et al.* 2008; Corrigan & Beheregaray in preparation). These past climatic changes are known to have caused significant alterations in marine ecosystems, including the creation of vast areas of new habitat (Hewitt 2000). The strong signal of spatial expansion along with a slightly more recent demographic expansion in the PC supports the idea of colonisation events from the GC to the Pacific. It has been suggested that the last glacial cycling had weaker effects inside the GC, allowing it to function as a warm refuge for temperate species during glaciation periods and as a centre of origin during interglacial periods (Jacobs *et al.* 2004).

The MIGRATE-N analysis detected strong gene flow between all regions, including those separated by the BCP and by 1,500 km. This could be interpreted as long distance migration in the species. Nevertheless, gene flow estimated using coalescent-based methods is strongly influenced by historical events (Kuhner 2009; Beerli 2009). Therefore, the high gene flow between Pacific coast and GC regions detected with MIGRATE-N, likely reflects historical rather than modern high connectivity. Historical connectivity between the Pacific coast and GC could be promoted by the presence of a seaway across the BCP around 3 Mya (Murphy & Aguirre-Leon 2002); however our data suggest a more recent process driving genetic structure in the species. It sees more likely that this historical connectivity is the fingerprint of a recent

colonization (~1Mya), as suggested by the mismatch distribution analysis and summary statistics. Thus, *M. henlei* appears to have a young age in the region of the GC and BCP, and shows no phylogeographic breaks related to the region's vicariant biogeographic history.

4.4.2 Contemporary genetic structure

The BCP has been reported as an important biogeographic barrier for several species of fish, invertebrates, mammals (Bernardi *et al.* 2003; Jacobs *et al.* 2004) and elasmobranchs (Sandoval-Castillo *et al.* 2004; Sandoval-Castillo & Rocha-Olivares 2011; Sandoval-Castillo and Beheregaray unpublished data). However, this barrier may not be equally efficient for epibenthic sharks with high dispersal capabilities, such as *M. henlei*. The species showed low levels of population genetic structure over the study region, both from a historical and contemporary perspective, which could be explained by its potentially high capacity to disperse. In some cases, this life history attribute was used to explain reduced genetic structure observed in coastal sharks over several hundred kilometres (Keeney *et al.* 2005; Mendonça *et al.* 2009). In addition, tag-based estimates of dispersal over 1,000 km have been reported for *Mustelus lenticulatus* in New Zealand (Francis 1988), suggesting a high migration capability in the genus. Despite the high dispersal potential of some congenerics, genetic structure over relatively short distances (<500 km) has been reported for several *Mustelus* species, including *M. antarcticus* from Australia (Gardner & Ward 1998), *M. manazo* from Japan (Yamaguchi *et al.* 2000; Chen *et al.* 2001), and *M. schmitti* from Uruguay (Pereyra *et al.* 2010).

A growing number of studies from other regions have reported that environmental discontinuities, including those related to fine-scale oceanographic processes, provide better explanations for patterns of population structure in coastal species than biogeographic history

(Galindo *et al.* 2006; Banks et al. 2007; Mendez *et al.* 2010; White *et al.* 2010). In the GC, studies have reported associations between the distribution of zooplankton (Brinton & Townsend 1980), phytoplankton (Round 1967) and bony fishes (Walker 1960; Thomson *et al.* 1979; Peguero-Icaza *et al.* 2008) with the Gulf's bioregions and their unique oceanographic patterns. The population genetic structure of several species of bony fishes has also been linked to these bioregions (Riginos 2005). Here, this theory was statistically assessed in an analysis of ecological isolation that compares data from seven key oceanographic variables with information about population genetic structure (Table 4.7). None of the correlations were significant after corrections for geographical distances, suggesting a more important role of geography rather than oceanography as a driver of population structure in *M. henlei*.

4.4.3 Isolation by distance and sex biased dispersal in M. henlei

The overall low genetic structure reported in *M. henlei* is better explained by isolation by geographic distance between populations, a result statistically supported by Mantel tests for both nuclear and mtDNA datasets (Table 4.8), and by spatial autocorrelation analysis (Fig. 4.4). Philopatric behaviour can have important impacts on the connectivity of populations, and it has been reported for several species of marine organisms with high dispersal capability (FitzSimmons *et al.* 1997; Lyrholm *et al.* 1999; Weimerskirch & Wilson 2000; Robichaud and Rose, 2001; Möller & Beheregaray 2004). For many species, females show stronger philopatry than males. In fact, for several species of sharks, local genetic structure has been associated to female philopatry to their natal area (Hueter *et al.* 2005). In addition, philopatry is a double selective behaviour; organisms "select" a geographic area with specific environmental conditions, where they are selected favourably. Therefore, in areas with structurally complex habitats, such as the GC-BPC region, the probability for philopatric behaviour is higher than in less complex ecosystems (Stacey & Ligon 1991). Also, since

coastal areas are environmentally more structured than the pelagic realm, it has been suggested that coastal shark species such as *M. henlei,* should show stronger philopatric behaviour than pelagic sharks (Hueter *et al.* 2005).

There is not tagging/tracking evidence of *M. henlei* female philopatry, but fisheries data in the GC show consistent interannual aggregations and existence of nursery sites; suggesting some geographic fidelity of the species (Pérez-Jiménez & Sosa-Nishizaki 2008; Salomón-Aguilar *et al.* 2009). The overall *M. henlei* genetic structure could thus be in part explained by some degree of female philopatric behaviour, whereas connectivity along the region could be mostly due to male dispersal. In fact, results from spatial autocorrelation shows different migration capabilities between sexes. Whilst females showed greater-than-random genetic similarity among individuals sampled in the same locality, this was not observed for males. In addition, the mean *AIc* value was negative for males and positive for females, suggesting that females are the philopatric sex (Fig. 4.6 and 4.7), although this difference was not significant. However, the latter could have been compromised by low power due to the small number of male samples (38 males compared to 82 females) and a weak sex-bias signal (Mossman & Waser 1999). Additional support for female philopatry comes from comparing levels of structure between mitochondrial and nuclear markers. If only modes of inheritance are taken into account (i.e. no differences in operational sex ratios and effective size of genomes considered), mitochondrial markers should show higher structure than nuclear markers under a scenario of dispersal mediated by males (Goudet *et al.* 2002; Prugnolle & de Meeus 2002). On average, mtDNA data revealed stronger differentiation in pairwise comparisons than microsatellites. Altogether, results from analyses of spatial autocorrelation, corrected assignment index and different signal between mitochondrial and nuclear markers support the proposal of male-biased dispersal in *M. henlei*.

4.4.4 Conservation implications for a highly exploited shark species

Despite that, historically, the Mexican elasmobranch fishery has been one of the most important around the world (FAO 2010), national regulation regarding elasmobranch management and conservation (NOM-29-PEC) was only recently implemented (2007). While this represents a significant improvement to shark management in Mexico, the legislation lacks species-specific guidelines and does not account for stock structure, geographic variation in population demography, or connectivity patterns. There are several examples of local population depletion and extirpation for elasmobranchs (Bonfil 1994; Field *et al.* 2009; Dulvy & Forrest 2010), underlining the importance of considering population structure in management policies.

Migrants have important demographic and ecological implications for a metapopulation (such as maintaining a stable effective population size and genetic variability; Tallmon *et al.* 2004). Isolation of populations with naturally high connectivity by habitat fragmentation therefore increases the extirpation and extinction probability of the species (Opdam *et al.* 2002). Reduced levels of connectivity among wild populations increases their susceptibility to habitat destruction, regional overfishing and climate change, because natural restocking of individuals and genes is not effectively occurring (Frankham *et al.* 2009). Therefore, for the correct management of *M. henlei*, it is important that conservation management initiatives preserve connectivity between its populations. Furthermore, even low levels of philopatric behaviour has substantial implications for shark conservation (Hueter *et al.* 2005). Shark fidelity to specific nursery areas increases vulnerability to human impacts. In general, shark nursery areas are located in high productivity ecosystems that are highly susceptible to habitat destruction (e.g. mangroves, coastal lagoons, estuaries) and functional deterioration. In addition, the seasonal congregation of several sharks in these areas (pregnant females included) attracts fishing activities and make populations more susceptible to overexploitation

(Hueter *et al.* 2005; Heupel *et al.* 2007; Kinney & Simpfendorfer 2009). Nursery areas are not stand-alone systems; they often depend of recruitment from other regions to maintain stable breeding populations (Kinney & Simpfendorfer 2009). Therefore, the creation of marine reserves that protect the integrity of nursery areas and their connectivity is primordial for the conservation of philopatric species like *M. henlei*. Inside the GC, several shark nursery areas, that are utilised by various species including *M. henlei* (Salomón-Aguilar *et al.* 2009), have been described. Unfortunately, most of these areas are exposed to intensive fishing regimes and habitat destruction (Salomón-Aguilar *et al.* 2009), stressing the need of a system of marine protected areas (MPAs) in the region. The few MPAs in the GC were designed based on local diversity levels (i.e. species diversty); however a network of MPAs that considers breeding sites and patterns of connectivity between them would better protect ecological processes that maintain diversity on broader temporal and spatial scales.

Chapter V Conclusions

5.1 Comparative Phylogeography, Seascape and Population Genetics.

Spatial patterns of genetic variability within a species can provide information about present and past connectivity among populations (Avise 2004). The analysis of phylogeographic and population genetic structure across multiple taxa is actually even more informative about major barriers to gene flow and the mechanisms responsible for genetic subdivision (Avise 2000; Kuo & Avise 2005).

The purpose of this concluding chapter is to integrate the major outcomes of the thesis. The study aimed at investigating population genetic structure of elasmobranchs in the context of their mobility, and historical and contemporary conditions of the Gulf of California (GC). It represents the first comparative genetic analysis in elasmobranchs from this iconic marine ecosystem. It sheds light on factors influencing genetic structure and speciation in elasmobranchs, it reports on previously unsuspected cryptic species and on well-delineated populations that should be managed separately, and it discloses key information about species biology.

5.1.1 High mobility vs Low Mobility

An aim of this work was to compare the effects of multiple barriers to gene flow in species with different mobility capacity. Event though there are no comparative tagging/recapture data to assess differences in mobility in *Rhinobatos, Squatina* and *Mustelus* species from the Gulf of California, Compagno (1990) and Musick *et al.* (2004) considered that vagility is

lower in demersal (i.e. *Rhinobatos* and *Squatina*) than in epibenthic species (i.e. *Mustelus*). The dispersal potential has been associated with evolutionary potential (Rosenblatt & Waples 1986; Chenoweth *et al.* 1998; Musick *et al.* 2004; Callens *et al.* 2011). High mobile species should maintain gene flow over larger geographic scales. It is expected that low mobile species show stronger genetic structure than high mobile species over the same geographic area. While population genetic studies in elasmobranchs are becoming more common, the different methods used and the range of biogeographic scenarios investigated make comparative analysis challenging. However, some general trends are becoming apparent. For instance, it appears that large pelagic sharks show low genetic structure at large geographic scales (e.g. > 5,000 km) and no structure over smaller scales (Pardini *et al.* 2001; Schrey & Heist 2003; Hoelzel *et al.* 2006; Castro *et al.* 2007). No apparent structure in scales of up to 500 km is common in epibenthic elasmobranchs (Heist *et al.* 1996; Chabot & Allen 2009; Mendonça *et al.* 2011). On the other hand, strong genetic structure at scales of less than 500km seems common in benthic sharks and rays (Dudgeon *et al.* 2009; Plank *et al.* 2010; Griffiths *et al.* 2010). There are several exceptions for these patterns, and these are potentially related to particular geological or ecological conditions of the study region (Schrey & Heist 2003; Lewallen *et al.* 2007; Schluessel *et al.* 2010; Sandoval-Castillo & Rocha-Olivares 2011). Nonetheless, the result of this thesis provides strong support to a negative correlation between vagility and genetic differentiation. While putatively low mobile taxa show strong genetic structure, including reproductively isolated populations (Chapters 2 and 3), high connectivity was found in the presumably high dispersing species (Chapter 4). This suggests a number of barriers to gene flow in the GC with stronger effects in low vagility elasmobranchs.

5.1.2 Historical Geological Barriers

The geological history of the GC and the Baja California Peninsula (BCP) has been active and complex (Chapter 1) and three main vicariant/dispersal events have been proposed for this region. Brusca (1973a) proposed that during the end of last Pleistocene glaciation, organisms from the GC recolonised the Pacific Coast of BCP when the temperature in the cape region was still cold. On the other hand, several authors have suggested that the closure of two seaways (one in the south and the other in mid BCP), interrupted gene flow in marine organisms from both coasts of BCP, resulting in sister allopatric populations or species inside and outside the Gulf (Walker 1960; Bernardi *et al.* 2003; Jacobs *et al.* 2004; Sandoval-Castillo *et al.* 2004). The glaciations and the south seaway theories require a warmer temperature in the cape region; thus, they are not only vicariant-based (see Bernardi *et al.* 2003 and Jacobs *et al.* 2004), but the closure of mid peninsula seaway does not require the physiological barrier.

In this work, strong genetic differentiation between PC and GC was found in *Rhinobatos productus* (Chapter 2) and *Squatina californica* (Chapter 3). The phylogenetic analysis in *Rhinobatos productus* clustered all samples from the PC and the central part of the GC in one lineage; for *Squatina californica* the highest values of historical gene flow were found between PC and the north part of the GC. Under the hypotheses of the southern seaway or the post-glacial recolonization it should be expected that patterns of structure would follow a stepping-stone model; however this model was rejected for both taxa. Overall, the results from chapters 2 and 3 support the mid BCP seaway theory, and also highlight the importance of the BCP as a vicariant barrier. However, these should not belittle the significance of oceanographic barriers in the cape region.

5.1.3 The Ecological Barriers

The GC has a very complex oceanography, with high geographic and temporal variation, that divides the sea in four bioregions: Upper Gulf (UG), Islands (IG), Lower Gulf (LG) and Open Gulf (OG) (Chapter 1). Oceanographic differences between regions could create ecological gradients and different niches, which over time would promote ecological isolation between populations. In this thesis, phylogenetic and population genetic analyses show that *R. productus* and *Squatina californica* from the GC have two highly congruent phylogeographic breaks that suggest common barriers to gene flow. Seascape analyses provided strong support for ecological isolation related to GC bioregions in both species. In addition, similar results of isolation by ecological distances in both taxa suggest that ecological barriers are mainly related with oxygen saturation, bathymetry and nutrients concentration. The first two have direct and more evident effects in elasmobranchs, but the effect of the latter is less evident. Oxygen concentration has important physiological implications in the mobility and metabolism of elasmobranchs (Heithaus *et al.* 2009; Froeschke *et al.* 2010) and bathymetry has been reported as an important barrier for dispersal of coastal sharks (Musick *et al.* 2004; Schultz *et al.* 2008; Speed *et al.* 2010). On the other hand, nutrients concentration has an important effect in the primary productivity, which in turn affects the abundance and diversity of herbivores (Korpinen *et al.* 2010; Murdock *et al.* 2011).

Genetic structure due to oceanographic factors has been reported in several high dispersal species (Banks *et al.* 2007; White *et al.* 2010; Mendez *et al.* 2010; Möller *et al.* 2011). It was expected that the oceanographic variables strongly affecting genetic structure in the two low mobility species would also affect connectivity in *M. helei*. However, this species showed moderate to high connectivity between localities (Chapter 4), suggesting that the selective pressure of the oceanographic parameters tested is weaker in high mobile elasmobranchs. It also suggests different ecological requirements for demersal and epibenthic elasmobranchs.

Even though both types of organisms have strong interactions with the benthos, demersal species spend longer time in the bottom of the sea and tend to be less active (Compagno 1990). In general, bottom waters have lower oxygen concentration and demersal organisms should therefore be adapted to such condition (Levin 2003; Levin *et al.* 2009). On the other hand, epibenthic sharks have higher activity and mobility, and are perhaps actively avoiding bathymetric barriers.

The PC of Baja California is divided in north and south regions in the midpoint of the peninsula (Punta Eugenia). This interface is characterized by abrupt temperature differences and the presence of eddies produced by the confluences of a warm northward and a cold southward current (Hewitt 1981). This area has been considered an important biogeographic (Briggs 1974a) and phylogeographic break (Bernardi *et al.* 2003). However, none of the species in this study show genetic structure related with this particular break. This could be explained by the irregularity of the barrier. The interface in Punta Eugenia is not constant throughout the year; the currents confluence has seasonal, inter-annual, and especially inter-decadal variations in strength and location (Durazo & Baumgartner 2002). These variations could make the barrier permeable over a few years, allowing sufficient migration to hamper genetic differentiation between northern and southern organisms.

5.2 Taxonomy and conservation

5.2.1 Cryptic species and taxonomy

This thesis has several implications for elasmobranch taxonomy. *Rhinobatos productus* was found to be a complex of species (Chapter 2). Here evidence of four lineages that are entirely or partially reproductively isolated by ecological factors was presented using genetic and

ecological data. This work provides support for a cryptic incipient species of angel shark in the GC (Chapter 3). Here, strong evidence was presented for historical and contemporary reproductive isolation between organisms from the PC and GC. These findings add to an increasing number of cryptic species recognised in elasmobranchs (e.g. Quattro *et al.* 2006; Castilho *et al.* 2007; Corrigan *et al.* 2008; Ward *et al.* 2008; Smith *et al.* 2008; Richards *et al.* 2009; Griffiths *et al.* 2010; Sandoval-Castillo & Rocha-Olivares 2011; Straube *et al.* 2011), supporting the idea that the diversity of sharks and rays is underestimated.

The radiation of the *R. productos* complex appears to have been recent, around 1.5 Mya and influenced by the final conformation of the GC. A combination of allopatric, parapatric and sympatric population arrangements influenced by ecological divergence appear as the most likely explanation for the rapid diversification of this complex. In *S. californica* the divergence appears as mostly allopatric and more recent, but also influenced by ecological isolation. These results overturn the supremacy of the allopatric speciation model (Coyne & Orr 2004) and the traditional notion about low evolutionary rate in elasmobranchs. Most families of elasmobranchs are thought to have appeared by the end of the Cretaceous, and contemporary species are considered to have descended without major adaptive changes (Underwood 2006; Kriwet *et al.* 2009). Nonetheless, several of the reported cryptic species of elasmobranchs appeared to have diverged in the last 3 Mya (Quattro *et al.* 2006; Castilho *et al.* 2007; Corrigan *et al.* 2008; Ward *et al.* 2008; Smith *et al.* 2008; Richards *et al.* 2009; Griffiths *et al.* 2010; Sandoval-Castillo & Rocha-Olivares 2011; Straube *et al.* 2011), including a case of rapid evolutionary radiation in the shark family Orectolobidae (Corrigan & Beheregaray 2009).

5.2.2 Conservation and management

This work originally reports on 4 distinct management units (MUs) in Californian angel shark (Chapter 3) and four evolutionarily independent units (ESUs) in the *R. productus* complex (Chapter 2). Remarkably, these units are narrow endemics with very similar distributions strongly delimited by oceanographic regions. The restricted distribution of these population units, combined with the inherently low fecundity and late sexual maturation of elasmobranchs, makes them extremely susceptible to overfishing, habitat degradation and climatic change, supporting the view they should be managed separately. In addition, low but significant population structure was found in *M. henlei* (Chapter 4). Different analyses suggest male-biased dispersal and considerable female phylopatry. The management plan for this species should consider the connectivity between localities outside and inside the Gulf and also areas to which females might be philopatric (i.e. breeding grounds).

These results are extremely useful for the conservation of these elasmobranchs and also for conservation management in the GC. This gulf is one of the most productive and diverse in the world but is also under substantial anthropogenic influence (Enríquez-Andrade *et al.* 2005). Marine protected areas (MPAs) are efficient management tools to protect marine diversity and the processes that maintain it (Lubchenco *et al.* 2003; Pomeroy *et al.* 2005). Despite ongoing debates about how much habitat should be protected to preserve biodiversity and connectivity (Cabeza & Moilanen 2001; Halpern 2003), the emergence of new technology and methods allow the design of efficient MPAs on the basis of integrative information and modelling (Palumbi *et al.* 2003; Leathwick *et al.* 2008). This thesis provides key information about conservation units relevant for the creation of a network of MPAs that integrate mechanisms to preserve genetic diversity and connectivity. This information can be summarized in a pattern in which distinct conservation units of apex predators are found in

different marine bioregions. Thus, the absolute minimum number of MPAs required to conserve biodiversity in these elasmobranchs would be one MPA per bioregion, and that should be of a sufficient size to cover the major breeding grounds of guitarfishes and angel sharks. These MPAs should be classified as sanctuary zones (i.e. no take zones, no activities from recreational or commercial fishermen allowed) to allow these narrow endemics to replenish their local populations. Ideally however, more than one MPA per bioregion would provide a more efficient tool by ensuring population connectivity between areas and perhaps the maintenance of metapopulations with appropriate levels of adaptive variation.

5.3 Future research

While this thesis provides important contributions to our understanding of the evolutionary history of elasmobranchs in the GC and the speciation process in the sea, it also identifies a number of future research avenues.

First, given the number of cryptic species suggested it is important that comparative morphological, demographic and physiological analyses be conducted. Meticulous comparative studies are necessary not only to identify diagnostic characters for species descriptions (i.e. morphology-based taxonomy), but also to identify possible morphological, physiological or behavioural adaptations (i.e. ecotypes) that evolved in ecological isolation. The latter should be done in combination with next generation sequencing projects capable of disclosing functional genomic variation and its spatial distribution. The identification of adaptive traits in elasmobranchs, both phenotypic and genetic, would allow a better understanding of the process of adaptive radiation in guitarfishes, as well as the resilience of species to climatic changes or other anthropogenic influences.

This study also indicates the need for further research assessing female phylopatry over fine spatial scales using tagging/recapture data. Such studies should help determining phylopatric behaviour, not only in female *M. henlei*, but also understand habitat preferences and habitat use in divergent lineages of *S. californica* and *R. productus*. Determining these preferences would allow us to better understand the mechanisms of ecological speciation that have apparently driven the evolution of reproductive isolation in these elasmobranchs.

Chapter VI Reference material

Aceves-Medina G, Jiménez-Rosenberg SPA, Hinojosa-Medina A, Funes-Rodríguez R, Saldierna-Martínez RJ, Smith PE (2004) Fish larvae assemblages in the Gulf of California. *Journal of Fish Biology* **65**, 832-847.

Almany G, Connolly S, Heath D, Hogan J, Jones G, McCook L, Mills M, Pressey R, Williamson D (2009) Connectivity, biodiversity conservation and the design of marine reserve networks for coral reefs. *Coral Reefs* **28**, 339-351.

Alvarez-Borreo S (1983) Gulf of California. In: *Estuaries and enclosed seas* (ed. Ketchum B). Elsevier.

Antao T, Beaumont MA (2011) Mcheza: A workbench to detect selection using dominant markers. *Bioinformatics*.

Applegate SP, Soltelo-Macias F, Espinosa-Arrubarrena L (1993) An overview of Mexican shark fisheries, with suggestions for shark conservation in Mexico, pp. 31-37. NOAA Technical Report.

Arnason U, Gullberg A, Janke A (2001) Molecular phylogenetics of gnathostomous (jawed) fishes: old bones, new cartilage. *Zoologica Scripta* **30**, 249-255.

Avise JC (1995) Mitochondrial DNA Polymorphism and a Connection Between Genetics and Demography of Relevance to Conservation. *Conservation Biology* **9**, 686-690.

Avise JC (2000) *Phylogeography: The History and Formation of Species* Harvard University Press.

Avise JC (2004) *Molecular markers, natural history and evolution* Chapman & Hall.

Avise JC (2009) Phylogeography: retrospect and prospect. *Journal of Biogeography* **36**, 3-15.

Banks SC, Ling SD, Johnson CR, Piggott MP, Williamson JE, Beheregaray LB (2010) Genetic structure of a recent climate change-driven range extension. *Molecular Ecology* **19**, 2011-2024.

Banks SC, Piggott MP, Williamson JE, Bové U, Holbrook NJ, Beheregaray LB (2007) Oceanic Variability and Coastal Topography Shape Genetic Structure in a Long-Dispersing Sea Urchin. *Ecology* **88**, 3055-3064.

Barraclough TG, Savolainen V (2001) Evolutionary rates and species diversity in flowering plants. *Evolution* **55**, 677-683.

Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology* **13**, 969-980.

Becks L, Ellner SP, Jones LE, Hairston Jr NG (2010) Reduction of adaptive genetic diversity radically alters eco-evolutionary community dynamics. *Ecology Letters* **13**, 989-997.

Beerli P (2006) Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. *Bioinformatics* **22**, 341-345.

Beerli P (2009) How to use Migrate or why are Markov chain Monte Carlo programs difficult to use? In: *Population genetics for animal conservation* (eds. Bertorelle G, Bruford MW, Hauffe HC, Rizzoli A, Vernesi C), pp. 42-74. Cambridge University Press, New York.

Beerli P (2010) Tutorial: Comparison of gene flow models using Bayes Factors.

Beerli P, Palczewski M (2010) Unified Framework to Evaluate Panmixia and Migration Direction Among Multiple Sampling Locations. *Genetics* **185**, 313-326.

Beheregaray LB (2008) Twenty years of phylogeography: the state of the field and the challenges for the Southern Hemisphere. *Molecular Ecology* **17**, 3754-3774.

Beheregaray LB, Sunnucks P (2001) Fine-scale genetic structure, estuarine colonization and incipient speciation in the marine silverside fish *Odontesthes argentinensis*. *Molecular Ecology* **10**, 2849-2866.

Bernardi G, Findley L, Rocha-Olivares A (2003) Vicariance and dispersal across Baja California in disjunct marine fish populations. *Evolution* **57**, 1599-1609.

Bernardi G, Powers D (1992) Molecular Phylogeny of the Prickly Shark, *Echinorhinus cookei*, Based on a Nuclear (18s rRNA) and a Mitochondrial (Cytochrome b) Gene. *Molecular Phylogenetics and Evolution* **1**, 161-167.

Bierne N, Welch J, Loire E, Bonhomme F, David P (2011) The coupling hypothesis: why genome scans may fail to map local adaptation genes. *Molecular Ecology* **20**, 2044-2072.

Bizzarro JJ, Smith WD, Márquez-Farías JF, Tyminski J, Hueter RE (2009) Temporal variation in the artisanal elasmobranch fishery of Sonora, Mexico. *Fisheries Research* **97**, 103-117.

Bonfil R (1994) *Overview of World Elasmobranch Fisheries* FAO, Rome.

Briggs JC (1974a) *Marine zoogeography* McGraw-Hill.

Briggs JC (1974b) Operation of Zoogeographic Barriers. *Systematic Zoology* **23**, 248-256.

Brinton E, Townsend A (1980) Euphausiids in the Gulf of California The 1957 Cruises, pp. 211-236. CalCOFI.

Brusca R (1973a) *Common intertidal invertebrates of the Gulf fo California* University of Arizona Press, Tucson.

Brusca RC (1973b) *A handbook to the common intertidal invertebrates of the Gulf of California* The Univ. of Arizona Pr.

Cabeza M, Moilanen A (2001) Design of reserve networks and the persistence of biodiversity. *Trends in Ecology & Evolution* **16**, 242-248.

Cailliet G, Musick J, Simpfendorfer CA, Stevens JD (2005) Ecology and Life History Characteristics of Chondrichthyan Fish. In: *Sharks, rays and chimaeras: the status of the Chondrichthyan fishes : status survey*

(ed. Fowler SL). IUCN.

Callens TOM, Galbusera P, Matthysen E, Durand EY, Githiru M, Huyghe JR, Lens LUC (2011) Genetic signature of population fragmentation varies with mobility in seven bird species of a fragmented Kenyan cloud forest. *Molecular Ecology* **20**, 1829-1844.

Camhi M, Fowler S, Musick J, Brautigam A, Fordham S (1998) *Sharks and their relatives: ecology and conservation* World Conservatio Union, Gland.

Camhi M, Pikitch EK, Babcock EA (2008) *Sharks of the open ocean: biology, fisheries and conservation* Blackwell Science.

Camhi M, Valenti S, Fordham S, Fowler S, Gibson C (2009) The conservation status of pelagic sharks and rays, p. 78. IUCN Shark Specialist Group, Newbury, UK.

Campbell D, Bernatchez L (2004) Generic Scan Using AFLP Markers as a Means to Assess the Role of Directional Selection in the Divergence of Sympatric Whitefish Ecotypes. *Molecular Biology and Evolution* **21**, 945-956.

Campos B, Fish M, Jones G, Riley R, Allen P, Klimley P, Cech J, Kelly J (2009) Movements of brown smoothhounds, (*Mustelus henlei*), in Tomales Bay, California. *Environmental Biology of Fishes* **85**, 3-13.

Cappetta H (2004) *Chondrichthyes: Mesozoic and Cenozoic Elasmobranchii* Pfeil.

Cappetta H, Ginter M, Hampe O (1987) *Chondrichthyes II: Mesozoic and Cenozoic Elasmobranchii* Gustav Fischer Verlag, Stuttgart.

Casey JM, Myers RA (1998) Near extinction of a large, widely distributed fish. *Science* **281**, 690-692.

Castilho R, Freitas M, Silva G, Fernandez-Carvalho J, Coelho R (2007) Morphological and mitochondrial DNA divergence validates blackmouth, *Galeus melastomus*, and Atlantic sawtail catsharks, *Galeus atlanticus*, as separate species. *Journal of Fish Biology* **70**, 346-358.

Castillo-Geniz JL, Marquez-Farias JF, Cruz MCRdl, Cortes E, Prado ACd (1998) The Mexican artisanal shark fishery in the Gulf of Mexico: towards a regulated fishery. *Marine and Freshwater Research* **49**, 611-620.

Castro-Aguirre JL, Balart EF, Arvizu-Martinez J (1995) Contribucion al conocimiento del origen y distribucion de la ictiofauna del Golfo de California, Mexico. *Hidrobiologica* **5**, 57-73.

Castro-Aguirre JL, Pérez HE (1996) *Catálogo sistemático de las rayas y especies afines de México: Chondrichthyes:Elasmobranchii:Rajiformes:Batoideiomorpha* Universidad Nacional Autónoma de México.

Castro ALF, Stewart BS, Wilson SG, Hueter RE, Meekan MG, Motta PJ, Bowen BW, Karl SA (2007) Population genetic structure of Earth's largest fish, the whale shark (*Rhincodon typus*). *Molecular Ecology* **16**, 5183-5192.

Cavalcanti M (2007) A Phylogenetic Supertree of the Hammerhead Sharks (Carcharhiniformes: Sphyrnidae). *Zoological studies* **46**, 6-11.

Chabot CL, Allen LG (2009) Global population structure of the tope (*Galeorhinus galeus*) inferred by mitochondrial control region sequence data. *Molecular Ecology* **18**, 545-552.

Chan LM, Brown JL, Yoder AD (2011) Integrating statistical genetic and geospatial methods brings new power to phylogeography. *Molecular Phylogenetics and Evolution* **In Press, Corrected Proof**.

Chen C-T, Huang S-Y, Lee S-C (2001) Genetic variation between populations of starspotted dogfish *Mustelus manazo* in central Japan and northern Taiwan. *Fisheries Science* **67**, 30-35.

Chenoweth S, Hughes J, Keenan C, Lavery S (1998) Concordance between dispersal and mitochondrial gene flow: isolation by distance in a tropical teleost, Lates carcarifer (*Australian barramundi*). *Heredity* **80**, 187-197.

Cheung WWL, Lam VWY, Sarmiento JL, Kearney K, Watson R, Pauly D (2009) Projecting global marine biodiversity impacts under climate change scenarios. *Fish and Fisheries* **10**, 235-251.

Chevolot M, Ellis JR, Hoarau G, Rijnsdorp AD, Stam WT, Olsen JL (2006) Population structure of the thornback ray (*Raja clavata* L.) in British waters. *Journal of Sea Research* **56**, 305-316.

Clarke S, Burgess G, Cavanagh R, Crow G, Fordham S, McDavitt M, Rose DA, Smith PE, Simpfendorfer CA (2005) Soc-economicsignificance of chondrichthyan fish. In: *Sharks, rays and chimaeras: the status of the Chondrichthyan fishes : status survey* (ed. Fowler S). IUCN/SSS Shark Specialist Group, Cambrige.

Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**, 1657-1659.

Compagno L (1999) Checklist of living elasmobranchs. In: *Sharks, skates, and rays: the biology of elasmobranch fishes* (ed. Hamlett WC), pp. 471–498. Johns Hopkins University Press, Baltimore.

Compagno LJV (1984) *Sharks of the world, an annotated and illustrated catalogue of shark species known to date: Carcharhiniformes* FAO.

Compagno LJV (1990) Alternative life-history styles of cartilaginous fishes in time and space. *Environmental Biology of Fishes* **28**, 33-75.

Compagno LJV (2005) Check list of living elasmobranches. In: *Reprodcutive iology and phylogeny of chondrichthyes: sharks, batoids and chimaeras.* (ed. Hamlett WC), pp. 501-548. Sciences Publishers, Endfield.

Corrigan S, Beheregaray LB (2009) A recent shark radiation: Molecular phylogeny, biogeography and speciation of wobbegong sharks (family: Orectolobidae). *Molecular Phylogenetics and Evolution* **52**, 205-216.

Corrigan S, Huveneers C, Schwartz TS, Harcourt RG, Beheregaray LB (2008) Genetic and reproductive evidence for two species of ornate wobbegong shark *Orectolobus spp*. on the Australian east coast. *Journal of Fish Biology* **73**, 1662-1675.

Cortés E (2002) Incorporating Uncertainty into Demographic Modeling: Application to Shark Populations and Their Conservation. *Conservation Biology* **16**, 1048-1062.

Cowen RK, Sponaugle S (2009) Larval Dispersal and Marine Population Connectivity. *Annual Review of Marine Science* **1**, 443-466.

Coyne JA, Orr HA (2004) *Speciation* Sinauer Associates, Sunderland, MA.

Darwin C (1859) *On the origin o fthe species by means of natural selection* John Murray, London.

De Queiroz K (2007) Species Concepts and Species Delimitation. *Systematic Biology* **56**, 879-886.

Dieckmann U, Doebeli M (1999) On the origin of species by sympatric speciation. *Nature* **400**, 354-357.

Douady CJ, Dosay M, Shivji MS, Stanhope MJ (2003) Molecular phylogenetic evidence refuting the hypothesis of Batoidea (rays and skates) as derived sharks. *Molecular Phylogenetics and Evolution* **26**, 215-221.

Drummond A, Rambaut A (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**, 214.

Dudgeon CL, Broderick D, Ovenden JR (2009) IUCN classification zones concord with, but underestimate, the population genetic structure of the zebra shark *Stegostoma fasciatum* in the Indo-West Pacific. *Molecular Ecology* **18**, 248-261.

Dulvy NK, Baum JK, Clarke S, Compagno LJV, Cortés E, Domingo A, Fordham S, Fowler S, Francis MP, Gibson C, Martínez J, Musick JA, Soldo A, Stevens JD, Valenti S (2008) You can swim but you can't hide: the global status and conservation of oceanic pelagic sharks and rays. *Aquatic Conservation: Marine and Freshwater Ecosystems* **18**, 459-482.

Dulvy NK, Forrest RE (2010) Life Histories, Population Dynamics, and Extinction Risks in Chondrichthyans. In: *Sharks and Their Relatives II: Biodiversity, Adaptive Physiology and Conservation* (eds. Carrier J, Musick J, Heithaus MR), pp. 639-379. CRC Press.

Dulvy NK, Reynolds JD (2002) Predicting Extinction Vulnerability in Skates

Predicción de la Vulnerabilidad de Extinción en Rayas. *Conservation Biology* **16**, 440-450.

Duncan KM, Martin AP, Bowen BW, De Couet HG (2006) Global phylogeography of the scalloped hammerhead shark (*Sphyrna lewini*). *Molecular Ecology* **15**, 2239-2251.

Dunn K, Morrissey J (1995) Molecular Phylogeny of Elasmobranchs. *Copeia* **1995**, 526-531.

Dunn KA, McEachran JD, Honeycutt RL (2003) Molecular phylogenetics of myliobatiform fishes (Chondrichthyes: Myliobatiformes), with comments on the effects of missing data on parsimony and likelihood. *Molecular Phylogenetics and Evolution* **27**, 259-270.

Durazo R, Baumgartner TR (2002) Evolution of oceanographic conditions off Baja California: 1997-1999. *Progress In Oceanography* **54**, 7-31.

Ebert D, Compagno L (2007) Biodiversity and systematics of skates (Chondrichthyes: Rajiformes: Rajoidei). *Environmental Biology of Fishes* **80**, 111-124.

Ebert DA, Squillante MD (2003) *Sharks, rays, and chimaeras of California* University of California Press.

Endler JA (1977) *Geographic variation, speciation, and clines* Princeton University Press.

Enríquez-Andrade R, Anaya-Reyna G, Barrera-Guevara JC, Carvajal-Moreno MdlÁ, Martínez-Delgado ME, Vaca-Rodríguez J, Valdés-Casillas C (2005) An analysis of critical areas for biodiversity conservation in the Gulf of California Region. *Ocean & Coastal Management* **48**, 31-50.

Epinosa-Pérez E, Castro-Aguirre JL, Campos LH (2004) *Catálogo sistemático de tiburones: elasmobranchii, selachimorpha* UNAM, Instituto de Biología.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. *Molecular Ecology* **14**, 2611-2620.

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564-567.

Field IC, Meekan MG, Buckworth RC, Bradshaw CJA (2009) Chapter 4 Susceptibility of Sharks, Rays and Chimaeras to Global Extinction. In: *Advances in Marine Biology* (ed. David WS), pp. 275-363. Academic Press.

Figueroa J, Marinone SG, Lavín MF (2003) A description of geostrophic gyres in the southern Gulf fo California. In: *Nonlinear processes in geophysical fluid dynamics: a tribute to the scientific work of Pedro Ripa* eds. Fuentes OUV, Sheinbaum J, Ochoa J), pp. 237-255. Springer.

Fischer W, Krupp F, Schneider W, Sommer C, Carpenter KE, Niem V (1995) *Guía FAO para la identificación para los fines de la pesca acifico centro-oriental. Volumen II. Vertebrados—Parte 1 Guía Pesca. Pacifico Centro Oriental* FAO, Roma.

FitzSimmons NN, Limpus CJ, Norman JA, Goldizen AR, Miller JD, Moritz C (1997) Philopatry of male marine turtles inferred from mitochondrial DNA markers. *Proceedings of the National Academy of Sciences* **94**, 8912-8917.

Folke C, Carpenter S, Walker B, Scheffer M, Elmqvist T, Gunderson L, Holling CS (2004) Regime Shifts, Resilience, and Biodiversity in Ecosystem Management. *Annual Review of Ecology, Evolution, and Systematics* **35**, 557-581.

Fowler S, Cavanagh R (2005) International Conservation and Management Initiatives for Chondrichthyan Fish. In: *Sharks, rays and chimaeras: the status of the Chondrichthyan fishes : status survey* (ed. Fowler SL). IUCN.

Fowler SL, Nature IUfCo, Resources N (2005) *Sharks, rays and chimaeras: the status of the Chondrichthyan fishes : status survey* IUCN.

Francis MP (1988) Movement patterns of rig (*Mustelus lenticulatus*) tagged in southern New Zealand. *New Zealand Journal of Marine and Freshwater Research* **22**, 259-272.

Frankham R, Ballou JD, Briscoe DA (2009) *Introduction to Conservation Genetics* Cambridge University Press.

Frisk M (2010) Life History Strategies of Batoids. In: *Sharks and their relatives II: Biodiversity, Adaptive Physiology and Conservation.* (eds. Carrier J, Musick J, Heithaus MR), pp. 283-316. CRC Press, London.

Froeschke J, Stunz GW, Wildhaber ML (2010) Environmental influences on the occurrence of coastal sharks in estuarine waters. *Marine Ecology Progress Series* **407**, 279-292.

Fu YX (1997) Statistical Tests of Neutrality of Mutations Against Population Growth, Hitchhiking and Background Selection. *Genetics* **147**, 915-925.

Gaida I (1997) Population structure of the Pacific angel shark *Squatina californica* (Squatiniformes: Squatinidae), around the California Channel Islands. *Copeia* **4**, 738-744.

Gaither MR, Toonen RJ, Robertson DR, Planes S, Bowen BW (2010) Genetic evaluation of marine biogeographical barriers: perspectives from two widespread Indo-Pacific snappers (*Lutjanus kasmira* and *Lutjanus fulvus*). *Journal of Biogeography* **37**, 133-147.

Galindo HM, Olson DB, Palumbi SR (2006) Seascape Genetics: A Coupled Oceanographic-Genetic Model Predicts Population Structure of Caribbean Corals. *Current Biology* **16**, 1622-1626.

Gardner MG, Ward RD (1998) Population structure of the Australian gummy shark (*Mustelus antarcticus*) inferred from allozymes, mitochondrial DNA and vertebrae counts. *Journal of Marine and Freshwater Research* **49**, 733-745.

Gastil R, Allison C, Phillips R (1975) Reconnaissance geologic map of the State of Baja California, . *Memorias geol. Soc. Am.* **140**, 170.

Gavrilets S, Li H, Vose MD (2000) Patterns of Parapatric Speciation. *Evolution* **54**, 1126-1134.

Ginter M, Hampe O, Duffin C (2010) *Handbook of Paleoichthyology / Chondrichthyes · Paleozoic Elasmobranchii: Teeth* Pfeil, Dr. Friedrich.

Goldstein DB, Schlötterer C (1999) *Microsatellites: evolution and applications* Oxford University Press.

Gonzalez-Suarez M, Flatz R, Aurioles-Gamboa D, Hedrick PW, Gerber LR (2009) Isolation by distance among California sea lion populations in Mexico: redefining management stocks. *Molecular Ecology* **18**, 1088-1099.

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology* **11**, 1103-1114.

Griffiths AM, Sims DW, Cotterell SP, El Nagar A, Ellis JR, Lynghammar A, McHugh M, Neat FC, Pade NG, Queiroz N, Serra-Pereira B, Rapp T, Wearmouth VJ, Genner MJ (2010) Molecular markers reveal spatially segregated cryptic species in a critically endangered fish, the common skate (*Dipturus batis*). *Proceedings of the Royal Society B: Biological Sciences* **277**, 1497-1503.

Grijalva-Chon J, Kaichi A, Numachi K (2002) Homogeneidad genética en tiburón angelito (*Squatina californica*) del Golfo de California, evidencia por análisis PCR-RFLP de la región control del ADN mitocondrial. *Ciencias marinas* **17**, 37-42.

Grismer LL (2000) Evolutionary biogeography on Mexico's Baja California peninsula: A synthesis of molecules and historical geology. *Proceedings of the National Academy of Sciences* **97**, 14017-14018.

Grismer LL (2002) *Amphibians and reptiles of Baja California, including its Pacific islands, and the islands in the Sea of Cortés* University of California Press.

Günther A (1870) Catalogue of the Fishes in the British Museum. (ed. History BMoN). British Museum, London.

Hall CA (2002) Nearshore marine paleoclimatic regions, increasing zoogeographic provinciality, molluscan extinctions, and paleoshorelines, California: Late Oligocene (27 Ma) to late Pliocene (2.5 Ma). *Geological Society of America Special Papers* **357**, 1-489.

Halpern BS (2003) The impact of marine reserves: do reserves work and does reserve size matter? *Ecological Applications* **13**, 117-137.

Harpending HC (1994) Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. *Human Biology* **66**, 591-600.

Hausback B (1984) Cenozoic volcanic and tectonic evolution of Baja California Sur, Mexico. In: *Geology of the Baja California Peninsula. Pacific* (ed. Frizzell VA). Univeirt.

Heist E (2004a) Genetics of Sharks, Skates, and Rays. In: *Biology of Sharks and Their Relatives* eds. Carrier J, Musick J, Heithaus MR). CRC Press.

Heist E (2004b) Genetics of Sharks, Skates, and Rays. In: *Biology of Sharks and Their Relatives*, pp. 471-485. CRC Press.

Heist EJ, Musick JA, Graves JE (1996) Mitochondrial DNA diversity and divergence among sharpnose sharks, *Rhizoprionodon terraenovae*, from the Gulf of Mexico and Mid-Atlantic Bight. . *Fishery Bulletin.* **94**, 664-668.

Heithaus MR, Delius BK, Wirsing AJ, Dunphy-Daly MM (2009) Physical factors influencing the distribution of a top predator in a subtropical oligotrophic estuary. *Journal Limnology and Oceanography* **54**, 472-482.

Helenes J, Carreño AL (1999) Neogene sedimentary evolution of Baja California in relation to regional tectonics. *Journal of South American Earth Sciences* **12**, 589-605.

Hellberg ME (2009) Gene Flow and Isolation among Populations of Marine Animals. *Annual Review of Ecology, Evolution, and Systematics* **40**, 291-310.

Herndon A, Gallucci VF, DeMaster D, Burke W (2010) The case for an international commission for the conservation and management of sharks (ICCMS). *Marine Policy* **34**, 1239-1248.

Heupel MR, Carlson JK, Simpfendorfer CA (2007) Shark nursery areas: concepts, definition, characterization and assumptions. *Marine Ecology Progress Series* **337**, 287-297.

Hewitt R (1981) Eddies and speciation in the California Current. , pp. 96-98. . California Cooperative Oceanic Fisheries

Hey J (2006) Recent advances in assessing gene flow between diverging populations and species. *Current Opinion in Genetics & Development* **16**, 592-596.

Hodgson JA, Thomas CD, Wintle BA, Moilanen A (2009) Climate change, connectivity and conservation decision making: back to basics. *Journal of Applied Ecology* **46**, 964-969.

Hoelzel A, Shivji MS, Magnussen J, Francis MP (2006) Low worldwide genetic diversity in the basking shark (*Cetorhinus maximus*). *Biology Letters* **2**, 639-642.

Hoelzer A (1997) Inferring Phylogenies From mtDNA Variation: Mitochondrial-Gene Trees Versus Nuclear-Gene Trees Revisited. *Evolution* **51**, 622-626.

Holsinger KE, Lewis PO (2007) Hickory: A package for analysis of population genetic data, version 1.1. Computer program and documentation. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs.

Howard DJ, Berlocher SH (1998a) *Endless forms: species and speciation* Oxford University Press.

Howard J, Berlocher H (1998b) *Endless Forms: Species and Speciation* Oxford University Press, New York.

Huang D, Bernardi G (2001) Disjunct Sea of Cortez–Pacific Ocean Gillichthys mirabilis populations and the evolutionary origin of their Sea of Cortez endemic relative, Gillichthys seta. *Marine Biology* **138**, 421-428.

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* **9**, 1322-1332.

Hudson RR, Turelli M (2003) Stochasticity overrules the "three-times rule": genetic drift, genetic draft, and coalescence times for nuclear loci versus mitochondrial DNA. *Evolution* **57**, 182-190.

Huelsenbeck JP, Ronquist F (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754-755.

Hueter R, Heupel M, Heist E, Keeney D (2005) Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science* **35**, 239–247.

Hughes AR, Inouye BD, Johnson MTJ, Underwood N, Vellend M (2008) Ecological consequences of genetic diversity. *Ecology Letters* **11**, 609-623.

Hughes TP, Bellwood DR, Folke C, Steneck RS, Wilson J (2005) New paradigms for supporting the resilience of marine ecosystems. *Trends in Ecology & Evolution* **20**, 380-386.

Human BA, Owen EP, Compagno LJV, Harley EH (2006) Testing morphologically based phylogenetic theories within the cartilaginous fishes with molecular data, with special reference to the catshark family (Chondrichthyes; Scyliorhinidae) and the interrelationships within them. *Molecular Phylogenetics and Evolution* **39**, 384-391.

Hurtado LA, Mateos M, Santamaria CA (2010) Phylogeography of Supralittoral Rocky Intertidal <italic>Ligia</italic> Isopods in the Pacific Region from Central California to Central Mexico. *PLoS ONE* **5**, e11633.

Hutchings JA (2000) Collapse and recovery of marine fishes. *Nature* **406**, 882-885.

Huxley J (1942) *Evolution: The Modern Synthsis* Allen and Uwin, London.

Iglésias SP, Lecointre G, Sellos DY (2005) Extensive paraphylies within sharks of the order Carcharhiniformes inferred from nuclear and mitochondrial genes. *Molecular Phylogenetics and Evolution* **34**, 569-583.

INAPESCA (2010) Carta Nacional Pesquera (ed. Secretario de Agricultura G, Desarrollo Rural, Pesca y Alimentación). Diario Oficila de la Federacion, Mexico.

Jacobs DK, Haney TA, Louie KD (2004) Genes, diversity, and geologic process on the Pacific Coast. *Annual Review of Earth and Planetary Sciences* **32**, 601-652.

Jensen J, Bohonak A, Kelley S (2005) Isolation by distance, web service. *BMC Genetics* **6**, 13.

Jensen J, Wong A, Aquadro CF (2007) Approaches for identifying targets of positive selection. *Trends in Genetics* **23**, 568-577.

Johnson M, Black R (1984) Pattern Beneath the Chaos: The Effect of Recruitment on Genetic Patchiness in an Intertidal Limpet. *Evolution* **38**, 1371-1383.

Karl S, Castro A, Lopez J, Charvet P, Burgess G (2011) Phylogeography and conservation of the bull shark (*Carcharhinus leucas*) inferred from mitochondrial and microsatellite DNA. *Conservation Genetics* **12**, 371-382.

Keeney DB, Heupel MR, Hueter RE, Heist EJ (2005) Microsatellite and mitochondrial DNA analyses of the genetic structure of blacktip shark (*Carcharhinus limbatus*) nurseries in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea. *Molecular Ecology* **14**, 1911-1923.

Kelly RP, Oliver TA, Sivasundar A, Palumbi SR (2010) A Method for Detecting Population Genetic Structure in Diverse, High Gene-Flow Species. *Journal of Heredity* **101**, 423-436.

Kelly RP, Palumbi SR (2010) Genetic Structure Among 50 Species of the Northeastern Pacific Rocky Intertidal Community. *PLoS ONE* **5**, e8594.

Kidd DM, Ritchie MG (2006) Phylogeographic information systems: putting the geography into phylogeography. *Journal of Biogeography* **33**, 1851-1865.

Kinney MJ, Simpfendorfer CA (2009) Reassessing the value of nursery areas to shark conservation and management. *Conservation Letters* **2**, 53-60.

Kirkpatrick M, Ravigné V (2002) Speciation by natural and sexual selection: Models and experiments. *The American Naturalist* **159**, S22-S35.

Klausmeyer KR, Shaw MR (2009) Climate Change, Habitat Loss, Protected Areas and the Climate Adaptation Potential of Species in Mediterranean Ecosystems Worldwide. *PLoS ONE* **4**, e6392.

Knowles LL (2009) Statistical phylogeography. *Annual Review of Ecology, Evolution, and Systematics* **40**, 593-612.

Knowles LL, Carstens BC (2007) Delimiting Species without Monophyletic Gene Trees. *Systematic Biology* **56**, 887-895.

Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC (1989) Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences* **86**, 6196-6200.

Korpinen S, Jormalainen V, Pettay E (2010) Nutrient availability modifies species abundance and community structure of Fucus-associated littoral benthic fauna. *Marine Environmental Research* **70**, 283-292.

Kriwet J, Kiessling W, Klug S (2009) Diversification trajectories and evolutionary life-history traits in early sharks and batoids. *Proceedings of the Royal Society B: Biological Sciences* **276**, 945-951.

Kuhner MK (2009) Coalescent genealogy samplers: windows into population history. *Trends in Ecology & Evolution* **24**, 86-93.

Kuo C-H, Avise J (2005) Phylogeographic breaks in low-dispersal species: the emergence of concordance across gene trees. *Genetica* **124**, 179-186.

Last PR (2007) The state of chondrichthyan taxonomy and systematics. *Marine and Freshwater Research* **58**, 7-9.

Lavín MF, Marinone SG (2003) An overview of the physical oceanography of the Gulf of California. In: *Nonlinear Processes in Geophysical Fluid Dynamics* (eds. Velasco-Fuentes OU, Sheinbaum J, Ochoa de la Torre JL), pp. 173-204. Kluwer Academic Publishers, Dordrecht.

Leaché AD, Crews SC, Hickerson MJ (2007) Two waves of diversification in mammals and reptiles of Baja California revealed by hierarchical Bayesian analysis. *Biology Letters* **3**, 646-650.

Leathwick J, Moilanen A, Francis M, Elith J, Taylor P, Julian K, Hastie T, Duffy C (2008) Novel methods for the design and evaluation of marine protected areas in offshore waters. *Conservation Letters* **1**, 91-102.

Levin L (2003) Oxygen minimum zone benthos: adaptation and community response to hypoxia. *Oceanography and Marine Biology* **41**, 1-45.

Levin LA, Ekau W, Gooday AJ, Jorissen F, Middelburg JJ, Naqvi SWA, Neira C, Rabalais NN, Zhang J (2009) Effects of natural and human-induced hypoxia on coastal benthos. *Biogeosciences* **6**, 2063-2098.

Lewallen E, Anderson T, Bohonak A (2007) Genetic structure of leopard shark (*Triakis semifasciata*) populations in California waters. *Marine Biology* **152**, 599-609.

Lin HC, SÁNchez-Ortiz C, Hastings PA (2009) Colour variation is incongruent with mitochondrial lineages: cryptic speciation and subsequent diversification in a Gulf of California reef fish (Teleostei: Blennioidei). *Molecular Ecology* **18**, 2476-2488.

Lluch-Cota SE, Aragón-Noriega EA, Arreguín-Sánchez F, Aurioles-Gamboa D, Jesús Bautista-Romero J, Brusca RC, Cervantes-Duarte R, Cortés-Altamirano R, Del-Monte-Luna P, Esquivel-Herrera A, Fernández G, Hendrickx ME, Hernández-Vázquez S, Herrera-Cervantes H, Kahru M, Lavín M, Lluch-Belda D, Lluch-Cota DB, López-Martínez J, Marinone SG, Nevárez-Martínez MO, Ortega-García S, Palacios-Castro E, Parés-Sierra A, Ponce-Díaz G, Ramírez-Rodríguez M, Salinas-Zavala CA, Schwartzlose RA, Sierra-Beltrán AP (2007) The Gulf of California: Review of ecosystem status and sustainability challenges. *Progress In Oceanography* **73**, 1-26.

López JA, Ryburn JA, Fedrigo O, Naylor GJP (2006) Phylogeny of sharks of the family Triakidae (Carcharhiniformes) and its implications for the evolution of carcharhiniform placental viviparity. *Molecular Phylogenetics and Evolution* **40**, 50-60.

Lubchenco J, Palumbi SR, Gaines SD, Andelman S (2003) Plugging a Hole in the Ocean: The Emerging Science of Marine Reserves. *Ecological Applications* **13**, S3-S7.

Luikart G, England P, Tallmon D, Jordan S, Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. *Nat Rev Genet* **4**, 981 - 994.

Lyrholm T, Leimar O, Johanneson B, Gyllensten U (1999) Sex–biased dispersal in sperm whales: contrasting mitochondrial and nuclear genetic structure of global populations. *Proceedings of the Royal Society of London. Series B: Biological Sciences* **266**, 347-354.

Maddison WP (1997) Gene trees in species trees. *Systematic Biology* **46**, 523-536.

Maddison WP, Maddison DR (2009) MESQUITE: a modular system for evolutionary analysis.

Manni F, Guerard E, Heyer E (2004) Geographic Patterns of (Genetic, Morphologic, Linguistic) Variation: How Barriers Can Be Detected by Using Monmonier's Algorithm,". *Human Biology* **76**.

Maravelias CD (1999) Habitat selection and clustering of a pelagic fish: effects of topography and bathymetry on species dynamics. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 437-450.

Márquez-Farías J (2007) Reproductive biology of shovelnose guitarfish *Rhinobatos productus* from the eastern Gulf of California México. *Marine Biology* **151**, 1445-1454.

Martin A (1993) Hammerhead shark origins. *Nature* **364**, 494-494.

Martin A (2001) The phylogenetic placement of Chondrichthyes: inferences from analysis of multiple genes and implications for comparative studies. *Genetica* **111**, 349-357.

Martin AP (1999) Substitution rates of organelle and nuclear genes in sharks: implicating metabolic rate (again). *Molecular Biology and Evolution* **16**, 996-1002.

Mascarenhas AS, Jr., Castro R, Collins CA, Durazo R (2004) Seasonal variation of geostrophic velocity and heat flux at the entrance to the Gulf of California, Mexico. *J. Geophys. Res.* **109**, C07008.

Mayr E (1942) *Systematics and the origin of species* Columbia University Press, New York.

McPhail JD (1994) Speciation and the evolution of reproductive isolation in the sticklebacks (Gasterosteus) of south-western British Columbia. In: *The Evolutionary Biology of the Threespine Stickleback* (eds. Bell MA, Foster SA). Oxford Science Publications, Oxford.

Mendez M, Rosenbaum HC, Subramaniam A, Yackulic C, Bordino P (2010) Isolation by environmental distance in mobile marine species: molecular ecology of franciscana dolphins at their southern range. *Molecular Ecology* **19**, 2212-2228.

Mendez M, Subramaniam A, Collins T, Minton G, Baldwin R, Berggren P, Sarnblad A, Amir OA, Peddemors VM, Karczmarski L, Guissamulo A, Rosenbaum HC (2011) Molecular ecology meets remote sensing: environmental drivers to population structure of humpback dolphins in the Western Indian Ocean. *Heredity*.

Mendonça F, Oliveira C, Gadig O, Foresti F (2011) Phylogeography and genetic population structure of Caribbean sharpnose shark *Rhizoprionodon porosus*. *Reviews in Fish Biology and Fisheries*, 1-16.

Mendonça FF, Oliveira C, Gadig OBF, Foresti F (2009) Populations analysis of the Brazilian Sharpnose Shark *Rhizoprionodon lalandii* (Chondrichthyes: Carcharhinidae) on the São Paulo coast, Southern Brazil: inferences from mt DNA sequences. *Neotropical Ichthyology* **7**, 213-216.

Meudt HM, Clarke AC (2007) Almost Forgotten or Latest Practice? AFLP applications, analyses and advances. *Trends in Plant Science* **12**, 106-117.

Möller L, Valdez F, Allen S, Bilgmann K, Corrigan S, Beheregaray L (2011) Fine-scale genetic structure in short-beaked common dolphins (*Delphinus delphis*) along the East Australian Current. *Marine Biology* **158**, 113-126.

Möller LM, Beheregaray LB (2004) Genetic evidence for sex-biased dispersal in resident bottlenose dolphins (*Tursiops aduncus*). *Molecular Ecology* **13**, 1607-1612.

Moritz C, Dowling TE, Brown WM (1987) Evolution of Animal Mitochondrial DNA: Relevance for Population Biology and Systematics. *Annual Review of Ecology and Systematics* **18**, 269-292.

Mossman CA, Waser PM (1999) Genetic detection of sex-biased dispersal. *Molecular Ecology* **8**, 1063-1067.

Murdock JN, Dodds WK, Gido KB, Whiles MR (2011) Dynamic influences of nutrients and grazing fish on periphyton during recovery from flood. *Journal of the North American Benthological Society* **30**, 331-345.

Murphy RW, Aguirre-Leon G (2002) Nonavian reptiles: origins and evolution. In: *A New Island Biogeography in the Sea of Cortes* (eds. Case T, Cody M, Ezcurra E), pp. 181–220. Oxford University Press, Oxford.

Musick J, Burgess G, Cailliet G, Camhi M, Fordham S (2000) Management of sharks and their relatives (Elasmobranchii). *Fisheries* **25**, 9-13.

Musick JA, Harbin MM, Compagno LJV (2004) Historical zoogeography of the Selachii. In: *Biology of Sharks and Their Relatives* (eds. Carrier JC, Musick JA, Heithaus MR), pp. 33-78. CRC Press, Boca Raton.

Myers RA, Baum JK, Shepherd TD, Powers SP, Peterson CH (2007) Cascading Effects of the Loss of Apex Predatory Sharks from a Coastal Ocean. *Science* **315**, 1846-1850.

Naylor GJP (1992) Tthe phylogenetic relationships among requiem and hammerhead sharks: inferring phylogeny when thousands of equally most parsimonious trees result *Cladistics* **8**, 295-318.

Naylor GJP, Martin AP, Mattison E, Brown W (1997) Interrelationship of Lamniform Sharks: Testing Phylogenetic Hypotheses with sequences data. In: *Molecular systematics of fishes* (eds. Kocher T, Stepien C). Academic Press.

Nielsen R (2005) Molecular signatures of natural selection. *Annual Reviews in Genetics* **39**, 197 - 218.

Norman JR (1926) A synopsis of the rays of the family Rhinobatidae, with a revision of the genus *Rhinobatus*. *Proceedings of the Zoological Society of London* **1926**, 941–982.

Nosil P (2008) Speciation with gene flow could be common. *Molecular Ecology* **17**, 2103-2106.

Nosil P, Crespi B, Gries R, Gries G (2007) Natural selection and divergence in mate preference during speciation. *Genetica* **129**, 309-327.

Nosil P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. *Molecular Ecology* **18**, 375-402.

Ochoa-Landin L, Ruíz J, Calmus T, Pérez-Segura E, Escandon F (2000) Sedimentology and stratigraphy of the Upper Miocene El Boleo Fm, Santa Rosalía, Baja California, Mexico. *Revista Mexicana de Ciencias Geologicas* **17**, 83-96.

Opdam P, Wiens JA, Norris K, Pain DJ (2002) *Fragmentation, habitat loss and landscape management: Conserving Bird Biodiversity* Cambridge University Press.

Ortega V, Álavarez-Borrego S, Arriaga S, Renner M, Bridge E (2010) Physical, Chemical and Biological Oceanography of the Gulf of California. In: *Biodiversity and Conservation in the Gulf of California* (ed. Brusca R). University of Arizona Press, Tucson.

Oskin M, Stock J (2003) Marine incursion synchronous with plate-boundary localization in the Gulf of California. *Geology* **31**, 23-26.

Palumbi SR (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics* **25**, 547-572.

Palumbi SR (2003) Population Genetics, Demographic Connectivity, and the Design of Marine Reserves. *Ecological Applications* **13**, S146-S158.

Palumbi SR, Gaines SD, Leslie H, Warner RR (2003) New wave: high-tech tools to help marine reserve research. *Frontiers in Ecology and the Environment* **1**, 73-79.

Pardini AT, Jones CS, Noble LR, Kreiser B, Malcolm H, Bruce BD, Stevens JD, Cliff G, Scholl MC, Francis M, Duffy CAJ, Martin AP (2001) Sex-biased dispersal of great white sharks. *Nature* **412**, 139-140.

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

Pastene LA, Goto M, Kanda N, Zerbini AN, Kerem DAN, Watanabe K, Bessho Y, Hasegawa M, Nielsen R, Larsen F, PalsbØLl PJ (2007) Radiation and speciation of pelagic organisms during periods of global warming: the case of the common minke whale, *Balaenoptera acutorostrata*. *Molecular Ecology* **16**, 1481-1495.

Peakall ROD, Smouse PE (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288-295.

Peguero-Icaza M, Sánchez-Velasco L, Lavín MF, Marinone SG (2008) Larval fish assemblages, environment and circulation in a semienclosed sea (Gulf of California, Mexico). *Estuarine, Coastal and Shelf Science* **79**, 277-288.

Pelc RA, Warner RR, Gaines SD (2009) Geographical patterns of genetic structure in marine species with contrasting life histories. *Journal of Biogeography* **36**, 1881-1890.

Pereyra S, García G, Miller P, Oviedo S, Domingo A (2010) Low genetic diversity and population structure of the narrownose shark (*Mustelus schmitti*). *Fisheries Research* **106**, 468-473.

Pérez-Jiménez JC, Sosa-Nishizaki O (2008) Reproductive biology of the brown smoothhound shark *Mustelus henlei,* in the northern Gulf of California, México. *Journal of Fish Biology* **73**, 782-792.

Pérez Jiménez JC, Nishizaki OS, Castillo Geniz JL, Buth D (2005) A New Eastern North Pacific Smoothhound Shark (Genus *Mustelus*, Family Triakidae) from the Gulf of California. *Copeia* **2005**, 834-845.

Pfenninger M, Véla E, Jesse R, Elejalde MA, Liberto F, Magnin F, Martínez-Ortí A (2010) Temporal speciation pattern in the western Mediterranean genus Tudorella P. Fischer, 1885 (Gastropoda, Pomatiidae) supports the Tyrrhenian vicariance hypothesis. *Molecular Phylogenetics and Evolution* **54**, 427-436.

Phillips SJ, Dudík M (2008) Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. *Ecography* **31**, 161-175.

Piggott MP, Banks SC, Tung P, Beheregaray LB (2008) Genetic evidence for different scales of connectivity in a marine mollusc. *Marine Ecology Progress Series* **365**, 127-136.

Pittenger G (1984) Movements, distributions, feeding, and growth of the pacific angel sharks, *Squatina californica* off Santa Barbara, California. *Copeia* **1986**, 987-994.

Plank SM, Lowe CG, Feldheim KA, Wilson RR, Brusslan JA (2010) Population genetic structure of the round stingray *Urobatis halleri* (Elasmobranchii: Rajiformes) in southern California and the Gulf of California. *Journal of Fish Biology* **77**, 329-340.

Polato NR, Concepcion GT, Toonen RJ, Baums IB (2010) Isolation by distance across the Hawaiian Archipelago in the reef-building coral Porites lobata. *Molecular Ecology* **19**, 4661-4677.

Polovina JJ, Balazs GH, Howell EA, Parker DM, Seki MP, Dutton PH (2004) Forage and migration habitat of loggerhead (*Caretta caretta*) and olive ridley (*Lepidochelys olivacea*) sea turtles in the central North Pacific Ocean. *Fisheries Oceanography* **13**, 36-51.

Pomeroy RS, Watson LM, Parks JE, Cid GA (2005) How is your MPA doing? A methodology for evaluating the management effectiveness of marine protected areas. *Ocean & Coastal Management* **48**, 485-502.

Posada D, Crandall KA (2001) Selecting the best-fit model of nucleotide substitution. *Systematic Biology* **50**, 580-601.

Pratt HL, Gruber SH, Taniuchi T (1990) *Elasmobranchs as living resources: advances in the biology, ecology, systematics, and the status of the fisheries : proceedings of the Second United States-Japan Workshop, East-West Center, Honolulu, Hawaii, 9-14 December 1987* U.S. Dept.

of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service.

Pringle JM, Wares JP (2007) Going against the flow: maintenance of alongshore variation in allele frequency in a coastal ocean. *Marine Ecology Progress Series* **335**, 69-84.

Prugnolle F, de Meeus T (2002) Inferring sex-biased dispersal from population genetic tools: a review. *Heredity* **88**, 161-165.

Quattro J, Stoner D, Driggers W, Anderson C, Priede K, Hoppmann E, Campbell N, Duncan K, Grady J (2006) Genetic evidence of cryptic speciation within hammerhead sharks (Genus *Sphyrna*). *Marine Biology* **148**, 1143-1155.

Rambaut A, Drummond A (2007) Tracer 1.4.

Randall JE, Compagno LJV (1995) A review of the guitarfishes of the genus *Rhinobatos* (Rajiformes: Rhinobatidae) from Oman, with description of a new species. *The Raffles Bulletin of Zoology* **43**, 289-298.

Renshaw GMC, Wise G, Dodd PR (2010) Ecophysiology of neuronal metabolism in transiently oxygen-depleted environments: Evidence that GABA is accumulated pre-synaptically in the cerebellum. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* **155**, 486-492.

Richards VP, Henning M, Witzell W, Shivji MS (2009) Species Delineation and Evolutionary History of the Globally Distributed Spotted Eagle Ray (*Aetobatus narinari*). *Journal of Heredity* **100**, 273-283.

Riddle BR, Hafner DJ, Alexander LF, Jaeger JR (2000) Cryptic vicariance in the historical assembly of a Baja California Peninsular Desert biota. *Proceedings of the National Academy of Sciences* **97**, 14438-14443.

Riginos C (2005) Cryptic vicariance in Gulf of California fishes parallels vicariant patterns found in Baja California mammals and reptiles. *Evolution* **59**, 2678-2690.

Rosenberg NA, Tao R (2008) Discordance of Species Trees with Their Most Likely Gene Trees: The Case of Five Taxa. *Systematic Biology* **57**, 131-140.

Rosenblatt R, Waples RS (1986) A Genetic Comparison of Allopatric Populations of Shore Fish Species from the Eastern and Central Pacific Ocean: Dispersal or Vicariance? *Copeia* **1986**.

Round FE (1967) The phytoplankton of the Gulf of California. Part I. Its composition, distribution and contribution to the sediments. *Journal of Experimental Marine Biology and Ecology* **1**, 76-97.

Rousset F (2008) Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources* **8**, 103-106.

Routley MH, Nilsson GE, Renshaw GMC (2002) Exposure to hypoxia primes the respiratory and metabolic responses of the epaulette shark to progressive hypoxia. *Comparative Biochemistry and Physiology - Part A: Molecular & Integrative Physiology* **131**, 313-321.

Rundle HD, Nosil P (2005) Ecological speciation. *Ecology Letters* **8**, 336-352.

Salomón-Aguilar C, Villavicencio-Garayzar C, Reyes-Bonilla H (2009) Shark breeding grounds and seasons in the Gulf of California: Fishery management and conservation strategy. *Ciencias marinas* **35**, 369-388.

Sandoval-Castillo J, Rocha-Olivares A (2011) Deep Mitochondrial Divergence in Baja California Populations of an Aquilopelagic Elasmobranch: The Golden Cownose Ray. *Journal of Heredity*.

Sandoval-Castillo J, Rocha-Olivares A, Villavicencio-Garayzar C, Balart E (2004) Cryptic isolation of Gulf of California shovelnose guitarfish evidenced by mitochondrial DNA. *Marine Biology* **145**, 983-988.

Schlitzer R (2010) Ocean Data View.

Schlotterer C (2004) The evolution of molecular markers just a matter of fashion? *Nat Rev Genet* **5**, 63-69.

Schluessel V, Broderick D, Collin SP, Ovenden JR (2010) Evidence for extensive population structure in the white-spotted eagle ray within the Indo-Pacific inferred from mitochondrial gene sequences. *Journal of Zoology* **281**, 46-55.

Schluter D (2000) *The Ecology of Adaptive Radiation* Oxford University Press, Oxford.

Schluter D (2001) Ecology and the origin of species. *Trends in Ecology & Evolution* **16**, 372-380.

Schluter D (2009) Evidence for ecological speciation and its alternative. *Science* **323**, 737-741.

Schrey AW, Heist EJ (2003) Microsatellite analysis of population structure in the shortfin mako (*Isurus oxyrinchus*). *Canadian Journal of Fisheries and Aquatic Sciences* **60**, 670-675.

Schultz JK, Feldheim KA, Gruber SH, Ashley MV, McGovern TM, Bowen BW (2008) Global phylogeography and seascape genetics of the lemon sharks (genus *Negaprion*). *Molecular Ecology* **17**, 5336-5348.

Schwartz MK, Luikart G, Waples RS (2007) Genetic monitoring as a promising tool for conservation and management. *Trends in Ecology & Evolution* **22**, 25-33.

Segura-Garcia I (2011) *Population genetics of species on the genera Tursiops and Delphinus within the Gulf of California and along the western coast of Baja California.*, Durham University.

Selkoe KA, Henzler CM, Gaines SD (2008) Seascape genetics and the spatial ecology of marine populations. *Fish and Fisheries* **9**, 363-377.

Sites JW, Marshall JC (2003) Delimiting species: a Renaissance issue in systematic biology. *Trends in Ecology & Evolution* **18**, 462-470.

Smith PJ, Steinke D, McVeagh SM, Stewart AL, Struthers CD, Roberts CD (2008) Molecular analysis of Southern Ocean skates (*Bathyraja*) reveals a new species of Antarctic skate. *Journal of Fish Biology* **73**, 1170-1182.

Smith W, Bizzarro J, Cailliet G (2009) The artisanal elasmobranch fishery on the east coast of Baja California, Mexico: Characteristics and management considerations. *Ciencias marinas* **35**, 209-236.

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity* **82**, 561-573.

Sobel JM, Chen GF, Watt LR, Schemske DW (2010) The biology of speciation. *Evolution* **64**, 295-315.

Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *J Exp Biol* **213**, 912-920.

Speed C, Field I, Meekan M, Bradshaw C (2010) Complexities of coastal shark movements and their implications for management. *Marine Ecology Progress Series* **408**, 275-293.

Stacey B, Ligon D (1991) The Benefits-of-Philopatry Hypothesis for the Evolution of Cooperative Breeding: Variation in Territory Quality and Group Size Effects. *The American Naturalist* **137**, 831-846.

Standora EA, Nelson DR (1977) A telemetric study of the behavior of free-swimming pacific angle sharks, *Squatina californica*. *Bulletin of Southern California Academy of Sciences* **76**, 193-201.

Stelbrink B, von Rintelen T, Cliff G, Kriwet J (2010) Molecular systematics and global phylogeography of angel sharks (genus Squatina). *Molecular Phylogenetics and Evolution* **54**, 395-404.

Stepien CA, Rosenblatt RH, Bargmeyer BA (2001) Phylogeography of the spotted sand bass, Paralabrax maculatofasciatus: divergence of Gulf of California and Pacific coast populations. *Evolution* **55**, 1852-1862.

Stevens JD, Bonfil R, Dulvy NK, Walker PA (2000) The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. *ICES Journal of Marine Science: Journal du Conseil* **57**, 476-494.

Storfer A, Murphy MA, Spear SF, Holderegger R, Waits LP (2010) Landscape genetics: where are we now? *Molecular Ecology* **19**, 3496-3514.

Storz JF (2005) Using genome scans of DNA polymorphism to infer adaptive population divergence. *Molecular Ecology* **14**, 671-688.

Straube N, Iglésias SP, Sellos DY, Kriwet J, Schliewen UK (2010) Molecular phylogeny and node time estimation of bioluminescent Lantern Sharks (Elasmobranchii: Etmopteridae). *Molecular Phylogenetics and Evolution* **56**, 905-917.

Straube N, Kriwet J, Schliewen UK (2011) Cryptic diversity and species assignment of large lantern sharks of the *Etmopterus spinax* clade from the Southern Hemisphere (Squaliformes, Etmopteridae). *Zoologica Scripta* **40**, 61-75.

Sunnucks P (2000) Efficient genetic markers for population biology. *Trends in Ecology & Evolution* **15**, 199-203.

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

Tajima F (1989) The Effect of Change in Population Size on DNA Polymorphism. *Genetics* **123**, 597-601.

Tallmon DA, Luikart G, Waples RS (2004) The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology & Evolution* **19**, 489-496.

Thomson DA, Findley LT, Kerstitch AN (1979) *Reef Fishes of the Sea of Cortez* University of Arizona Press.

Underwood CJ (2006) Diversification of the Neoselachii (Chondrichthyes) during the Jurassic and Cretaceous. *Paleobiology* **32**, 215-235.

Upton DE, Murphy RW (1997) Phylogeny of the side-blotched lizards (Phrynosomatidae:*Uta*) based on mtDNA sequences: Support for a midpeninsular seaway in Baja California. *Molecular Phylogenetics and Evolution* **8**, 104-113.

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-Checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.

Vekemans X (2002) AFLP-SURV. Laboratoire de Génétique et Ecologie Végétale, Université Libre de Bruxelles, Bruxelles, Belgium.

Vélez-Zuazo X, Agnarsson I (2011) Shark tales: A molecular species-level phylogeny of sharks (Selachimorpha, Chondrichthyes). *Molecular Phylogenetics and Evolution* **58**, 207-217.

Via S (2001) Sympatric speciation in animals: the ugly duckling grows up. *Trends in Ecology & Evolution* **16**, 381-390.

Via S (2002) The ecological genetics of speciation. *The American Naturalist* **159**, S1-S7.

Via S (2009) Natural selection in action during speciation. *Proceedings of the National Academy of Sciences* **106**, 9939-9946.

Via S, West J (2008) The genetic mosaic suggests a new role for hitchhiking in ecological speciation. *Molecular Ecology* **17**, 4334-4345.

Vidal OF, L. Leatherwood, S. (1993) Annotated checklist of the marine mammals of the Gulf of California. *Proceedings of the San Diego Society of Natural History* **(1993)**, 14.

Villavicencio-Garayzar C (1993) Biología reproductiva de *Rhinobatos productus* (Pisces:Rhinobatidae), en Bahia Almejas, Baja California Sur, México. *Revista de Biologia Tropical* **41**, 777-782.

Vos P, Hogers R, Bleeker M, Reijans M, Lee Tvd, Hornes M, Friters A, Pot J, Paleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**, 4407-4414.

Walker BW (1960) The Distribution and Affinities of the Marine Fish Fauna of the Gulf of California. *Systematic Biology* **9**, 123-133.

Walker TI (1998) Can shark resources be harvested sustainably? A question revisited with a review of shark fisheries. *Marine and Freshwater Research* **49**, 553-572.

Ward RD, Holmes BH, White WT, Last PR (2008) DNA barcoding Australasian chondrichthyans: results and potential uses in conservation. *Marine and Freshwater Research* **59**, 57-71.

Warren DL, Glor RE, Turelli M (2008) Environmental niche equivalency versus conservatism: quantitative approaches to niche evolution. *Evolution* **62**, 2868-2883.

Warren DL, Glor RE, Turelli M (2010) ENMTools: a toolbox for comparative studies of environmental niche models. *Ecography* **33**, 607-611.

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

Weimerskirch H, Wilson RP (2000) Oceanic respite for wandering albatrosses. *Nature* **406**, 955-956.

Wetherbee B, Cortés E (2004) Food Consumption and Feeding Habits. In: *Biology of Sharks and their relatives.* (eds. Carrier J, Musick J, Heithaus MR), pp. 223-244. CRC Press, Boca Raton.

White C, Selkoe KA, Watson J, Siegel DA, Zacherl DC, Toonen RJ (2010) Ocean currents help explain population genetic structure. *Proceedings of the Royal Society B: Biological Sciences* **277**, 1685-1694.

Whitlock R, Hipperson H, Mannarelli M, Butlin RK, Burke T (2008) An objective, rapid and reproducible method for scoring AFLP peak-height data that minimizes genotyping error. *Molecular Ecology Resources* **8**, 725-735.

Wiens JJ (2007) Species Delimitation: New Approaches for Discovering Diversity. *Systematic Biology* **56**, 875-878.

Wilga CD, Motta PJ, Sanford CP (2007) Evolution and ecology of feeding in elasmobranchs. *Integrative and Comparative Biology* **47**, 55-69.

Winchell CJ, Martin AP, Mallatt J (2004) Phylogeny of elasmobranchs based on LSU and SSU ribosomal RNA genes. *Molecular Phylogenetics and Evolution* **31**, 214-224.

Wolf JBW, Lindell J, Backström N (2010) Speciation genetics: current status and evolving approaches. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 1717-1733.

Wourms JP, Demski LS (1993) The reproduction and development of sharks, skates, rays and ratfishes: introduction, history, overview, and future prospects. *Environmental Biology of Fishes* **38**, 7-21.

Würtz M (2010) *Mediterranean pelagic habitat : oceanographic and biological processes, an overview* IUCN.

Yamaguchi A, Taniuchi T, Shimizu M (2000) Geographic Variations in Reproductive Parameters of the Starspotted Dogfish, (*Mustelus manazo*), from Five Localities in Japan and in Taiwan. *Environmental Biology of Fishes* **57**, 221-233.

Zangerl R (1981) *Chondrichthyes I: Paleozoic Elasmobranchii* G. Fischer.

Zenger KR, Stow AJ, Peddemors V, Briscoe DA, Harcourt RG (2006) Widespread utility of highly Informative AFLP molecular markers across divergent shark species. *Journal of Heredity* **97**, 607-611.