TITLE

Population genomics informs conservation and management of the Galapagos shark (*Carcharhinus galapagensis*) at local, regional and oceanic scales

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Abstract

Elasmobranchs (sharks and rays) have increasingly experienced human pressures over recent decades, ranging from overfishing to habitat loss. Addressing these pressures is the main challenge for elasmobranch conservation. Specific life-history traits (including slow growth, late maturation, long gestation periods, and small litters) have resulted in high susceptibility to excessive mortality, and have limited their recovery ability. A better understanding of aspects of their biology, population connectivity, habitat use, adaptation and demographic patterns is an important step towards improved management and conservation of elasmobranchs. Yet there is insufficient information to understand the status of almost 50% of elasmobranch species, according to the IUCN Red List of Threatened species. The advent of Next Generation Sequencing (NGS) technologies has seen a transition from conservation genetics to genomics (from gene to genome scales), which is informing our understanding of species and improving conservation outcomes based on these insights. This technology has provided access to thousands of genome-wide markers, collectively capable of providing reliable inferences about demographic patterns and enabling detection of local adaptation. Developing and applying genomics tools to investigate elasmobranch biology and ecology is therefore an important step towards improving their management and conservation.

This thesis focuses on the Galapagos shark (*Carcharhinus galapagensis*), a species categorized as “Near Threatened” by the IUCN in 2003, with a circumtropical distribution and a preference for isolated oceanic islands in tropical and warm temperate waters. Importantly, information about population structure and connectivity across most of the species’ geographic range is lacking, and current knowledge of habitat use and population structure is limited to specific areas. Hence, *C. galapagensis* is a good elasmobranch case study to apply comprehensive genomics tools to detect stock structure, inter-population connectivity, intra-population self-replenishment and to estimate effective population sizes. Informed by these measures, population status at different geographic scales can be used to prioritize populations

in need of improved management. Galapagos sharks also co-occur with a closely related carcharhinid, *Carcharhinus obscurus* in parts of their respective distribution ranges. Although the Galapagos shark shows some level of site fidelity**,** there have been reports of individuals traveling long distances (>2,000 km). Despite their ecological importance in ecosystems as high-level predators, limited genetic and genomic resources are available for Galapagos sharks worldwide. Therefore, the main objective of this thesis was to develop genetic and genomic resources for the Galapagos shark, and to use these to confirm species status, identify inter-specific hybridisation if present and investigate population structure at different geographic scales across the Pacific Ocean, in order to inform and improve conservation efforts of the species at local and regional scales.

By sequencing the mitochondrial control region (947bp in length) and developing thousands of genome-wide Single Nucleotide Polymorphisms (SNPs), I was able to detect population structure at a relatively small geographic scale within the Galápagos Islands (Ecuador). While mitochondrial DNA did not identify structure among these Islands, clustering and network analyses using 8,103 neutral SNPs congruently indicated that two well-differentiated stocks exist in the southern Galápagos Marine Reserve - western (Isabela Island) and eastern (San Cristobal and Santa Cruz Islands). Effective population size (N_e) estimation of both populations was relatively low (approximately 200 for each population), highlighting their vulnerability to harvest (including by-catch) and habitat loss. Regional environmental differences across the archipelago or in their behaviors may underlie the observed population structure, but the extent of the influence of these and other factors are still to be investigated. This is the first effort to include genomic tools to assess genetic structure and connectivity of elasmobranchs within the archipelago. Implications of these findings are important for appropriate management of Galapagos sharks in the Galápagos islands, where previous management plans have based conservation strategies on acoustic and satellite tagging information of other species with different ecological characteristics, such as the scalloped hammerhead shark.

Abstract

The population structure and connectivity of *C. galapagensis* across the Pacific Ocean using a combination of mtDNA and nuclear genome-wide SNPs for 229 individuals was also examined. At least two genetically discrete geographic groups were delineated by analysing 7,274 neutral SNPs: an East Tropical Pacific (including samples from Mexico and the Galápagos Islands) and a central-west Pacific group (including samples from Hawaii, New Zealand (the Kermadec Islands), Australia (Elizabeth and Middleton Reefs, Lord Howe and Norfolk islands)), along with a few South African samples from the Indian ocean). Additional population structure was suggested using outlier SNPs, potentially under selective pressure, at the within region level, with four putatively adaptive conservation units identified: the west Pacific (Australia and New Zealand), the central Pacific (Hawaii), Mexico and the east Pacific (Galápagos). The identification of management and adaptive units at various spatial scales is particularly important for overharvested large predatory organisms, often characterized by smaller, localized populations. I highlight the importance of including both regional and global scale assessments, as well as the use of putative adaptive loci to accurately inform conservation at different geographic scales.

Finally, aiming to understand the relationship between Galapagos and dusky (*Carcharhinus obscurus*) sharks, given the debate in the literature regarding their validity as independent species, this thesis addressed the taxonomic status of the Galapagos shark and investigated possible hybridisation between these two shark species. Appropriate taxonomic identification, as well as a comprehensive understanding of hybridisation (if detected) in ecologically important species like sharks, is an important step towards achieving effective long-term conservation goals. With a single documented case of inter-species hybridisation in sharks to date - between the Australian *Carcharhinus tilstoni* and common *C. limbatus* (blacktip shark) - hybridisation amongst sharks remains poorly investigated. Morphological similarities between the Galapagos and dusky sharks have previously resulted in misidentifications in areas where they co-occur, indicating the need for appropriate tools to distinguish these species in the first instance. Although a recent study helped define them as valid independent species, no

evidence of ongoing hybridisation was found, probably due to small sample sizes from contact zones. Therefore, I proposed that hybridisation and introgression between Galapagos and dusky sharks be examined by sampling more individuals of both species from contact zones (specifically, along the western Mexican coast in the east Pacific), and by using an approach capable of detecting low levels of admixture, such as SNPs. Using empirical analytical approaches and simulations, I first identified a subset of 1,873 highly informative and reliable discriminatory loci for these two species. These discriminatory SNPs were able to identify the extent and direction of hybridisation and introgression between Galapagos and dusky sharks, indicating the presence of four individuals corresponding to various hybrid generations. Given the morphological similarities between these, and other closely related *Carcharhinus* species leading to mislabelling/misidentification cases, I emphasise the importance of a robust and broad sampling strategy across the Pacific distribution of both species. Furthermore, I highlight the need for a complete phylogenomic study of the whole genus, together with comprehensive quality assessment and data filtering in order to accurately define species relationships and detect rare hybridisation and introgression cases.

Overall, this thesis presents the most comprehensive set of genomic resources for the Galapagos shark to date and results provide important novel insights into the species conservation genetic status across the Pacific Ocean, as well as within regional and local geographic areas. This will better inform what needs and challenges are faced by Galapagos shark populations and will ultimately help inform improved conservation and management efforts of more shark species as well.

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Abbreviations

AUs	Adaptive Units
AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of Molecular Variance
COI	Cytochrome Oxidase I
CR	Control Region
CUs	Conservation Units
Cyt-b	Cytochrome b
DAPC	Discriminant Analysis of Principal Components
DArT	Diversity Array Technology
ESUs	Evolutionary Significant Units
EPB	Eastern Pacific Barrier
FDR	False Discovery Rate
F_{ST}	Fixation Index
GNP	Galápagos National Park
GMR	Galápagos Marine Reserve
GSI	Genetic Stock Identification
H_e	Expected Heterozygosity
H_{nb}	Unbiased Expected Heterozygosity
H_o	Observed Heterozygosity
HWE	Hardy-Weinberg Equilibrium
IUCN	International Union for Conservation of Nature
LD	Linkage Disequilibrium
MAF	Minor Allele Frequency
ML	Maximum Likelihood
MPA	Marine Protected Area
mtDNA	Mitochondrial DNA
MUs	Management Units
N_e	Effective Population Size
NGS	Next Generation Sequencing
NMO	Non-model Organism
RAD	Restriction-Associated Digestion
RAPD	Random Amplification of Polymorphic DNA
RE	Restriction Enzyme
SNP	Single Nucleotide Polymorphism
TEP	Tropical Eastern Pacific

Chapter 1 - General introduction

1. Conservation status of Elasmobranchs

Chondrichthyes, or cartilaginous fishes, are one of the most ecologically diverse vertebrate groups, and includes two subclasses: elasmobranchs (sharks and rays) and holocephalans (chimaeras) (Compagno 1999). The following assessment is focused on the first and most speciose lineage within Chondrichthyes, the elasmobranch group. Sharks and rays are particularly susceptible to population declines due to specific life-history traits. Such characteristics (including slow growth, late maturation, long gestation periods, and reduced number of young) make elasmobranchs susceptible to population declines and limits their recovery capacity (Hoenig & Gruber, 1990; Stevens, Bonfil, Dulvy, & Walker, 2000). Currently, overfishing (targeted and incidental catches) and habitat loss have been identified as the two main conservation problems for this group (Dulvy et al., 2014). Despite the important ecological role that elasmobranchs play in ecosystem dynamics and health, their role as top-level predators is not yet well understood (Heupel, Knip, Simpfendorfer, & Dulvy, 2014). Additionally, obtaining information and/or experimentally manipulating elasmobranchs in order to comprehensively understand the consequences of their depletion and accurately assess species and population status is a complex, if not impossible, task (Heupel et al., 2014).

Elasmobranchs have species-specific ecological and biological characteristics, resulting in different threats. Consequently, responses to human pressures can vary enormously, even among conspecifics and it becomes necessary to acknowledge this in order to achieve conservation goals (Shivji et al., 2002). Appropriate long-term management strategies must consider anthropogenic, biological, ecological and evolutionary factors to achieve species-specific sustainability in the long-term (Barbieri, Maltagliati, Roldán, & Castelli, 2014). Importantly, with the move into the genomics era and next generation sequencing (NGS) methods, a new range of opportunities to study elasmobranch populations arises, including accurate taxonomic identification, fisheries and trade monitoring, stock assessments, and importantly, identification and prioritization of conservation units (Dudgeon et al., 2012)..Although the concept of Conservation Units (CUs) and the approaches used to

define them can be flexible (Fraser & Bernatchez, 2001), we refer to the framework provided by Funk et al. (2012), which defines a CU as a group of organisms that is considered distinct from others for conservation purposes. This framework acknowledges adaptive differences between various types of CUs, such as management units (MUs) and evolutionary significant units (ESUs), and suggests different classes of markers should be used to delineate each. Importantly, genomics data has been used to accurately identify species, genetic stocks, and even to successfully assign individuals to the origin biological population in some marine organisms, including mussels (*Mytilus chilensis*, Araneda, Larraín, Hecht & Narum, 2016), the American eel (*Anguilla rostrata*, Bernatchez et al., 2017) and the American lobster (Bernatchez et al., 2017).

2. From conservation genetics to conservation genomics of Elasmobranchs

Molecular markers have been used for nearly three decades in conservation studies, making important and positive contributions to the conservation of marine populations, including elasmobranchs (Feutry et al., 2014; Karl, Castro, Lopez, Charvet, & Burgess, 2010; Keeney, Heupel, Hueter, & Heist, 2003). Despite almost one quarter of elasmobranchs being considered threatened by the IUCN Red List for threatened species and almost fifty per cent being Data Deficient (Dulvy et al., 2014), insufficient attention is paid to this group considering what is required to ensure adequate management and to reduce depletion risks.

A wide range of molecular markers, including allozymes, mitochondrial DNA genes, microsatellites, amplified fragment length polymorphisms (AFLPs), and random amplification of polymorphic DNAs (RAPDs), are commonly used in conventional conservation genetic and forensic studies (Dudgeon et al., 2012; Ouborg, Pertoldi, Loeschcke, Bijlsma, & Hedrick, 2010). Phylogeographic studies of globally distributed sharks have traditionally used a combination of mitochondrial DNA (mtDNA) sequences and nuclear microsatellites to investigate genetic variation and population structure (Daly-Engel et al., 2012; Karl et al., 2010; Keeney & Heist, 2006; Portnoy, Mcdowell, Heist, Musick, & Graves, 2010) in order to identify MUs or ESUs. The combination of both molecular markers has allowed the

identification of historic and current population demographic patterns, genetic diversity, and connectivity at the intra-specific level. Some of these studies have also found evidence for differential dispersal patterns between the sexes (Daly-Engel et al., 2012; Portnoy et al., 2010). Even though mtDNA evolution is relatively slow in sharks and rays, some mitochondrial genes, such as the NADH dehydrogenase subunits 2 and 4 (NADH2 and NADH4, Naylor et al. 2012), Cytochrome oxidase 1(COI), Cytochrome b (Cyt-b) and especially the non-coding mitochondrial Control Region (CR) have been informative for shark population structure analyses (Duncan et al. 2006; Chabot and Allen 2009; Vélez-Zuazo and Agnarsson 2011; Boomer et al. 2012; Clarke et al. 2015; Camargo et al. 2016). This is because the control region encompasses variable regions of the mtDNA genome that are less constrained by selection than other coding mitochondrial genes (Portnoy & Heist, 2012). Additionally, because mtDNA is inherited maternally, it has been useful and informative about sex-biased migration/reproductive behavior, when used in combination with bi-parentally inherited nuclear markers. Note that sex-biased behavior can only be evaluated if the nuclear data used (generally microsatellite markers) have sufficient power to identify population genetic differentiation, which is not generally the case (e.g. Pardini et al. 2001; Schrey and Heist 2003). To have sufficient power, large sample sizes (ideally at least 50 individuals per location) and/or numbers of unlinked microsatellites (at least six and more if sample size <50) are required to detect the presence/absence of genetic structure (e.g. see Horne et al. 2013). This is particularly relevant for elasmobranch studies as large sample sizes are rarely available for these species. Furthermore, microsatellites are widely used due to bi-parental co-dominant inheritance, selective neutrality (largely) and high mutation rates (Daly-Engel et al., 2012; Karl et al., 2010; Pardini et al., 2001). However, despite their advantages, microsatellites have additional limitations such as homoplasy, null alleles, and shifts in allele size due to mutations in flanking regions (Francois Balloux, Brunner, Lugon-Moulin, Hausser, & Goudet, 2000; Portnoy & Heist, 2012).

In light of these limitations in commonly used molecular markers and given the continual emergence of advanced sequencing technologies, accessing and applying 100's to 1000's of genome-wide markers, capable of increasing the power and resolution of CUs, can improve management outcomes and achieve long-term conservation goals for non-model organisms in a time and cost effective manner (da Fonseca et al., 2016; Shafer et al., 2015). The transition from gene to genome scale delivers enormous advantages at a time when it is increasingly important to stem Chondricthyan biodiversity losses globally (e.g. see Dulvy et al 2014). By increasing the number of markers used from tens to thousands, genome-wide data will lead to reliable inferences of demographic patterns and detection of local adaptation (Luikart, England, Tallmon, Jordan, & Taberlet, 2003; Luikart, Ryman, Tallmon, Schwartz, & Allendorf, 2010) and hence CUs. Accurately defining species and their conservation units, detecting hybridisation and introgression processes and forensic identification of market products is crucial to improve elasmobranch conservation (Ogden, 2011). Furthermore, future work should investigate potential impacts of environmental and anthropogenic factors on exploited shark and ray populations, and their reproductive biology to improve management efforts (Devlin & Nagahama, 2002).

Next generation sequencing techniques have been available for over a decade, and have become widespread in many biological disciplines, including conservation (Dudgeon et al., 2012; Shendure & Ji, 2008). While studies have been biased towards economically important species, such as Chinook salmon (*Oncorhynchus tshawytscha*, Larson et al., 2014) and Pacific salmon (*Oncorhynchus* spp, Seeb et al. 2011), genomic approaches to improve elasmobranch conservation are still very limited (Dudgeon et al., 2012). As challenges associated with NGS are overcome (e.g. large computational resources), and costs continue to decrease, the number of studies using genomics to inform conservation has increased in recent years, especially studies focusing on taxonomic identification and stock assignment (Bowden, Vargas-Caro, Ovenden, Bennett, & Bustamante, 2016; Bustamante, Barría, Vargas-Caro, Ovenden, & Bennett, 2016; Portnoy et al., 2015; Vargas-Caro, Bustamante, Lamilla, Bennett,

& Ovenden, 2016). The first elasmobranch studies using NGS technology included those from Chabot and Nigenda (2011), and Chabot (2012), who used Roche 454 pyro-sequencing to discover microsatellites for tope (*Galeorhinus galeus*), and smoothhound (*Mustelus henlei*) sharks, respectively. Although these pioneer studies opened the doors to a new-generation of technology and its applications, they were still hampered by a limited number of available markers. Subsequently, studies have transitioned to genome-wide data use with a wider range of applications.

3. Genomic approaches for elasmobranch conservation

Despite increasing efforts to include NGS technologies and genomic tools in marine conservation over the past decade, the use of genomic approaches to inform elasmobranch conservation is still limited. Some of the genomic tools currently used in elasmobranch conservation include mitogenomes, whole genome sequences and whole genome markers (SNPs and microsatellites).

3.1 Mitogenomes

Complete mitochondrial genomes (mitogenomes), are powerful tools to define species relationships (Alam, Petit III, Read, & Dove, 2014; Díaz-Jaimes, Bayona-Vásquez, Adams, & Uribe-Alcocer, 2016). Previous studies in different taxa, including elasmobranchs, highlighted the advantage of using the whole mitogenome to increase the resolution of phylogenetic analyses and divergence time estimates (Feutry, Kyne, & Chen, 2016; Foote et al., 2011; Shamblin et al., 2012), primarily due to the incongruence of results when using single genes (short variable regions compared to the entire mitogenome) (Duchêne, Archer, Vilstrup, Caballero, & Morin, 2011; Jacobsen et al., 2012). Recent mitogenome studies defining the phylogenomic position of sharks (e.g. *Eusphyra blochii*, Feutry et al. 2016) and rays (e.g. *Mobula mobular*, Bustamante et al. 2016; *Dipturus trachyderma*, Vargas-Caro et al. 2016) emphasized the importance of mitogenomes to correctly identify cryptic species, define species relationships, and ultimately to enforce conservation measurements and prevent the disappearance of species due to overexploitation (Blower, Hereward, & Ovenden, 2013).

Before 2013, only two shark mitogenomes were available: the small-spotted catshark (*Scyliorhinus canicula*, Delarbre et al. 1998), and the smoothhound shark (*Mustelus manazo*, Cao et al. 1998). With the advent of next-generation sequencing, and the development of new cost-effective sequencing platforms, accessibility to a wide range of elasmobranch complete mitogenomes has drastically increased (Figure 1.1, Supplemental Tables 1 and 2). Currently, more than thirty elasmobranch families, including sharks and rays, have been successfully sequenced and published. Importantly, access to cost-effective sequencing methods has increased the availability of genomic data from non-model organisms, an important step to improve evolutionary inferences and to address Data Deficiency-related problems within these high-level oceanic predators. Shark mitogenome studies include representatives from 23 families, the most speciose family (Carcharhinidae) being most represented (37%), with mitogenomes sequenced for 22 Carcharhinids (Figure 1.2). For rays, most mitogenomes are from members of the Dasyatidae family (40%) (Figure 1.3).

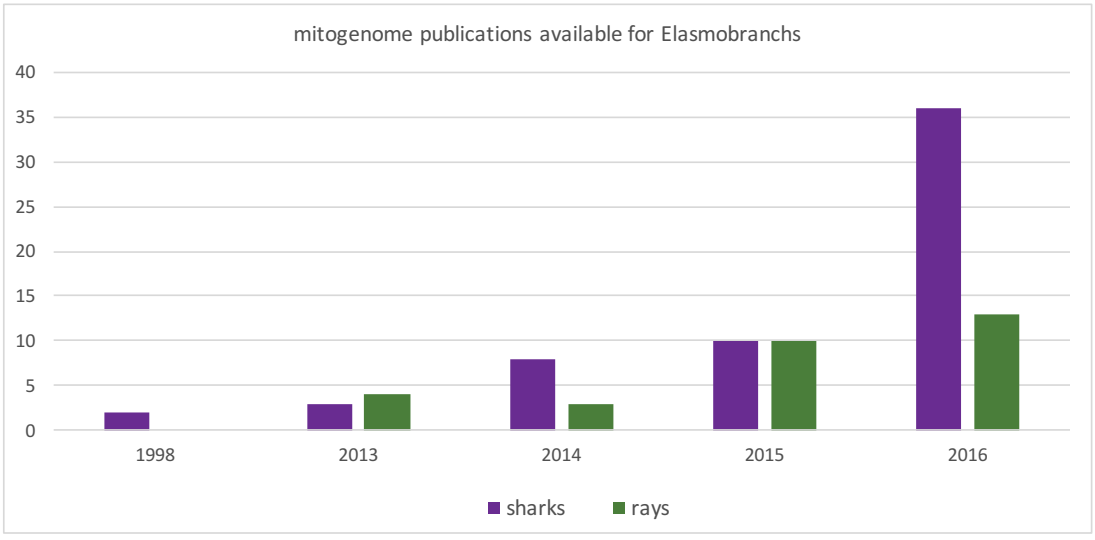

Fig. 1.1 Elasmobranch mitogenome publications to date.

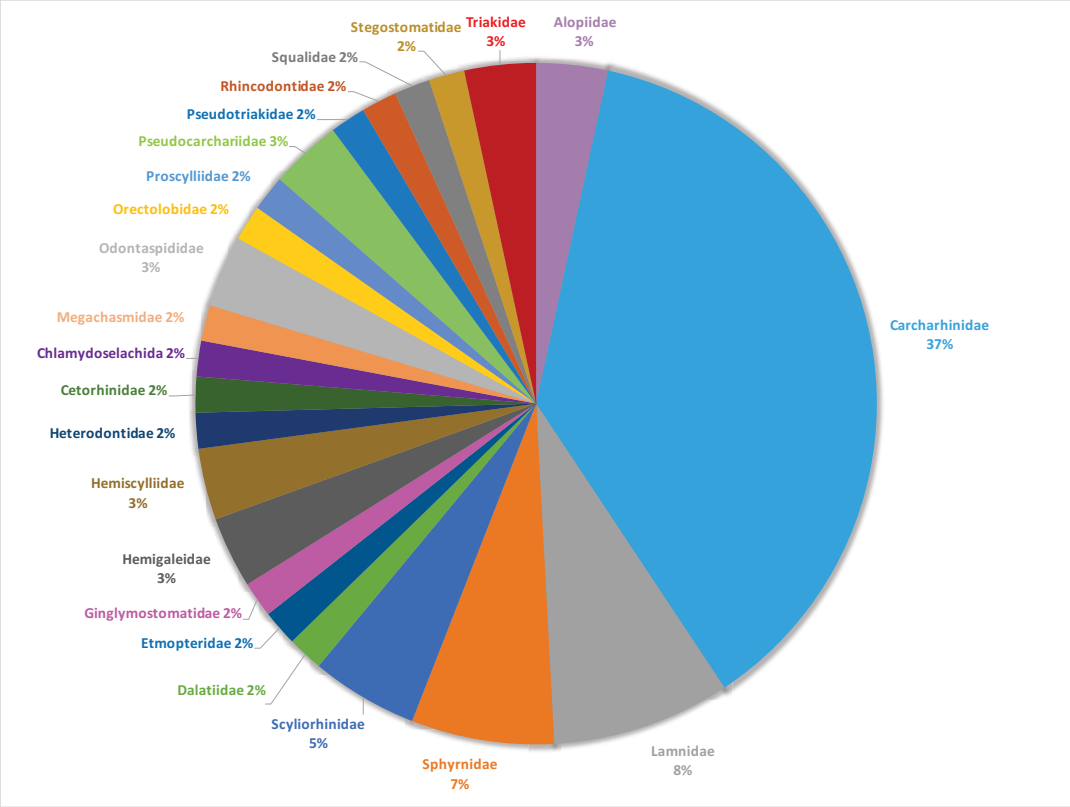

Fig. 1.2 Percentage composition of all mitogenomes available, by shark family. (Supplemental Table 1)

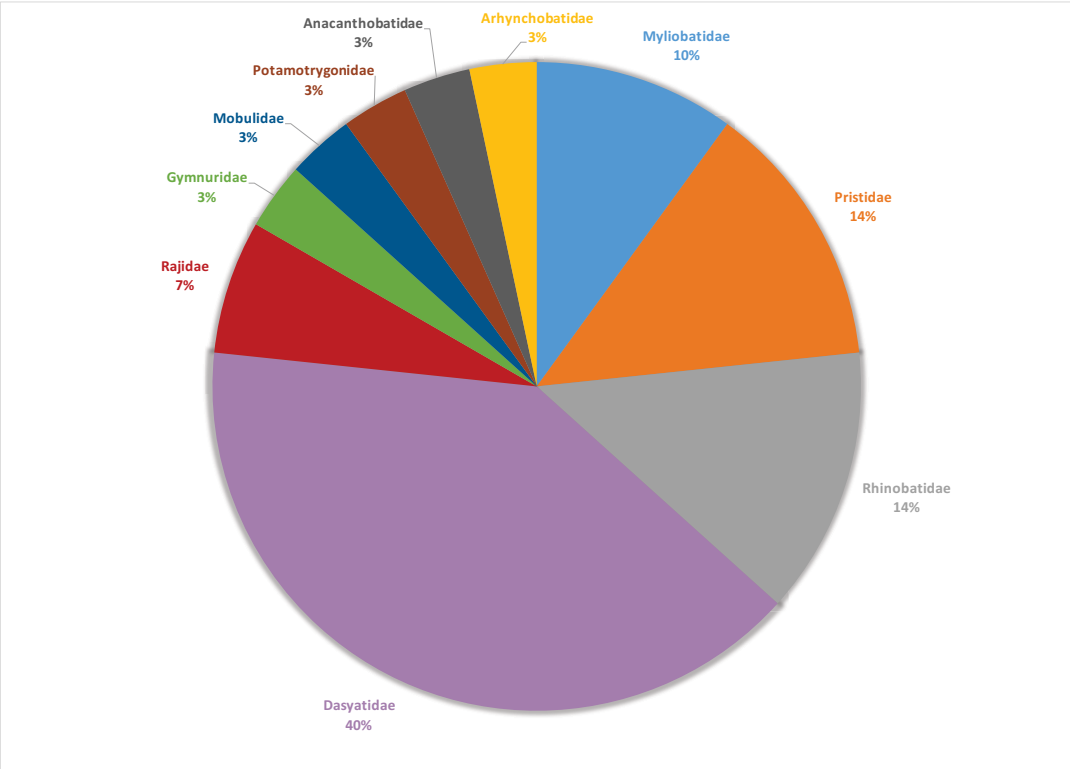

Fig. 1.3 Percentage of all mitogenomes available, by ray family. (Supplemental Table 2)

3.2 Whole-genome sequencing

Sequencing a full genome can be challenging and time consuming, especially if the target species has a large genome and computing capacity is limited (Willette et al., 2014). Whole genome sequencing is probably among the least cost-effective approaches to respond to conservation questions. First, it requires high DNA quality; secondly it requires increased sequencing and bioinformatics resources; finally, the amount of data generated exceeds that required for stock assessments, landscape genetics, or marker-assisted restoration (Allendorf, Hohenlohe, & Luikart, 2010; Lemmon & Lemmon, 2013). Alternative affordable methods, including whole-genome resequencing are becoming popular alternatives to investigate intra population variation. Such alternative strategies present multiple advantages, depending on the availability of genomic resources for the species under investigation (Therkildsen & Palumbi, 2017). For example, in species with an available reference genome, it is possible to target and sequence specific (highly informative) regions of the genome at higher depths (Hong Xia et al., 2015). Furthermore, although their application in non-model species with limited or no genome resources is recent, they can still be useful and provide high quality information (Therkildsen & Palumbi, 2017). Although conservation studies can be performed in the absence of a reference genome, full nuclear genome data can provide important insights and contribute to our understanding of evolution, metabolism, immunology, and local adaptation (Venkatesh et al., 2014; Willette et al., 2014). The chances of identifying and annotating functionally/biologically important adaptive regions across the genome for example, increase when a reference genome is available for the targeted, or a closely related species (Allendorf et al., 2010; Willette et al., 2014).

In the case of elasmobranchs, conservation genomics using full genome information is just emerging. Until recently, the only fully annotated genome available within the Chondrichthyes was the elephant shark (*Callorhinchus milii*, Venkatesh et al. 2014). This species is in the holocephalan subclass, sister to elasmobranchs, and was selected as a chondrichthyan genome model because of its small genome size (approximately 1 Gb). The

elephant shark genome has provided useful information to better understand cartilaginous fish evolution, and important insights about the mechanism of bone formation and the origin of adaptive immunity within this group (Venkatesh et al., 2007, 2014). However, due to the phylogenetic distance between holocephalans and elasmobranchs, genomes from elasmobranchs are needed to further our knowledge on elasmobranch evolution, and therefore, to identify the most unique taxa in greatest need of conservation action.

To the best of our knowledge, there is not yet a complete nuclear genome fully sequenced and assembled for an elasmobranch species. However, some projects, such as the whale shark (*Rhincodon typus*) genome project, have been under development for several years now (Read et al., 2016). This project used a combination of different sequencing platforms, including 454 pyrosequencing, Illumina sequencing and Pacific BioSciences (PacBio) technology, to successfully sequence and assemble a genome estimated to be 3.44 Gb in size. Although the full assembly has not yet been published, researchers leading the investigation have made a draft genome available at http://whaleshark.georgiaaquarium.org/. Similarly, other whole genome sequencing projects have been initiated for other sharks and rays, including great white sharks (*Carcharhodon carcharias*) and the little skate (*Raja erinacea*). We posit information from these genomes will be a valuable resource for other non-model species with limited or non-existent genomic resources, particularly for identifying possible adaptive loci associated with ESUs and CUs.

3.3 Genome-wide nuclear markers: Single Nucleotide Polymorphisms (SNPs)

Single Nucleotide Polymorphisms (SNPs) are single base pair changes distributed across the entire nuclear genome (Vignal, Milan, SanCristobal, & Eggen, 2002), representing coding and non-coding regions and permitting examination of both neutral and adaptive genetic variation. These mostly biallelic markers exhibit slow mutation rates and low homoplasy, which makes SNP scoring more efficient and reliable than microsatellite scoring, providing access to much larger numbers of loci for conservation genomics (Portnoy & Heist, 2012). The most commonly used genomic approaches to develop SNPs are the Restriction-site Associated DNA

(RAD) sequencing methods. These approaches, useful for non-model organisms lacking genome resources, reduce the complexity of the genome by fragmenting genomic DNA using restriction enzymes (RE) with different cutting frequencies (common and rare cutters), while discovering, sequencing and genotyping thousands of markers (Davey et al., 2011). SNPs developed through RAD sequencing methods have significantly improved the resolution of Conservation Unit (CU) delimitation and population assignment of commercially important fish species such as Chinook salmon (*O. tshawytscha*), ultimately informing fishing practices to avoid overharvest and depletion of weak stocks (Dann, Habicht, Baker, & Seeb, 2013; Larson, Seeb, Pascal, Templin, & Seeb, 2014). Moreover, SNPs are a valuable tool to detect introgression between farmed and wild populations in species such as Atlantic salmon (*Salmo salar*) (Glover et al. 2013) and fine population structure and stock assessment of migratory species such as American lobster (*Homarus americanus*) (Benestan et al., 2015; 2016). However, as noted for other genomic methods, SNPs have only recently been incorporated into elasmobranch conservation research, with limited examples showing the potential applications of SNPs for this purpose (Table 1.1).

1.3.3.1 Stock assessment and conservation unit identification

One of many advantages of using genome-wide SNPs in conservation is the opportunity to identify and compare neutral versus adaptive genomic differentiation in wild populations. This approach permits the identification of functionally important genome regions in order to study the genetic basis of local adaptation at the population and species level (Allendorf et al., 2010). Identifying neutral and adaptive genomic variation concurrently enables comprehensive insights into the processes affecting population and species differentiation: from genetic drift and gene flow (inferred from neutral loci) to local selection and adaptation (inferred from adaptive loci). This is important, since financial resources generally limit conservation decisions and greater insights allow limited resources to be directed towards higher conservation priorities (Funk et al., 2012; Willette et al., 2014). Although identifying the exact genomic regions where SNPs come from is often difficult when using RAD sequencing in organisms without a

reference genome (Davey et al., 2011, 2013), different approaches have been developed to detect loci under adaptive selection (so-called outliers). A common method to detect these putative adaptive loci is based on a common metric of genetic differentiation (F_{ST}) (Allendorf et al., 2010; Beaumont, 2005; Bierne, Roze, & Welch, 2013). This approach uses a large number of loci to infer the F_{ST} distribution expected under neutrality, and then tests for loci that are outliers to this neutral distribution (Bierne et al., 2013; Candy et al., 2015; Steane et al., 2014). Theoretically, outlier loci can be under selection, or linked to genomic regions under selection and are much less common than neutral loci. F_{ST} outlier SNPs have shown substantially increased power and resolution to identify conservation units when compared to neutral markers such as neutral SNPs and microsatellites (Vincent, Dionne, Kent, Lien, & Bernatchez, 2013). However, debate remains regarding the importance of correct identification and use of adaptive loci to inform conservation (de Guia & Saitoh, 2007; Garner et al., 2016; Shafer et al., 2015), with a current trend towards increased use of putative adaptive (outlier) loci and inferred local adaptation to inform conservation (Nielsen et al., 2009; Candy et al., 2015). Once identified, neutral and outlier loci can be independently analysed to define populations and identify those with priority for conservation. In high gene flow systems, outlier loci can be used to identify the provenance of illegally trafficked material (Luikart et al., 2003), due to their greater resolution of spatially or ecologically discrete population structure than neutral loci, which in turn measure slower evolutionary forces due to isolation and subsequent genetic drift, but not selection. While some authors highlight the importance of combining landscape genetics with population genomics in an effort to better explain possible drivers of local adaptation, as well as validation studies that confirm the adaptive nature of outliers (Shafer et al., 2015; Steane et al., 2014; Vincent et al., 2013; Willette et al., 2014), other researchers believe the use of outlier loci alone, and interpretation of results based on such loci is justifiable provided a rigorous outlier identification method has been used (Candy et al., 2015). This is particularly important, given the lack of whole genome resources in non-model species, which prevents mapping (and therefore validating) outlier loci to functionally relevant genomic regions. In order to avoid power overestimation for example, SNP discovery and discriminatory accuracy assessments

should be performed for a different set of samples (Candy et al., 2015). Also, some authors recommend using independent outlier detection methods than F_{ST} alone, in order to increase statistical confidence in F_{ST} outliers (Lal, Southgate, Jerry, Bosserelle, & Zenger, 2017).

Portnoy et al. (2015), were first to provide insight into the power of genome-wide markers to detect local adaptation signals within elasmobranchs, by using 49 putatively adaptive SNPs to assess sex-biased dispersal and its effects on locally adaptive variation in the bonnethead shark (*Sphyrna tiburo*) from the northeastern coast of the Gulf of Mexico. More recently, Momigliano et al. (2017) used a combination of mtDNA and 5517 nuclear SNPs to characterize patterns of genetic structure and investigate signatures of selection in the grey reef shark (*Carcharhinus amblyrhynchos*) from the Indo Pacific, with results suggesting cryptic genetic structure and strong signals of local adaptation in the region. Besides studies focusing on regional population genetic structure, a new approach using genome-wide SNP data was used to study genetic connectivity of the restricted range endemic Speartooth shark (*Glyphis glyphis*) within a contemporary context, by comparing and contrasting cohorts of juveniles and adults from within and between nurseries in three river systems. Using both SNPs and mitogenomics the authors identified juvenile cohort fidelity to their nursery, female philopatry and male dispersal. Such contemporary, rather than evolutionary time scale studies are particularly useful for effective management and conservation (Feutry et al., 2017), because low levels of connectivity - normally captured by sampling adults spatially - may suggest long-term (evolutionary) connectivity that is inadequate to sustain demographically independent (having less than 10% inter-population migration) populations, as demonstrated for a restricted range endemic anemone fish using evolutionary and contemporary approaches (Steinberg et al., 2016).

Table 1.1 Studies using SNPs to inform shark conservation.

Species	Common name	Reference	Study site	Genomics approach	# markers used	Application
Sphyrna tiburo	Bonnethead shark	Portnoy et al. 2015	North-eastern coast of the Gulf of Mexico	Double-digest (dd) RAD sequencing	5865 neutral and 49 outlier SNPs	Assessment of geographic patterns of variation and local adaptation
Carcharhinus amblyrhynchos	Grey reef shark	Momigliano et al. 2017	Indo Pacific	Diversity Array Technologies (DArT) SNP sequencing	5517 neutral SNPs	Investigating population structure and signatures of selection to inform conservation
Glyphis glyphis	Speartooth shark	Feutry et al. 2017	Northern Territory, Australia	Diversity Array Technologies (DArT) SNP sequencing	1330 neutral SNPs	Inferring contemporary genetic connectivity to inform management practices
Carcharhinus galapagensis and *C.obscurus*	Galapagos and dusky sharks	Corrigan et al. 2017	Worldwide	Target gene capture	2152 neutral SNPs	Detecting genetic admixture to validate species distinctiveness
Carcharhinus galapagensis	Galapagos sharks	Pazmiño et al. 2017, this study	Galápagos Islands	Diversity Array Technologies (DArT) SNP sequencing	7934 neutral SNPs	Stock assessment, and effective population size estimation
Carcharhinus galapagensis	Galapagos sharks	This study (Chapter 3)	Pacific Ocean	Diversity Array Technologies (DArT) SNP sequencing	8368 neutral and 13-27 outlier SNPs	Phylogeographic assessment and investigation of local adaptation to inform conservation
Carcharhinus galapagensis and *C. obscurus*	Galapagos and dusky sharks	This study (Chapter 4)	East Tropical Pacific	Diversity Array Technologies (DArT) SNP sequencing	1873 neutral SNPs	Detecting hybridisation and introgression signals

1.3.3.2 Taxonomic identification, hybridisation and introgression assessment

Although many studies have focused on understanding the effects of anthropogenic hybridisation (resulting from human intervention such as habitat alteration) to enforce conservation of wild populations, hybridisation can be an important, natural evolutionary force contributing to adaptability and diversification (Mallet, 2005). Correct taxonomic identification,

together with a comprehensive understanding of the taxonomic and evolutionary consequences of hybridisation is crucial within the field of conservation and evolutionary biology (Frankham, 2010), and a priority for elasmobranch conservation (Ovenden, Morgan, Kashiwagi, Broderick, & Salini, 2010; Portnoy & Heist, 2012). Conventional molecular techniques that discriminate sharks and rays at the species level have been used for a long time, and mostly rely on short mitochondrial sequences or DNA-barcoding (Fields, Abercrombie, Eng, Feldheim, & Chapman, 2015; Holmes, Steinke, & Ward, 2009; Naylor et al., 2012; Naylor, Yang, Corrigan, & Carvalho, 2016; White & Last, 2012). Furthermore, assessment of hybridisation and introgression signals in elasmobranchs remain poorly investigated, with a single documented case of ongoing interspecific hybridisation between sharks - Australian *Carcharhinus tilstoni* and common *C. limbatus* (blacktip shark) in eastern Australia (Morgan et al., 2012). Combined mitochondrial DNA sequence data and nuclear microsatellite genotypes can be used to detect hybridisation as for blacktip sharks. However, assessing introgression patterns (gene flow between hybridising species) is complex, both morphologically and genetically. Hybrids beyond first generation (beyond F1) are often morphologically indistinguishable from parental species, and share unequal proportions of their two parental species genomes (Nussberger, Greminger, Grossen, Keller, & Wandeler, 2013). Consequently, a panel of diagnostic markers capable of detecting the presence of species-specific loci is needed (Amish et al., 2012). Due to low homoplasy and mostly biallelic nature, SNPs have been successfully used to detect introgression and hybridisation patterns in fish taxa such as Atlantic salmon (*Salmo salar*, Glover et al. 2013) and rainbow trout (*Oncorhynchus mykiss*, Amish et al. 2012). Maes et al. (in prep), used genome-wide SNPs to assess levels of introgression between the two blacktip shark species reported to hybridise along the east coast of Australia, and successfully defined a species-diagnostic panel of loci capable of detecting hybrids up to the third (F_3) generation, demonstrating the potential of these markers to detect elasmobranch hybridisation.

Importantly, emerging techniques capable of generating and analyzing genome-wide data in a quick and cost-effective manner hold promise within the conservation field, especially

for sharks and rays, which are subject to over exploitation, and whose conservation enforcement relies on species level taxonomic identification, especially when body parts (specifically fins) are the only material available for identification (Shivji et al., 2002; Shivji, Chapman, Pikitch, & Raymond, 2005). Identifying the most common species caught by fisheries, coupled with a good understanding of population status and introgression patterns, can lead to identifying vulnerability and prioritizing species for conservation.

4. Focal species

4.1 The Galapagos shark (*Carcharhinus galapagensis*)

The Galapagos shark (*Carcharhinus galapagensis*, Snodgrass and Heller 1905) is a circumtropically distributed species in tropical and warm temperate waters with preference for isolated oceanic islands and seamounts (Compagno, 1984; Wetherbee et al., 1996). They mature slowly (10 years), produce few offspring and have gestation periods of 12 months (Wetherbee et al., 1996). The global Galapagos shark population was assessed as “Near Threatened” by the IUCN Red List of Threatened species (Bennett, Gordon, & Kyne, 2003). However, despite its widespread oceanic island distribution, current understanding of habitat use and population structure is limited to specific areas, mostly Hawaii and Mexico where the species movements have been monitored using acoustic telemetry (Kohler, Casey, & Turner, 1998; Lowe, Wetherbee, & Meyer, 2006; Meyer, Papastamatiou, & Holland, 2010; Papastamatiou, Meyer, Kosaki, Wallsgrove, & Popp, 2015). *Carcharhinus galapagensis* is a good case study to examine how a lack of comprehensive information about inter-population connectivity, intra-population self-replenishment and effective population sizes can lead to uncertainty of a species’ extinction risk and potentially result in inappropriate conservation and management of the species. Hence we have selected this species to assess population structure, resilience and viability, along with population genetic diversity in order to define appropriate and effective management practices to support persistence of the species in the long term across its distribution range.

4.2 Previous relevant studies

Although molecular tools have been widely used for other shark species, research of *C. galapagensis* remains limited, with few studies restricted to localized areas of its distribution. The first study assessing genetic structure of Galapagos sharks was that of van Herwerden et al. (2008). Based on mitochondrial control region sequences (822bp) from 86 individuals from Elizabeth and Middleton Reefs Marine National Nature Reserve, and Lord Howe Island Marine Park, the authors identified two genetic stocks: one comprising individuals from Elizabeth and Middleton Reefs, the other Lord Howe Island (F_{ST}= 0.5416, P<0.0001). These results highlighted the importance of regional and global assessments to better understand population dynamics. In 2014, Green (2014) conducted the first study combining mtDNA (1029 bp of the control region) and nuclear genome-wide SNPs for these, plus one New Zealand location, and suggested Galapagos sharks from Australia and New Zealand may form a single Evolutionary Significant Unit (ESU).

Furthermore, in 2012 a global phylogenetic analysis including 305 shark species, and aiming to provide a framework for elasmobranch identification, included four individuals of *C. galapagensis* (Naylor et al., 2012). The results, based on NADH2, a mitochondrial gene, produced an unexpected result by placing the four *C. galapagensis* individuals from Hawaii together with *C. obscurus* (a morphologically cryptic species). This raised questions regarding the validity of the species and lead the authors to suggest *C. galapagensis* may be an oceanic phenotype of *C. obscurus*. This hypothesis was later discarded by a combination of mitochondrial NADH2 sequences and 2152 SNPs from independent autosomal regions (Corrigan et al., 2017). While mtDNA again failed to differentiate the two species, purportedly due to historical mitochondrial introgression, nuclear SNPs successfully separated them (F_{CT} = 0.27), thereby confirming their status as distinct species (Corrigan et al., 2017) whilst suggesting that the two species may be hybridizing or may have hybridised historically.

5. Thesis aims and outline

The overarching goal of this study was to apply advanced genomics techniques to develop novel genomic resources to inform conservation and management of the Galapagos shark (*Carcharhinus galapagensis*) throughout its geographic range, by evaluating three hypotheses: 1) Population structure can be identified within regional stocks using genome-wide SNPs. This was done by developing and screening genome-wide nuclear SNP markers for *C. galapagensis* capable of informing improved conservation actions at local scales, 2) Population structure can be identified at ocean-wide scales using genome-wide SNPs. This was done by assessing large-scale levels of divergence and patterns of connectivity at regional and ocean-wide scales, while estimating the potential level of adaptive divergence of *C. galapagensis* across the Pacific Ocean, and 3) Diagnostic SNP markers are capable of detecting introgression between *C. galapagensis* and *C. obscurus* in the Pacific. This requires developing a panel of diagnostic SNP markers capable of detecting introgression between *C. galapagensis* and *C. obscurus* in the Pacific. These three objectives are presented as independent data chapters and publications, as outlined below.

Chapter 2 describes SNP sequencing through DArT sequencing methods for *C. galapagensis* and the power of genome-wide SNP markers to assess population structure and genetic diversity at a small geographic scale amongst several islands in the southern part of the Galápagos Islands. Genetic parameters were estimated and compared between nuclear and mitochondrial DNA, and effective population size was calculated for each identified genetic stock in order to define conservation units and inform conservation and management of the species within the Galapagos Marine Reserve.

Chapter 3 applies the genomic resources developed in Chapter 2 to assess Pacific Ocean-wide phylogeographic divergence of Galapagos sharks. By including samples from both sides of the Pacific Ocean, the central Pacific, and a few Indian Ocean (South Africa) individuals, it was possible to get a comprehensive understanding of connectivity and population structure of the species through much of it’s range. This chapter includes the

evaluation of putative adaptive (outlier) markers as a tool to identify differentiation likely driven by local adaptation, with additional conservation relevance within regions.

Chapter 4 assesses the level of species divergence and the discrimination power of SNPs to investigate hybridisation and introgression between *C. galapagensis* and its conspecific, the dusky shark (*C. obscurus*). To achieve this, a broader overview of species misidentification within the genus *Carcharhinus* is also presented, together with the implications of species misidentification for shark conservation. The importance of appropriate molecular tools, to correctly define taxonomic identification is also discussed. This chapter revises some important considerations of correctly selecting a diagnostic panel of SNPs capable of detecting different levels of introgression between species, by identifying backcrosses to parental species and highlights the power of genome-wide markers to detect hybrids beyond the first generation, F1.

Finally, **chapter 5** synthesises the major findings of this thesis and presents an overview of the implications of these findings for improved management and conservation of the species, while identifying future research directions.

6. Publication plan

Journal articles produced from chapter 2 to 4 have been either published, submitted or are currently in preparation for submission, as detailed below. Published articles are included in appendix 1.

Pazmiño DA, Maes GE, Simpfendorfer CA, Salinas-de-León P, van Herwerden L (2017). Genome-wide SNPs reveal low effective population size within confined management units of the highly vagile Galapagos shark (*Carcharhinus galapagensis*). *Conservation Genetics*. doi:10.1007/s10592-017-0967-1.

Pazmiño DA, Maes GE, Green ME, Simpfendorfer CA, Hoyos-Padilla EM, Duffy CAJ, Meyer CG, Kerwath SE, van Herwerden L (In Press). Genome-wide SNP markers reveal strong Trans-

Pacific break and local conservation units in the Galapagos shark (*Carcharhinus galapagensis*). *Heredity*.

Pazmiño DA, van Herwerden L, Simpfendorfer CA, Junge C, Donnellan S, Hoyos-Padilla EM, Duffy CAJ and Maes GE (In Prep). Introgressive hybridisation between *Carcharhinus galapagensis* and *Carcharhinus obscurus* in the east Pacific. Target journal is *Molecular Ecology.*

Pazmiño, D.A., van Herwerden, L., Simpfendorfer, C.A., Donnellan, S., Ovenden, J., Feutry, P., and Maes, G.E. (In Prep). Identification and application of sex-linked SNP markers in carcharhinid sharks. *Marine Genomics.*

Chapter 2 - Population structure assessment of the Galapagos shark (*Carcharhinus galapagensis*) within the Galápagos Marine Reserve

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Contribution:

Diana Pazmiño and Lynne van Herwerden: Sample tissue collection

Diana Pazmiño: data analysis, writing and editing chapter.

Lynne van Herwerden, Gregory Maes, Colin Simpfendorfer, Pelayo Salinas de León: Supervision and editing of manuscript.

Published: Pazmiño, D.A., Maes, G.E., Simpfendorfer, C.A. Salinas-de-León, P., van Herwerden, L. (2017). **Genome-wide SNPs reveal low effective population size within confined management units of the highly vagile Galapagos shark (*Carcharhinus galapagensis*)**. *Conservation Genetetics*. doi:10.1007/s10592-017-0967-1.

1. Abstract

The Galapagos shark (*Carcharhinus galapagensis*) is one of over thirty shark species inhabiting the Galápagos Marine Reserve (GMR), where it is a priority species for conservation. Identifying stock structure and effective population size for species-specific management and effective conservation of this top predator is important. We examined stock structure, connectivity and effective population size of Galápagos sharks among GMR locations using genome wide neutral Single Nucleotide Polymorphism (8,103 SNP) and mtDNA markers. Potential historical gene flow and/or sex-biased dispersal were also examined using the mitochondrial control region (997bp). Cluster analyses of neutral SNPs revealed two differentiated stocks in the GMR - a western (Isabela Island) and eastern (San Cristobal and Santa Cruz Islands) stock. Effective population size (N_e) estimates of approximately 200 suggest these populations are susceptible to ongoing natural and anthropogenic stressors and are of concern for long term resilience of populations. Mitochondrial DNA failed to identify distinct stocks, with AMOVA analyses indicating most genetic variation occurs within, rather than among locations. This pattern of genome-wide nuclear (but not mtDNA) discrimination among neighbouring islands either points to possible sex-biased dispersal by females or identifies limitations of the single organelle mtDNA marker at such small spatial scales. Regional differences across the archipelago or in behaviour may be implicated in the observed population structure. Further research focusing on a larger, Pacific wide analysis of population connectivity and effective population size at a broader spatial scale is required, to estimate the extent of discreteness and potential local adaptation. Potential adaptive units (AUs) in Galapagos sharks can provide valuable information and should ultimately be identified to leverage adaptive management as part of conservation of the species and future fisheries forensics applications.

2. Introduction

Sharks as high-level predators play an important role in marine ecosystem dynamics (Heupel et al., 2014). Unfortunately, one in four shark species have an elevated risk of extinction due to overfishing and other anthropogenic pressures (Dulvy et al., 2008, 2014).

Sharks are considered vulnerable to overfishing as most species are slow growing, late maturing and have low fecundity compared to bony fishes (Camhi, Fowler, Musick, Bräutigam, & Fordham, 1998). These characteristics result in low rates of juvenile recruitment and hence population recovery. Appropriate management strategies must consider anthropogenic, biological, ecological and evolutionary factors to achieve species-specific sustainability in the long-term (Barbieri et al., 2014). Information provided by molecular tools, such as identification of genetic groups is useful for this purpose, considering that each stock may require different management strategies (Ward, 2000). Previous studies on sharks using coastal areas for breeding have reported fine-scale genetic population structure (e.g. *Carcharhinus plumbeus*; Portnoy et al., 2010), whereas open-ocean distributed species have only exhibited weak genetic population structure, even at inter-oceanic scales (e.g. *Cetorhinus maximus*; Rus Hoelzel, Shivji, Magnussen, & Francis, 2006).

With the emergence of next-generation sequencing (NGS) technologies, ecological and evolutionary studies using genomic approaches have become more powerful and informative (Allendorf et al., 2010; Garvin, Saitoh, & Gharrett, 2010). Novel reduced-representation sequencing methods have facilitated the development and production of large genome-wide data sets, such as single nucleotide polymorphisms (SNPs) for non-model organisms. Information provided by both neutral and adaptive SNPs is highly relevant for conservation and management (Helyar et al., 2011). Therefore, inferences about connectivity, mechanisms directing gene flow, effective population size (N_e), levels of introgression and local adaptation (Palstra, O'connell, & Ruzzante, 2007) can now be addressed at increasingly fine spatial scales. Importantly, effective population size, a measure of genetic health in populations, is an important parameter to evaluate conservation status and improve management (Hare et al., 2011; Palstra & Ruzzante, 2008). Low N_e indicates a high probability of deleterious allele fixation and loss of adaptive variation through genetic drift (Hare et al., 2011; Newman & Pilson, 1997). Interpreting N_e can be challenging, as there is no established fixed population size threshold. However, N_e values above 500 may reduce the effects of inbreeding and maintain

evolutionary potential in marine organisms (Franklin & Frankham, 1998; Lynch & Lande, 1998; Palstra & Ruzzante, 2008). As a multi-use Marine Protected Area (MPA) where shark fishing is completely banned, the Galápagos Marine Reserve (GMR) offers a tool for protecting high level predators and an opportunity to test the utility of MPAs and the concept of management units (MUs) for recovering shark populations by increasing the abundance of breeding age individuals (Jennings, 2000). Improving knowledge about shark status within the GMR where the Galapagos shark occurs could assist managers to make informed decisions and therefore improve management measures, maximizing conservation benefits.

The geographical setting and geological history of the Galápagos islands is well understood (White, McBirney, & Duncan, 1993). These volcanic islands were formed as the Nazca plate moved over a hot spot in an eastward direction. Therefore, the age of the islands increases from west to east. It constitutes the only tropical archipelago where major warm and cool water currents intersect, providing a contrast of biogeographic and environmental conditions at a relatively small spatial scale (Edgar, Banks, Fariña, Calvopiña, & Martínez, 2004; Houvenaghel, 1978). Despite the significance of the Galápagos Islands, little is known about the distribution of sharks and other top predators within the archipelago (Bensted-Smith, 2002). This is of concern, as socio-economic pressures in the region may negatively affect their populations. For example, despite the banning of shark fishing and trading by Ecuadorian law more than 20 years ago (Jacquet, Alava, Pramod, Henderson, & Zeller, 2008), current fishing practices still impact shark populations as they are commonly incidentally captured on fishing lines targeting other species (Zimmerhackel, Schuhbauer, Usseglio, Heel, & Salinas-de-León, 2015) and during recent experimental long-lining efforts (Reyes, Salinas-de-León, Banda, Sevilla, & Revelo, 2014), a practice banned in the GMR since 2005 due to the high levels of shark by-catch (Murillo, Reyes, Zárate, Banks, & Danulat, 2004). One of the most common large shark species in the GMR is the Galapagos shark (*Carcharhinus galapagensis*). This species has a circum-global distribution in both tropical and temperate waters, but most commonly occurs at isolated oceanic islands (Compagno, 1984). Galapagos sharks are currently

listed by the IUCN as Near Threatened globally (2003 assessment; Bennett, Gordon, & Kyne, 2003). Quantifying inter-population connectivity, intra-population self-replenishment and effective population size—hence providing insights into population resilience and viability under exploitation pressure of Galapagos shark stocks, along with population genetic diversity, will provide important information about their potential extinction risk in the GMR and identify potential changes to management practices to ensure sustainability. This study aimed to develop tools to quantify or estimate various genetic parameters useful to inform conservation management of *C. galapagensis* in the Galápagos Islands as follows: (1) develop and screen genome-wide nuclear SNPs, complemented with mtDNA sequence data; (2) compare and contrast nuclear genomic diversity and population structure estimates with mitochondrial DNA diversity and population structure estimates to identify possible environmental and/or sex-biased behavioral factors (e.g. dispersal) of relevance for conservation; (3) estimate the effective population size (N_e) of stock(s) identified to gauge population resilience of the populations in the GMR; (4) translate the information on genetic diversity, population structure and Ne to inform for improved management of Galapagos sharks in the GMR, so that populations of this iconic top predator may persist in the longer term.

3. Materials and Methods

3.1 Sample collection and DNA extraction

I sampled Galapagos sharks from three locations in the southern GMR: Santa Cruz (SCz), San Crist.bal (SCr) and Isabela (ISA) islands. I collected at least 22 shark fin clips from three different islands (Table 2.1; Fig. 2.1), using barbless hooks while line fishing to catch and release sharks (Animal Ethics Permit Number A1988). Samples were preserved in 80% ethanol. I obtained additional samples from the northern Galápagos (Darwin and Wolf islands) and another two localities in SCz islands (Seymour Norte and Seymour Surroundings) from the Galápagos National Park and Charles Darwin Foundation collections, with 85 *C. galapagensis* samples collected in total (Table 2.1; Fig. 2.1).

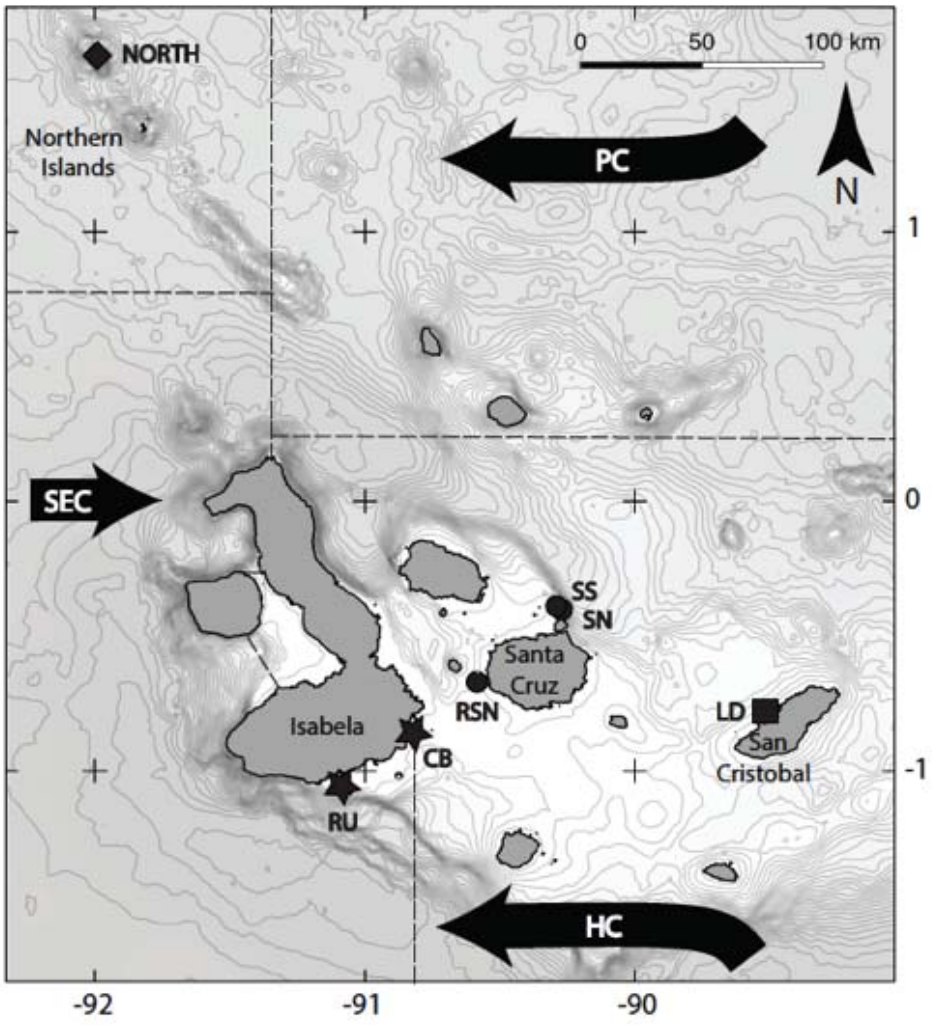

Fig. 2.1 Map of the Galápagos archipelago indicating sample collection sites, currents and bioregions defined after fish and macro-invertebrate abundance and species richness (Harris, 1969; Palacios, 2003; Edgar et al., 2004). Star, circle, square and diamond symbols indicate sites where samples were collected from each island: ISA (n = 27), SCz (n = 29), SCr (n = 25) and NI, respectively. Arrows indicate the direction of the three main oceanic currents affecting the archipelago: warm South Equatorial Current (SEC), cool Panama Current (PC) and cool Humboldt Current (HC). Different biogeographic zones are indicted with dashed lines.

Table 2.1 Genetic diversity, determined for mtDNA control region, and neutral SNPs (8103) of *C. galapagensis* and *C. limbatus* collected in the Galápagos Islands.

Species	Island	Code	Sampling location	Code	n	*n**	**COORDINATES**	Diversity Indices			
								SNPs		mtDNA	
								$H_{n.b}$ [a]	H_o [b]	H [c]	π [d]
C. galapagensis	ISABELA	ISA	Cerro Ballena	CB	8	6	00°50'S, 90°49'W	0.212	0.188 p>0.05	0.585 SD 0.07	0.018 SD 0.01
			Roca Unión	RU	19	18	01°02'S, 91°06'W				
	SANTA CRUZ	SCz	Roca Sin Nombre	RSN	22	22	00°40'S, 90°35'W	0.211	0.190 p>0.05	0.555 SD 0.09	0.074 SD 0.04
			Seymour Norte	SN	5	4	00°24'S, 90°17'W				
			Seymour surroundings	SS	2	2	00°23'S, 90°17'W				
	SAN CRISTOBAL	SCr	León Dormido	LD	25	22	00°47'S, 89°31'W	0.214	0.193 p>0.05	0.361 SD 0.14	0.007 SD 0.00
	NORTHERN ISLANDS	NORTH	Darwin & Wolf	NI	4	3	01°39'N, 91°59'W	-	-	1.000 SD 0.27	0.053 SD 0.04
C. limbatus	OUTGROUP		Cerro Ballena	CB	3	2	00°49'S, 90°50'W	-	-	-	-
Total					88	79					

n Number of individuals that successfully amplified for mtDNA control region, [a] Expected heterozygosity corrected for population sample size , [b] Observed heterozygosity, [c] Haplotype diversity, [d] Nucleotide diversity*

I extracted genomic DNA from fin clips using a salting out protocol (Sunnucks & Hales, 1996). Additionally, for nuclear (SNP) marker development, I performed DNA quality assurance, including a restriction enzyme digest using EcoRI (New England Biolabs). Restriction digests were performed at 37 °C for 3 hours in a total volume of 22 µl containing 5 µl neat DNA, 2 µl NEBuffer [final concentration 1X], 0.2 µl Eco RI and 14.3 µl DNase/RNase-free distilled water. Digestion controls were prepared as were digests, but in the absence or Eco RI. Digestion was terminated by a 65 °C incubation for 20 minutes and for tested samples, 12.5 µl digested, undigested and neat DNA were electrophoresed on an 0.8% agarose gel in 1 x TBE for 45 minutes at 100V and visualized using Biotium Gel-Green. Finally, I determined DNA concentrations spectrophotometrically (NanoDrop 1000, Thermo Scientific) and electrophoretically to verify DNA integrity using agarose gel electrophoresis (0.8% agarose in 1X TBE buffer).

3.2 Nuclear SNP development by DArT sequencing

SNPs were jointly developed and genotyped in all sampled individuals using standard procedures applied by Diversity Array Technology Pty Ltd (DArT, Canberra-Australia) as per Sansaloni et al. (2010) and (2011). Following DNA quality evaluation, samples were digested using two methylation-sensitive restriction enzymes: the frequent cutter PstI (5′ -CTGCA^G-3′), and the rare cutter SphI (5′ -GCATG^C-3′), to digest 150–200 ng of gDNA. Small fragments (<200 bp) of digested DNA were ligated to a barcoded adaptor (6–9 bp in length) and amplified using PCR. PCR products were standardised in concentration and pooled for sequencing on a single HiSeq 2500 (Illumina) lane at a 2.5 million read depth. The generated raw reads were processed through Illumina CASAVA v.1.8.2 software for an initial quality assessment. Subsequent FASTQ files generated by this software were further filtered to produce DArT scores and SNP reports using the DArTtoolbox bioinformatics pipeline, consisting of primary and secondary workflows. During the primary workflow, the DArTSoft14 package was used to remove low quality score reads (<25) and stringently filter barcode region of sequences. Single end sequences (~ 70 bp) were subsequently de-multiplexed by barcode, aligned, and

BLASTed to existing data in the DArTdb database and to viral/bacterial sequences in GenBank to identify possible contamination. The secondary workflow aligned identical reads into clusters across individuals in order to identify polymorphisms, and discard monomorphic clusters, ensuring variant calling. Additionally, 15% of randomly selected samples were used as replicates for genotyping reproducibility assessments. Loci with reproducibility higher than 95% were retained. Loci were identified as SNP or reference allele according to the occurrence frequency. Over-represented sequences were removed to reduce possible gene duplication. Based on Mendelian inheritance patterns, sequences that are not statistically plausible to be allelic (i.e. paralogous regions or sequencing errors) were discarded. Downstream SNP quality control included filtering according to the following criteria: Minor Allele Frequencies (MAF) > 2%, Hardy–Weinberg Equilibrium (HWE), Linkage disequilibrium (LD) r2 > 0.5, discarding monomorphic markers, and a call rate threshold > 85% (i.e . a total of 15% missing data, random with respect to genotype, was allowed in the final filtered data set, and no imputation of missing data was done during analyses) in order to reduce low-quality and uninformative data (Larson, Seeb, Everett, et al., 2014).

3.3 Outlier detection

I discarded putative loci under selection and kept a purely neutral dataset for demographic connectivity analyses; population outlier tests were run using a coalescent based simulation approach in Lositan Selection Detection Workbench (Antao, Lopes, Lopes, Beja-Pereira, & Luikart, 2008). This software identifies loci with unusually high or low F_{ST} values compared with values expected under neutrality using a frequency-based approach to assess the relationship between F_{ST} and H_e. Three independent runs were computed within a 95% confidence interval; an infinite alleles model was used with 100,000 iterations evaluating False Discovery Rate (FDR). One hundred and seventy-four outliers identified by FDR tests as outliers (using the Benjamin-Hochberg, 1995 method) were manually removed.

3.4 Genetic structure analysis

I converted neutral SNP loci obtained from DArT sequencing manually into a Genepop format file to be transformed into various formats according to software requirements using PGDSpider v2.0.6.0 (Lischer & Excoffier, 2012). Populations were pre-defined based on a preliminary population assignment analysis using Structure v2.3.4 (Pritchard, Stephens, & Donnelly, 2000). I performed a relatedness analysis to prevent biased intra-population diversity results. Considering that most of the individuals collected in 2014 were juveniles and sub-adults, and in order to avoid biased gene diversity, a relatedness analysis was performed using MLRelate (Kalinowski, Wagner, & Taper, 2006). A total of 500 random genotypes were simulated to run the statistical test with a confidence interval of 0.95. I defined the minimum number of loci and sample size required in order to accurately estimate N_e using NeoGen v1.3.0.5 b2 (Blower, Riginos, & Ovenden, in preparation). This software includes fundamental species-specific life history, demographic and genetic priors to simulate the genetic and demographic composition of a specific population. Additionally, we calculated the contemporary effective population size based on the LD method (N_{eLD}) for each genetic stock using NeEstimator v.2.01 (Do et al., 2014). For contemporary estimations of N_e (informed by population genetic structure analyses) a minimum of 27 (ISA) and maximum of 54 (SCr-SCz) individuals were used (Table 2.1; Antao, Pérez-Figueroa, & Luikart, 2011). Amongst several demographic and genetic methods available to estimate temporal and contemporary N_e, the linkage disequilibrium (LD) method (Hill, 1981) is the most evaluated single-sample estimator of contemporary effective population size (Hare et al., 2011; Luikart et al., 2010), and performs best, even with small population sizes (Gilbert & Whitlock, 2015). Average observed (H_o) and expected heterozygosities corrected for population sample size ($H_{n.b.}$), and pairwise F_{ST}, were calculated for each locus using the Adegenet package in R Studio v0.98.977 (Jombart & Ahmed, 2011; R Development Core Team, 2008) and were also independently evaluated using Genetix v4.05 (Belkhir et al., 2004). Deviation from HWE was assessed using Adegenet and Genetics R Packages with 1000 permutations. Loci were removed if HWE deviations were

significant across all populations ($p < 0.01$). Plink v.2.050 (Purcell et al., 2007) was used to test for LD between each pair of loci by calculating the correlation coefficient (r^2) of alleles at two loci, independent of allele frequency. Loci were removed if $r^2 > 0.5$.

Genetically distinct populations were identified using Structure v2.3.4 (Pritchard et al., 2000), a clustering based tool which evaluates the likelihood that a sample belongs to K populations (K representing any number of clusters) based on allele frequencies at each locus. Data was analysed using K values from 1 to 4, with 10 independent iterations, one million Markov Chain Monte Carlo (MCMC) repetitions and an independent allele frequency burn-in of 100,000. The most likely number of populations (K) was defined according to the DeltaK statistic as calculated in Structure Harvester webv0.6.93 (Earl & vonHoldt, 2011). Additionally, considering that the DeltaK method does not test the hypothesis that K = 1, I tested this scenario by manually comparing posterior probabilities of the data for all K values tested (Evanno, Regnaut, & Goudet, 2005; Pritchard & Wen, 2003). I performed a population network analysis using NetViewP v0.5.3 (Steinig, Neuditschko, Khatkar, Raadsma, & Zenger, 2016), visualised in Cytoscape (Smoot, Ono, Ruscheinski, Wang, & Ideker, 2011). The NetView P implementation consists of three components: An identity by similarity (IBS) distance matrix (which relies on allele-sharing distance, ASD) construction using Plink v.2.050, a minimum spanning tree construction, a nearest neighbor graph and network visualization. A total of four runs varying in number, based on the nearest neighbor graph (kNN from 10 to 40) were carried out in order to capture both fine and large-scale genetic structure. Finally, the direction and magnitude of gene flow between populations was assessed using the Nei's Gst method implemented in the divMigrate function of the diveRsity R package (Sundqvist, Keenan, Zackrisson, Prodöhl, & Kleinhans, 2016). This software uses directional measures of genetic differentiation in order to calculate relative migration and identify gene flow patterns.

3.5 mtDNA amplification, sequencing and alignment

I used the mitochondrial (mtDNA) Control Region (CR) for phylogenetic analysis as detailed in van Herwerden et al. (2008). Polymerase chain reaction (PCR) was carried out to

amplify the CR using GoTaq Flexi DNA polymerase (Promega). PCR was performed using light strand ProL2 (5′ -CTG CCC TTG GCT CCC AAA GC-3′, Pardini et al., 2001) and heavy strand 282H (5′ -AAG GCT AGG ACC AAA CCT-3′) primers (Keeney et al., 2003). Reactions were carried out in a total volume of 25 µl containing 5.0 µl PCR buffer (5X), 1.5 µl MgCl2 (25 mM), 0.5 µl 2 mM deoxynucleotide triphosphates (dNTPs), 0.5 µl each of the Forward and Reverse primers, each at 10 pmol/µl, 0.2 µl Taq DNA polymerase (5 Units, PROMEGA) and 1 µl of diluted DNA (at a concentration of 10–25 ng/µl). PCR cycling conditions consisted of an initial denaturation at 95 °C for 2.0 min, 30 cycles at 95 °C for 30 s, 53 °C for 30 s, and 72 °C for 1.5 min. Finally, an extension of 72 °C for 10 min was performed. PCR products were cleaned using the Sephadex G50 spin column protocol (GE Life Sciences) and sent to Georgia Genomic Facility (http://dna.uga.edu, USA) for sequencing in forward and reverse directions. Forward and reverse sequences were assembled into contigs, trimmed, and aligned in Geneious v5.4.7 (http://www.geneious.com, Kearse et al., 2012).

3.6 Phylogenetic and population genetic analysis

A phylogeny of mtDNA CR sequences under Maximum Likelihood (ML) and Bayesian Criterion was constructed using default settings of the software Mega 6.06 (Tamura, Dudley, Nei, & Kumar, 2007) and MrBayes 4.0 (Huelsenbeck, Ronquist, Nielsen, & Bollback, 2001), respectively. The model of sequence evolution for phylogenetic and population genetic analyses was estimated using Partition Finder v1.1.0 (Lanfear, Calcott, Ho, & Guindon, 2012) and posterior parameter distributions were examined using Tracer v.1.6 (Rambaut, Suchard, Xie, & Drummond, 2014). An analysis of molecular variance (AMOVA) was performed in Arlequin v.3.5 (Excoffier, Smouse, & Quattro, 1992; Excoffier, Laval, & Schneider, 2005) using 10,000 permutations to estimate F-statistics in order to detect population genetic partitioning between sampling locations and to estimate mtDNA genetic diversity (h , π). These indices were calculated using equations from Nei (1987), and Nei and Li (1979) and were used as indicators of population viability (along with N_e estimates) and resilience within identified management units (MUs). Additionally, I tested for demographic population expansion or reduction by

performing Tajima's D (Tajima, 1989), Fu and Li's D* and F* (Fu & Li, 1993) neutrality tests. Neutrality and population expansion tests can also be useful to identify evidence for selection; Tajima's D, and Fu and Li's D* and F* were estimated in DnaSP 4.10 (Rozas, Sánchez-DelBarrio, Messeguer, & Rozas, 2003) and Arlequin ver. 3.5 (L. Excoffier et al., 1992) using 1000 bootstrap pseudo-replicates. All positions containing gaps and missing data were eliminated for these tests.

4. Results

4.1 SNP filtering and quality control

A total of 9692 markers were genotyped for 85 Galapagos shark individuals (Table 2.1). After filtering steps based on SNP call-rate, MAF, HWE and LD between SNPs, a total of 8187 SNPs were retained. Lositan identified 232 outlier SNPs after three independent runs, which were removed from the data set. Subsequent analyses were performed using 7934 neutral SNPs.

4.2 Population structure analysis based on neutral SNPs

A total of six full and two half siblings at SCr, and two full and one-half sibling within ISA were identified and excluded from Bayesian population structure analyses in order to prevent bias caused by family structure (Rodríguez-Ramilo & Wang, 2012). No Full or half siblings were detected at SCz. However, sibs were retained for LDN_e analysis to increase precision of N_e estimations while keeping the evolutionary signal of population sizes (Waples & Anderson, 2017). Genome wide SNP average expected heterozygosity corrected for population sample size ($H_{n.b}$) varied between 0.211 (ISA) and 0.214 (SCr), while observed heterozygosity (H_o) ranged from 0.188 (ISA) to 0.193 (SCr). Pairwise F_{ST} values between sampling locations ranged from 0.005 between SCz and SCr to 0.25 between ISA and the other two sampled sites (Table 2.2). This difference was supported by AMOVA results, which indicated significant differentiation between ISA in the west (also defined in chapter 3 as WGAL) and the remaining sites (SCr and SCz, also defined in chapter 3 as EGAL) to the east (Table 2.3).

Table 2.2 Pairwise F_{ST} values calculated using 8,103 neutral SNPs (above diagonal) and mtDNA control region using 997 bp (below diagonal).

	Isabela	Santa Cruz	San Cristobal
ISA	-	0.252*	0.251*
SCz	0.012 ^{ns}	-	0.005*
SCr	0.000 ^{ns}	0.004 ^{ns}	-

* *Significance at P < 0.05,* ^{ns} *Not Significant*

Table 2.3 AMOVA summarized results for *C. galapagensis* groups using SNP data and mtDNA. Fixation index were F_{ST}: 0.255* and F_{ST}: 0.000^{NS} respectively (p<0.05 Significance).

Variation source	**Sum of squares**		**Variance component**		**Percentage variation**	
Marker	SNPs	mtDNA	SNPs	mtDNA	SNPs	mtDNA
Among groups	21266	0.185	273.25	0.000	25.54*	0.00
Within groups	70766	31.670	66.39	0.427	6.21	100.00
Within individuals	61337	-	730.20	-	68.25	-
Total	153369	31.855	1069.86	0.427	100.00	100.00

P < 0.05* Significance

Individual cluster analyses using Structure were performed for two scenarios: including or excluding northern island samples. The most likely number of populations was identified by a DeltaK value of 2 in both scenarios, suggesting two differentiated populations: ISA was distinct from SCz and SCr, which grouped together; the northern islands samples also grouped with the eastern SCz-SCr population (Fig. 2.2A), but note very few specimens were available from the north (n = 4). Likelihood of K = 1 was low, and this scenario was discarded based on manual comparisons between values of posterior probability of data for all K tested, confirming K = 2. Likewise, the relative migration network indicated 96% or more bidirectional gene flow

between SCz and SCr, but less than 10% between either of these eastern locations and ISA (Fig. 2.2B). Similarly, the NetView P network analysis of Galapagos sharks (k-NN = 20) resolved and identified the same two main clusters: ISA and a single SCz-SCreastern cluster (Fig. 2.2C). This arrangement did not change throughout the range of k-NN values and network construction was not dependent on a priori population information. NeOGen simulations (Supplementary Fig.1) showed accurate estimations of LDN_e for sample sizes greater than 50 with a minimum of 100 loci. Finally, estimates of effective population sizes, LDN_e with 95% confidence were 171 and 205 for the western (ISA) and eastern (SCr and SCz) genetic stock, respectively.

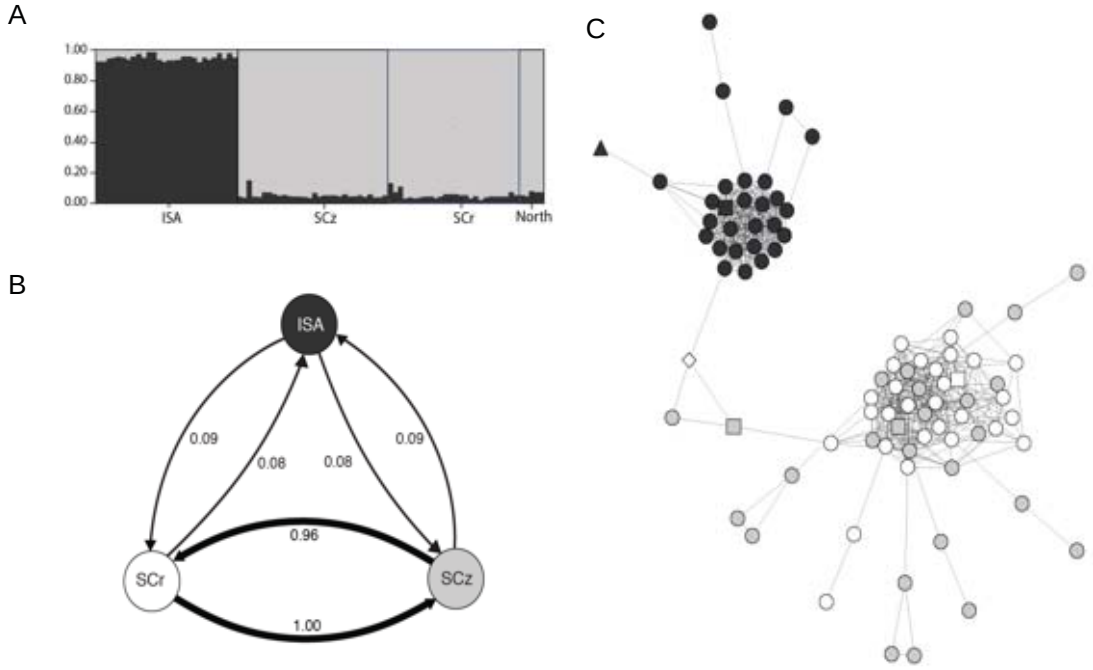

Fig. 2.2. Population structure analysis based on 8103 SNPs (A) Bar plots resulting from STRUCTURE analysis using all sampled populations - Isabela (ISA), Santa Cruz (SCz), San Cristóbal (SCr), and Darwin and Wolf (North). Each colour (dark and light grey) represents a population identified. (B) Relative migration network, indicating less than 10% gene flow between ISA and the eastern population (SCr, SCz), and at least 96% (bidirectional) gene flow between SCr and SCz. (C) Network from NetView P analysis using neutral SNPs of the Galápagos shark (*C. galapagensis*). Visualisation of 20 nearest neighbours (k-NN) from Isabela (dark grey), San Cristobal (white) and Santa Cruz (light grey), respectively. Mitochondrial haplotypes are represented by different shapes: circles and squares correspond to the most common haplotypes H1 and H2, respectively, triangles and diamonds indicate unique haplotypes for ISA (H4) and SCz (H9), respectively.

4.3 Mitochondrial DNA variation and structure

Mitochondrial control region sequences (997 bp) were analysed for 77 *Carcharhinus galapagensis* and two *Carcharhinus limbatus* individuals (outgroup) using phylogenetic analysis. We detected 51 variable sites (~7%), 47 of which were parsimony informative. An A–T base pair bias was evident (T = 30.3%, C = 13.7%, A = 35.0% and G = 21.0%) as reported for marine fish mtDNA (Mcmillan & Palumbi, 1995). The phylogenies produced under Maximum Likelihood and Bayesian inference criteria had congruent topologies. Neither tree contained spatially resolved *C. galapagensis* clades and depicted clades lacked bootstrap and posterior probability support (not shown). Maximum sequence divergence between *C. galapagensis* and *C. limbatus* was 5.03%. Twelve mtDNA haplotypes (*h*) were identified, 10 of which were *C. galapagensis* specific. Haplotype (*h*) and nucleotide (π) diversities of $h = 0.549$ ($\pm$0.060 standard deviation) and $\pi = 0.4\%$ were obtained for the sampled population as a whole. Two haplotypes (H1 and H2) dominated and occurred in 21.5% (17) and 63.3% (50) of individuals. Seven unique haplotypes (found in a single individual) were identified: two from each of the main sampled islands ISA, SCz, SCr, and one from the northern islands (H11). Another haplotype (H5) was shared between ISA and SCz (Fig. 2.3). Genetic distances based on mtDNA sequences ranged from 0.1 to 1% between the four locations. Within population genetic distances varied from 0.1 to 0.7% in ISA, 0.1 to 0.4% in SCr, and 0.1 to 0.9% in SCz. An AMOVA of mtDNA sequences indicated no variation among locations (NORTH, SCr, SCz and ISA), while most of the total variation was within locations. Pairwise F_{ST} values for the southern populations were not significant ($p < 0.05$) and ranged from 0.00 to 0.19. Intra-population diversity was lower for SCr (n = 22) than for SCz population (n = 29), while ISA (n = 24) had higher diversity (Table 2.1). Neutrality tests for population expansion were non-significant for Tajima's "D". However, Fu and Li's D* and F*, which detects an excess of old mutations were significant for the SCr population (-6.763 and -5.693, $p < 0.02$, respectively).

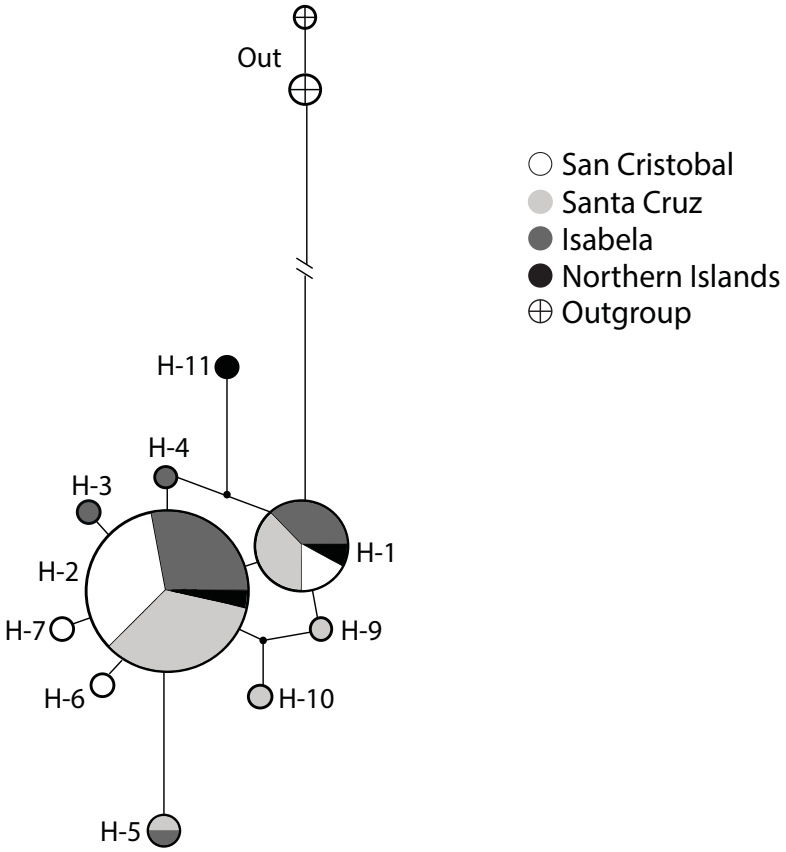

Fig. 2.3 *C. galapagensis* haplotype network, based on informative variable characters of the mtDNA control region. Each circle represents a haplotype, where circle size is proportional to haplotype abundance. Branch lengths represent the number of mutations between haplotypes.

5. Discussion

5.1 Connectivity and genetic differentiation in the Galápagos Islands

For this study, non-neutral (adaptive candidate) loci were removed in order to improve neutrality parameters and N_e estimates, and results presented here were obtained using loci conforming to neutral expectations only. Neutral genomic data provided for the first time small-scale population structure for Eastern Pacific *C. galapagensis*, and allowed us to identify two significantly divergent (25%) genetic groups between the south-western location (ISA), and the rest of the GMR archipelago. Cluster and Network analysis confirmed this differentiation

pattern, indicating that genome-wide single nucleotide polymorphisms (SNPs) are informative markers for Galapagos shark population structure within the Galápagos Islands.

We posit that this genetic structure may result from an intersection of ocean currents, which may shape the geographic distribution of this species. Waters from three different surface and subsurface currents affect the islands: (i) the warm Panama current, primarily influencing the northern islands; (ii) the cool Humboldt current, influencing southern and central islands (both aforementioned currents flow westward along the equator); (iii) the eastward flowing South Equatorial current, which encounters the western part of the archipelago, generating a productive upwelling system in the westernmost islands: Fernandina and Isabela (Houvenaghel, 1978). These marked changes in oceanographic conditions across the archipelago were reported by Edgar et al. (2004), who divided Galápagos coastal waters into five major marine bioregions based on faunal abundance and species richness data: (a) far northern, (b) northern, (c) south-eastern, (d) western, and (e) Elizabeth (Fig. 2.1). The southeastern and western regions each contain one of the two genetically differentiated populations identified here. Galapagos sharks could have specialized to the biota within each bioregion, and sharks' genetic structuring pattern might follow that of other fish they feed on (Salinas-de-León, pers. Comm). Additionally, Wolf et al. (2008) suggested divergence of the highly mobile Galapagos sea lions (*Zolophus wollebaeki*) between the western and eastern parts of the archipelago, based on ecological and genetic traits. Whilst water depth may be a factor affecting the separation of the archipelago into two groups, we do not favor this scenario, because the maximum depth between ISA and SCz (100–250 m), is less than that between SCr and SCz (250–500 m, Wolf et al., 2008). Other factors that may affect the population structure include geological history of the islands, demography, and colonization history of some locations, but the extent of the influence of these factors has yet to be examined.

Previous studies using neutral SNPs described low levels of genetic differentiation for other fish taxa, e.g. Atlantic herring, *Clupea harengus* (Lamichhaney et al., 2012; Nielsen et al., 2009). Importantly, Portnoy et al. (2015) found weak differentiation between populations of

bonnethead sharks (*Sphyrna tiburo*) along the north-eastern coast of the Gulf of Mexico using neutral compared to outlier loci. Similarly, when analysing Galapagos shark outlier loci (not shown), further stratification within the archipelago seemed to emerge, with SCr forming a potential separate cluster from SCz, illustrating greater sensitivity to detect fine-scale variation and potential for identifying local adaptation. However, in the absence of a reference genome to map and identify the functional significance of outliers, and the need to define reliable and well-supported conservation units, we base our conclusions and recommendations solely on neutral loci.

5.2 mtDNA phylogeography

There was no Galapagos shark population genetic structure within the GMR based on mtDNA, which was also supported by phylogenetic analyses. Lack of genetic structure using mtDNA was also reported for Pacific blue sharks (*Prionace glauca*, Taguchi, King, Wetklo, Withler, & Yokawa, 2015) and basking sharks (*Cetorhinus maximus*, Rus Hoelzel et al., 2006) between Pacific and Atlantic populations. Low mutation rates in elasmobranch mtDNA (Martin, Naylor, & Palumbi, 1992) or difference in statistical power between types of markers might underlie the relatively low nucleotide diversity and lack of population structure based on mtDNA data, as observed in this study. observed in this study. However, when compared to other widespread, pelagic marine species, including sharks and bony fishes (*Carcharhinus limbatus* $\pi = 0.0021 \pm 0.0013$, $h = 0.805 \pm 0.018$, Keeney, Heupel, Hueter, & Heist, 2005; *Carcharodon carcharias* $\pi = 0.0203$; Pardini et al., 2001; *Acanthocybium solandri* $\pi = 0.053$, $h = 0.999$; Garber, Tringali, & Franks, 2005), nucleotide diversity based on the control region was highly variable.

Discrepancies can occur between inferences from mtDNA and nuclear markers due to stochastic factors affecting mtDNA evolution (Moura et al., 2014). Also, sex biased migratory patterns, previously reported for highly migratory marine species (Pilot, Dahlheim, & Hoelzel, 2010) and some carcharhinid sharks such as *C. limbatus* (Keeney et al., 2003) can affect

genetic structure. However, unlike our findings, previous studies show stronger mtDNA structure and weaker nuclear structure, suggesting male-biased dispersal may be occurring in other sharks, but not in Galapagos sharks, where female biased dispersal is more likely, notwithstanding the vagaries of this marker. The lack of genetic differentiation using microsatellite markers in the aforementioned studies could result from either a lack of power due to small sample sizes or few loci, as well as homoplasy in microsatellites when compared with mtDNA (Carreras-Carbonell, Macpherson, & Pascual, 2006). In contrast, SNPs have low levels of homoplasy and coupled with the many genome-wide markers used in these analyses, allowed us to identify structure that was not evident from mtDNA. Such a pattern was also reported for yellowfin tuna (*Thunnus albacares*) using SNP markers (Grewe et al., 2015).

5.3 Conservation status of Galapagos sharks in the GMR

The GMR is a multiple use reserve based on zonation, including no take zones (Heylings, Bensted-Smith, & Altamirano, 2002) and has protected sharks since its creation in 1998. This protection has resulted in the largest recorded global shark biomass in the northern Galápagos Islands of Darwin and Wolf (Salinas-de-León et al., 2016). Local and international efforts are building on the development of knowledge to better understand the role of this MPA in protecting species and marine ecosystems (Hearn et al., 2014). Previous tagging studies and underwater surveys have improved our understanding of the ecology and dynamics of species such as hammerhead sharks in the Galápagos (Ketchum, Hearn, Klimley, Espinoza, et al., 2014). Additionally, a theoretical trophic model developed for the reserve suggests that shark populations might be recovering after a population size reduction due to a combination of overfishing and two of the strongest El Niño events (1982–1983 and 1997–1998) on record (Wolff, Ruiz, & Taylor, 2012). However, little is known about Galapagos shark ecology, and integrative studies including genetic approaches can help to better understand their conservation status.

By identifying at least two genetic stocks in the archipelago and estimating another three parameters relevant to their long-term viability: genetic diversity, gene flow levels between stocks and effective population size of stocks, I aim to improve management strategies of the Galapagos shark within the GMR. The Ecuadorian Government and responsible authorities can use such information for re-zoning the GMR, in an effort to enhance protection. Haplotype diversity for the sampled Galapagos sharks was low overall (h = 0.549) based on mtDNA, compared to other circumtropical distributed shark species (e.g. h = 0.75 for the shortfin mako shark *Isurus oxyrinchus*, Heist, Musick, & Graves, 1996; h = 0.80 for the scalloped hammerhead shark *Sphyrna lewini*; Duncan et al., 2006; and h = 0.89 for the Pacific blue shark *Prionace glauca*; Taguchi et al., 2015). Amongst stocks within the GMR, the ISA stock showed a lower haplotype and nucleotide diversity (h = 0.585, π = 0.018) than the SCr-SCz stock (h = 0.739, π = 0.053), suggesting ISA is a population of particular concern. Low levels of mitochondrial variation using the control region have been reported for species that have undergone extreme declines, such as *Carcharhinus sorrah* (Ovenden, Kashiwagi, Broderick, Giles, & Salini, 2009) and *Carcharias taurus* (Stow et al., 2006) in east and west Australia. This is relevant to management, because low genetic diversity increases extinction risk and reduces recovery rates of populations (Walsh, Munch, Chiba, & Conover, 2006) along with concerns of inbreeding depression, another factor that increases extinction risk, in the absence of immigration from elsewhere. Low levels of gene flow (<10%) between ISA and the SCz-SCr stock, suggest the ISA shark population might be experiencing demographic isolation and its management may require review. This is of less concern for the SCz-SCr stock, given strong levels of bidirectional gene flow (96–100%) between these sub-populations. The small effective population size of both *C. galapagensis* stocks in the southern Galápagos further highlight this concern, N_e being less than half of the minimum (N_e =500) value considered necessary for populations to persist in the long term (Franklin & Frankham, 1998; Palstra & Ruzzante, 2008). Indeed, the presence of kinship at several sampled locations is consistent with a risk of inbreeding associated with small populations. I acknowledge the possibility that juveniles (which represent most of the sampling effort presented here) and adults can show

different geographic patterns due to differential dispersal capabilities, and encourage the monitoring of both reproductive stages in future studies. I also acknowledge that the small sample size for the ISA population (n = 24) may reduce confidence in the N_e estimate obtained and that it should therefore be interpreted carefully. Nevertheless, when taken together the estimates of genetic diversity, level of gene flow and effective population size all point towards Galapagos sharks having an elevated risk of extinction from the southern Galápagos islands in the long term, particularly the ISA population. This information indicates that a differentiated management of both Galapagos shark stocks is required to reduce risk factors. Particularly worrying for the long-term survival of this species in the Archipelago is the high level of by-catch mortality associated with recent experimental long-lining efforts in the GMR, where hundreds of Carcharinid sharks were captured by a dozen vessels in under a year (Reyes et al., 2014). If this long-line fishery is to be approved for the entire Galapagos artisanal fleet (≈100 vessels), this could have catastrophic consequences for the population viability of Galapagos sharks in the archipelago. Samples from the northern islands were only obtained opportunistically and further directed sampling in this region is required for a genetic assessment. Although methodologically challenging, including individuals from different reproductive capacities (juveniles and adults) combined with the use of tagging and genetic information is required to better understand population structure patterns and to develop a more comprehensive management plan for Galapagos sharks throughout the GMR.

5.4 Implications

Worldwide, some coastal and oceanic shark species have suffered major population declines over the past 25 years (Baum et al., 2003; Dulvy et al., 2008, 2014), with by-catch accounting for 50% of estimated global chondrichthyan catches (Stevens et al., 2000). This, coupled with elasmobranch life histories makes them very susceptible to depletion and limits their recovery capability (Cortés, 2000; Simpfendorfer, 2000). The Galápagos Marine Reserve Management Plan recognizes that the zoning scheme in many cases lacks information on the distribution of local biodiversity, which makes it difficult to design no-take areas using

scientific criteria. Scalloped hammerhead has been recommended as an indicator species, mostly due to the increasing amount of information on its ecology and dynamics within the GMR. However, species-specific features of sharks highlight the importance of including data from other species and developing integrative analyses in order to provide more effective protection within the reserve. To date, little is known about Galapagos sharks in the archipelago, with most information coming from tagging and tracking research (Ketchum, Hearn, Klimley, Peñaherrera, et al., 2014). This study presents the first use of genomic data to investigate population structure of a shark species in the Galápagos Islands. Low (< 10%) levels of gene flow between the genetic stocks previously defined suggest they are demographically independent and should be considered as different Management Units. In order to maintain their genetic diversity, we recommend they should be managed as discrete stocks. Unequal pressure in different zones within the reserve, either due to fishing or oceanographic conditions, coupled with the presence of well-differentiated bioregions (Edgar et al., 2004) in the GMR, underline the need for better informed and thus improved management and conservation of marine species in the GMR. Based on low haplotype and nucleotide diversity, the ISA population is more susceptible to anthropogenic/environmental pressures when compared to the SCz–SCr population, indicating that each stock requires an individual management strategy. These two stocks are demographically independent and have relatively low N_e, along with relatively low genetic diversity, suggesting vulnerability to ongoing anthropogenic and environmental stresses in the long term. Such low effective population size (N_e) estimates either suggests recent colonization of the archipelago or that populations have recently experienced a bottleneck (Antao et al., 2011; Grant & Bowen, 1998). Our results advise against experimental long-lining practices within the Galápagos Marine Reserve, since high levels of associated shark by-catch and mortality will further increase vulnerability of these two stocks.

The use of genomic data to study geographically distinct populations may improve accuracy and quality of inferences about population structure, history, levels of gene flow, effective population size and demography (Ouborg et al., 2010). Analyses using thousands instead of tens

of markers have allowed fine-scale genetic structure to be delineated between two populations of Galapagos shark in the GMR, with clear implications for their management to prevent further population declines. Further genetic analyses including sampling at intermediate locations, and additional samples from the northern Islands are needed to identify how many Management Units occur across the archipelago. Finally, to improve management of Galapagos sharks worldwide, further work including connectivity and adaptive variation analyses are required at larger scales across the Pacific. Such analyses can be used to study footprints of selection and local adaptation, which will not only better inform the conservation of *C. galapagensis* across its range, but also enable the delineation of genetically distinct lineages. This is an important step for conservation, as it should enable management to preserve adaptive genetic variation and to maintain the evolutionary potential within Galapagos shark populations.

Chapter 3 – Phylogeography of the Galapagos shark (*Carcharhinus galapagensis*) across the Pacific Ocean

Contribution:

Diana Pazmiño and Madeline E. Green: data collection

Mauricio Hoyos-Padilla, Clinton A.J. Duffy, Carl G. Meyer, and Sven E. Kerwath collaborated with tissue samples and edition of the manuscript

Diana Pazmiño: data analysis, writing and editing chapter.

Lynne van Herwerden, Gregory Maes, Colin Simpfendorfer, Pelayo Salinas de León: Supervision and editing.

This chapter has been prepared as a publication entitled "**Strong trans-Pacific break and local conservation units in the Galapagos shark (*Carcharhinus galapagensis*) revealed by genome-wide cytonuclear markers**" and is currently submitted to *Heredity* with the following authors: Diana A. Pazmiño, Gregory E. Maes, Madeline E. Green, Colin A. Simpfendorfer, E. Mauricio Hoyos-Padilla, Clinton A.J. Duffy, Carl G. Meyer, Sven E. Kerwath, Pelayo Salinas-de-León, Lynne van Herwerden.

1. Abstract

The application of genome-wide cytonuclear molecular data to identify management and adaptive units at various spatio-temporal levels is particularly important for overharvested large predatory organisms, often characterized by smaller, localized populations. Despite being "near threatened", current understanding of habitat use and population structure of *Carcharhinus galapagensis* is limited to specific areas within its distribution. We evaluated population structure and connectivity across the Pacific Ocean using genome-wide Single Nucleotide Polymorphisms (~7200 SNPs) and mitochondrial Control Region sequences (945bp) for 229 individuals. Neutral SNPs defined at least two genetically discrete geographic groups: an East Tropical Pacific (Mexico, east and west Galapagos Islands), and another central-west Pacific (Lord Howe Island, Middleton Reef, Norfolk Island, Elizabeth Reef, Kermadec, Hawaii and Southern Africa). More fine-grade population structure was suggested using outlier SNPs: west Pacific, Hawaii, Mexico, and Galapagos. Consistently, mtDNA pairwise Φ_{ST} defined three regional stocks: east, central and west Pacific. Compared to neutral SNPs (F_{ST}=0.023-0.035), mtDNA exhibited more divergence (Φ_{ST}=0.258-0.539) and high overall genetic diversity (h=0.794 ±0.014; π=0.004 ±0.000), consistent with the longstanding Eastern Pacific Barrier between the east and central-west Pacific. Hawaiian and Southern African populations group within the west Pacific cluster. Effective population sizes were moderate/high for east/west populations (738 and 3421, respectively). Insights into the biology, connectivity, genetic diversity, and population demographics informs for improved conservation of this species, by delineating three to four conservation units across their Pacific distribution. Implementing such conservation management may be challenging, but is necessary to achieve long-term population resilience at basin and regional scales.

2. Introduction

The transition from conservation genetics to conservation genomics has lead to the development and increasing use of genome-wide genetic data capable of responding to complex ecological and evolutionary questions (Shawn R. Narum, Buerkle, Davey, Miller, & Hohenlohe, 2013; Pujolar, Jacobsen, Als, Frydenberg, Munch, et al., 2014; Savolainen, Lascoux, & Merilä, 2013). Identifying conservation units (CU), including Evolutionary Significant Units (ESU) and Management Units (MU), is an essential task for improved conservation of wild populations and to guarantee their evolutionary potential and long term persistence (Funk et al., 2012;

Savolainen et al., 2013). Importantly, the tremendous increase in number of markers facilitated through genotyping-by-sequencing (GBS) methods has enabled reliable assessments of genetic variation, relatedness, effective population size, and has increased the statistical power and resolution of population adaptation and phylogenetic structure analyses (Benestan et al., 2016; Larson, Seeb, Everett, et al., 2014; Portnoy et al., 2015; Portnoy & Heist, 2012). Accordingly, effective delimitations of ESUs and MUs enabling improved and informed management practices, especially for non-model organisms, is now possible (Hamon et al., 2017; Ouborg et al., 2010; Shafer et al., 2015; Willette et al., 2014). New molecular tools, including Single Nucleotide Polymorphisms (SNPs) now provide the opportunity to better understand and compare random versus adaptive genomic differentiation in wild populations. This in turn facilitates identification of functionally important genome regions to study the genetic basis of local adaptation at the population and species level (Vignal *et al.* 2002; Allendorf *et al.* 2010). Despite delimitation of CUs being a standard practice in conservation genetics/genomics, debate remains regarding the importance of correct identification of adaptive loci and their use to inform conservation (de Guia & Saitoh, 2007; Garner et al., 2016; Shafer et al., 2015), with a growing trend towards investigating statistical outlier loci and local adaptation (Candy et al., 2015; Steane et al., 2014; Vincent et al., 2013).

Documenting genetic differences in marine environments is challenging due to limited evident gene flow barriers (Selkoe, Henzler, & Gaines, 2008; Waples, 1998), especially for highly migratory species such as sharks (Portnoy et al., 2014; Portnoy & Gold, 2012). Barriers such as ocean currents, geographic distance, habitat discontinuity, or differential dispersal ability can be responsible for population structure in marine organisms (Dawson, Louie, Barlow, Jacobs, & Swift, 2002; Baums, Boulay, Polato, & Hellberg, 2012). A good example of this within the Pacific Ocean is the Eastern Pacific Barrier (EPB) - a 4000-7000 km stretch of ocean lacking intermediate islands - that separates the eastern from the central and west Pacific (Briggs, 1974; Lessios & Robertson, 2006; Gaither, Bowen, Rocha, & Briggs, 2016). Whilst the advance of Next Generation Sequencing (NGS) techniques has increased access to these cost-effective genome-wide markers (Allendorf et al., 2010; Willette et al., 2014), they have not yet been

widely used for Chondrichthyan studies (Portnoy et al., 2015). Conservation genetics studies of globally distributed sharks have traditionally used a combination of mitochondrial DNA (mtDNA) sequences and nuclear microsatellites to investigate population structure (Daly-Engel et al., 2012; Karl et al., 2010; Keeney & Heist, 2006; Portnoy et al., 2010). The combination of both marker types has permitted identification of historic and current population demographic patterns, genetic diversity, and connectivity at the intra-specific level. These studies also have provided evidence for differential dispersal patterns between sexes (Daly-Engel et al., 2012; Portnoy et al., 2010). However, despite being widely used, conventional nuclear markers such as microsatellites present limitations (including homoplasy, null alleles, and shifts in allele size caused by mutations in flanking regions) to population structure investigations (Balloux *et al.* 2000; Portnoy & Heist 2012).

Chondrichthyans have experienced increasingly intensive fishing and habitat degradation pressure over recent decades. It is estimated that a hundred million sharks are killed annually and over-fishing has resulted in the loss of over 90% of sharks and large predatory fishes across all ocean basins (Dulvy et al., 2014; Myers & Worm, 2003; Polidoro et al., 2012; Worm et al., 2013). Currently, the IUCN Red List for Threatened Species estimates one quarter of all shark and ray species are at risk of extinction (Dulvy *et al.* 2014). Common biological characteristics of chondrichthyans such as slow growth, late maturation and low fecundity (Hoenig & Gruber, 1990) limit their recovery from anthropogenic pressure and lead to low resilience. These characteristics make it challenging to define a single conservation strategy for sharks, and proper management requires individual species assessments (Clarke et al., 2015). Understanding aspects of the biology, habitat use and population demographics is the first step towards improved conservation and management of sharks and rays, yet 50 percent are IUCN listed as Data Deficient (Dulvy et al., 2014), highlighting the need for more shark population structure and monitoring data for most species. Information on distribution patterns and population connectivity is crucial to avoid local depletion when a species is composed of more than one breeding unit (Clarke et al., 2015; Shivji, 2010).

The Galapagos shark (*Carcharhinus galapagensis*, Snodgrass & Heller 1905) is a circumtropically distributed species with preference for isolated oceanic islands and seamounts in tropical and warm temperate waters (Compagno, 1984; Wetherbee et al., 1996). However, there have been some reports of individuals in open ocean habitats (Kohler et al., 1998). Studies of Galapagos shark behavior associate different depth preferences to different life history stages: adults preferring deeper - and juveniles shallower habitats (Lowe et al., 2006; Meyer et al., 2010). Others have shown reverse diel vertical movements by juveniles, which prefer deeper waters at night and shallower waters during daytime; seasonal changes in horizontal and vertical movements (Papastamatiou et al., 2015). Previous studies of Galapagos shark movements in Hawaii, based on acoustic telemetry, have shown that individuals remain within a range of approximately 30 km for periods of up to four years (Lowe et al., 2006; Meyer et al., 2010; Papastamatiou et al., 2015). Most acoustic tagging from Hawaii and mark-recapture data from the Atlantic are congruent, indicating considerable site attachment in the species. However, occasionally some individuals migrate long distances >2000 km (Kohler *et al.* 1998; C. Meyer unpublished data). A recent Galapagos shark population genetic assessment in the southern Galápagos Islands identified two management units separated by only 50-60 km using mtDNA and SNPs (Pazmiño, Maes, Simpfendorfer, & van Herwerden, 2017).

The Galapagos shark is “Near Threatened” according to the IUCN Red List of Threatened species (Bennett et al., 2003). However, information about population structure and connectivity across most of its distribution range is still lacking, and current knowledge of habitat use and population structure is limited to specific areas (Kohler *et al.* 1998; Meyer *et al.* 2010; Papastamatiou *et al.* 2015; Pazmiño *et al.* 2017). We performed a large-scale genetic assessment of Galapagos sharks across the Pacific Ocean using both nuclear genome-wide SNPs and mtDNA control region sequences. I aimed to: 1) assess the phylogeographic patterns and potential sex-biased dispersal signals of Galapagos sharks across the Indo-Pacific, 2) estimate the level of divergence within regionally defined populations using statistical outliers, and 3) estimate the effective population sizes (N_e) for each defined genetic population to inform conservation and management of the Galapagos shark.

3. Material and Methods

3.1 Tissue collection and DNA extraction

This study examined samples from nine locations across the Pacific Ocean and also included a few individuals from the southwestern Indian Ocean. Five southwest Pacific locations were sampled: Elizabeth (ELZ) and Middleton (MID) Reefs, Lord Howe (LHW), and Norfolk Islands (NOR) from Australia; Raoul Island (Kermadec Islands, KER) from New Zealand. One central Pacific location, Hawaii (HAW) and three east Pacific locations were sampled: the Revillagigedo Islands, Mexico (MEX) and the Galápagos Islands in Ecuador (EGAL and WGAL). Samples from the Galápagos Islands are those from Pazmiño et al. (2017). Additionally, a token sample (3 individuals) representing the southwest Indian Ocean was obtained from an isolated shallow seamount (Walters Shoals), 600 km east of South Africa, (SAF) (Fig. 3.1A). Given the small sample, SAF could not be included in a range of population genetic analyses. Genomic DNA was extracted from fin clips using a modified salting out protocol (Sunnucks & Hales, 1996), DNA concentrations were spectrophotometrically (NanoDrop 1000, Thermo Scientific) estimated and DNA integrity was electrophoretically verified using 0.8% agarose in 1x TBE buffer. Neat and diluted aliquots of extracted DNA were stored at -20°C.

3.2 Nuclear SNP marker development using Genotype by Sequencing.

Following DNA extractions, a quality control step involving a test restriction digest was performed at 37 °C for 3 hours in a volume of 22uL containing 5uL neat DNA, 2uL NEBuffer, 0.2uL *EcoRI* enzyme and 14.8 uL DNase/RNase-free distilled water. Digestion controls contained all reagents as above, except *EcoRI*. Digestion was terminated by a 20 minute incubation at 65 °C. Finally, 12.5uL of digested, undigested and neat DNA were electrophoresed through a 0.8% agarose gel in 1 x TBE for 45 minutes at 100V and visualized using Biotium Gel-Green. Only high quality DNA obtained from this trial was sent for library preparation and sequencing at Diversity Arrays Technology (DArT PL) in Canberra, Australia.

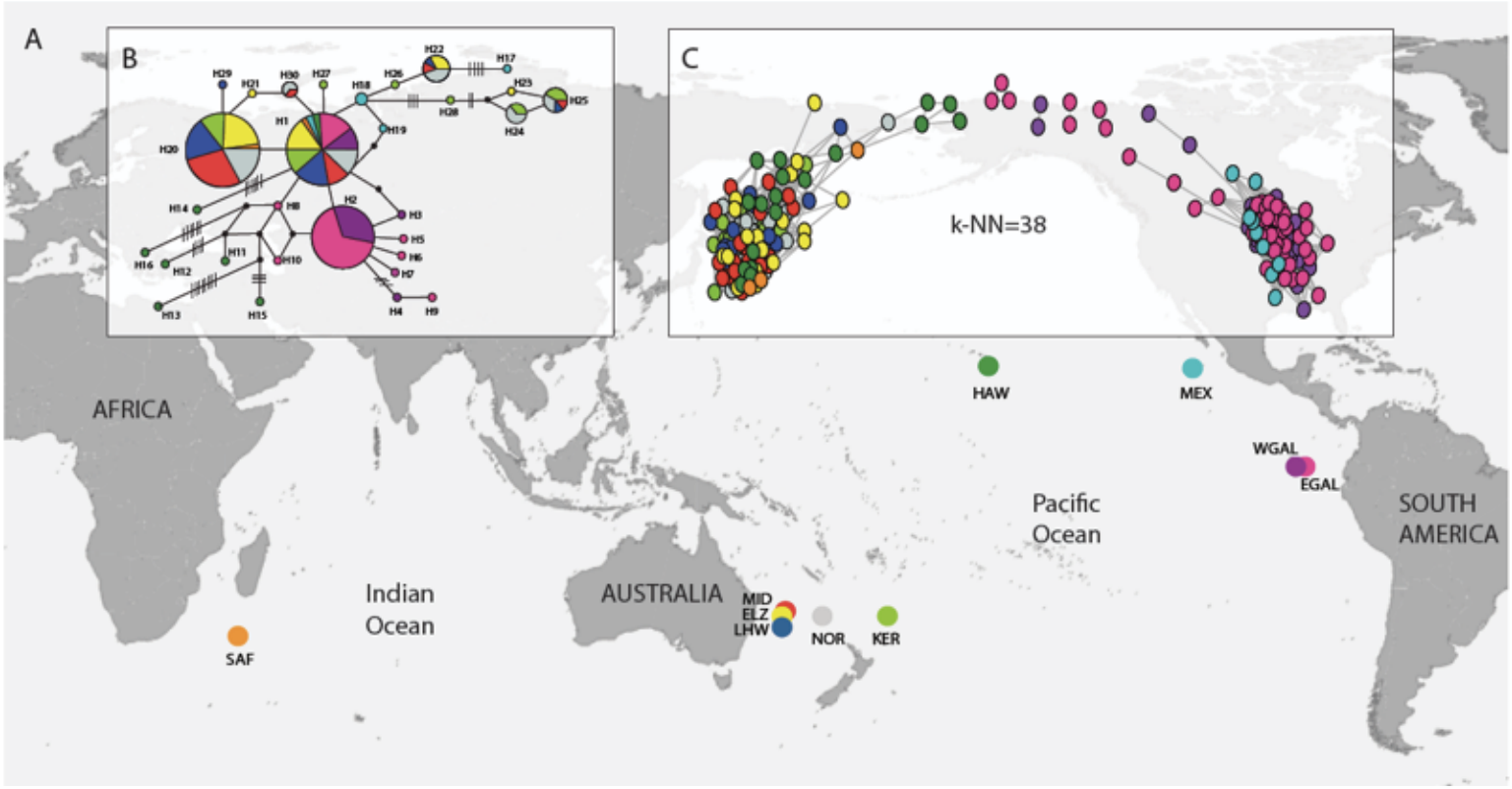

Fig. 3.1 [A] Sampling locations of Galapagos sharks across the Pacific and Indian Ocean as follows: west Pacific locations - Elizabeth (ELZ) and Middleton (MID) Reefs, Lord Howe Island (LHW), Norfolk Island (NOR), Kermadec Island (KER); east Pacific locations - Revillagigedo Islands in Mexico (MEX), east and west Galapagos Islands (EGAL and WGAL); central Pacific - Hawaii (HAW); and west Indian Ocean - Walters Shoal, South Africa (SAF). [B] Haplotype network of 229 mt control region sequences of Galapagos sharks. Sizes of circles are proportional to haplotype frequencies. Each dash crossing a branch represents one mutation between haplotypes. [C] Population network of 200 individuals and 7,274 neutral SNPs using the NetViewP pipeline. The network reconstruction is based on an Identity by Similarity (IBS) distance matrix and visualized at a maximum number of nearest neighbor (k-NN) threshold of 38.

Following a second DNA quality evaluation by DArT, double digestion was performed using methylation-sensitive restriction enzymes to digest 150-200 ng of gDNA. The resultant fragments were ligated to barcoded adaptors and amplified using PCR. PCR products were then standardised in concentration and pooled for sequencing on a single HiSeq 2500 (Illumina) lane to yield approximately 2.5 million reads per sample. Preparation and sequencing of libraries was performed by DArT as per Sansaloni et al. (2010) and Kilian et al. (2012). SNPs were jointly developed and genotyped following standard procedures applied by DArT. For a comprehensive description of SNP calling and DArT quality filtering processes, I refer to Pazmiño *et al.* (2017). The initial dataset consisted of 57,341 polymorphic SNP loci, and downstream SNP quality control steps were performed before further analysis in order to reduce low-quality and uninformative data (Larson, Seeb, Everett, et al., 2014). Only loci with a call rate >85% were retained; the threshold for Minor Allele Frequencies (MAF) was determined at 2%. Linkage disequilibrium was tested using PLINK v2.050 (Purcell et al., 2007) by calculating the correlation coefficient of alleles at two loci, independent of allele frequency. Finally, I tested Hardy-Weinberg equilibrium (HWE) using GENODIVE v2.0 (Meirmans & Van Tienderen, 2004). Loci displaying significant deviation from HWE expectations in all populations ($p<0.01$) were removed.

Output files obtained from the afore-mentioned procedures were first converted manually into a GENEPOP format file to be transformed into various other formats using PGDSpider v2.0.6.0 (Lischer & Excoffier, 2012). In order to identify statistical outlier loci I used two simulation approaches for the whole data set, the first approach was implemented in LOSITAN Selection Detection Workbench (Antao et al., 2008), and the second was implemented in ARLEQUIN. For analyses at the “within region level”, where hierarchical genetic structure is no longer required, the ARLEQUIN approach was replaced by an approach implemented in PCADAPT R package (Luu, Bazin, & Blum, 2016). LOSITAN uses a coalescent-based simulation approach to identify loci with unusually high or low pairwise F_{ST} values compared with pairwise F_{ST} values expected under neutrality to assess the relationship between F_{ST} and

expected heterozygosity (H_e). Three independent runs were computed within a 95% confidence interval; an infinite alleles model was used with 100,000 iterations evaluating the Benjamin-Hochberg (1995) False Discovery Rate (FDR). ARLEQUIN performs coalescent simulations examining the joint null distribution of hierarchical F_{ST} and H_e and estimates p-values for each locus, while considering the hierarchical genetic structure of the data using a hierarchical island model (Slatkin & Voelm, 1991). Hierarchical genetic structure was determined based on neutral variation and p-values were corrected using the Benjamin-Hochberg (1995) FDR method. The statistical method implemented using the PCADAPT R package detects outlier loci based on Principal Component Analysis (PCA) by assuming that markers excessively related with population structure are candidates for local adaptation. P-values were adjusted with a Benjamin-Hochberg (1995) FDR correction as implemented in the QVALUE R package (Storey, 2015). To define the Neutral data set, all detected putative outliers were removed from the data. Loci were then divided into two data sets: one including the neutral SNPs only, the other including outlier SNPs only.

Pairwise F_{ST} and expected (H_e) heterozygosity values were calculated for each locus of both data sets using ARLEQUIN, and were also independently evaluated using GENETIX v4.05 (Belkhir et al., 2004). Significance of pairwise F_{ST} values was assessed by running 10,000 permutations. Discriminant Analysis of Principal Components (DAPC; Jombart, Devillard, & Balloux, 2010) was also performed as an initial analysis of population structure for neutral and outlier loci independently, using the ADEGENET package in R Studio v0.98.977 (Jombart & Ahmed, 2011; R Development Core Team, 2008). Each individual was assigned to a predefined population (based on geographic location) for this analysis an α-score optimisation was used to determine the number of principal components to retain.

The partitioning into putative genetically distinct populations was performed using the clustering approach implemented in STRUCTURE v2.3.4 (Pritchard et al., 2000), which investigates the likelihood that a sample belongs to K populations (K representing any number) based on allele frequencies at each locus. Data was analyzed for both neutral and outlier data sets using K values ranging from 1 to 10, with 10 independent iterations, one million Markov

Chain Monte Carlo (MCMC) repetitions and an independent allele frequency burn-in of 100000. The most likely number of populations (K) was defined according to the DeltaK statistic as calculated using STRUCTURE HARVESTER webv0.6.93 (Earl & vonHoldt, 2011). This was validated by hand in order to test for K=1 specifically, since this is not otherwise evaluated (Evanno et al., 2005; Pritchard & Wen, 2003) and was followed by population network analysis using NETVIEW P (Neuditschko, Khatkar, & Raadsma, 2012; Steinig et al., 2016) in order to reveal fine and large scale genomic structure between and within populations. After performing an identity by similarity (IBS) distance matrix reconstruction using PLINK (which relies on allele-sharing distance, ASD), the NETVIEW P implementation calculates a minimum spanning tree based on the matrix, and finally the nearest neighbor network is constructed for different thresholds of the maximum numbers of nearest neighbors that can be connected by edges during construction of the network (k-NN) (Steinig et al., 2016) ranging from 10 to 100.

A phylogenetic analysis was performed to examine any underlying phylogenetic partitions in the data. This was done using the maximum likelihood (ML) criterion and required SNP data to be formatted into a hapmap file using a customized R script, which was analyzed using SNPHYLO (Lee, Guo, Wang, Kim, & Paterson, 2014), a pipeline specifically developed for large SNP data sets. The tree reconstruction was performed on a subset of 15 individuals from each sampling location (if available) in order to reduce computational time. Elizabeth and Middleton Reefs were considered as a single location due to proximity and genetic similarity observed based on F_{ST} values in the present and previous analyses (van Herwerden *et al.* 2008). The Galápagos Islands population was split into two: east (EGAL) and west (WGAL) Galápagos according to Pazmiño *et al.* (2017). Three samples of the sister species, *Carcharhinus obscurus,* were used as outgroup in the analysis. A total of 1,000 bootstrap replicates were performed to gauge support for identified phylogenetic structure.

Finally, contemporary effective population size was calculated based on the Linkage disequilibrium method (N_{eLD}) for each population using NEESTIMATOR v.2.01 (Do et al., 2014) following an initial power assessment using NEOGEN software (Blower et al., in

preparation). This software incorporates life-history characteristics specific to the Galapagos shark to estimate the appropriate number of loci and individuals in order to accurately calculate N_e. Alleles with frequencies below critical values (P_{Crit} of 0.02 and 0.05) were removed. Each population was previously filtered for linked loci in PLINK. Based on a standard measure of linkage disequilibrium (r^2), I selected two thresholds (r^2 = 0.10 and 0.20), and all loci above those thresholds were removed from the data to prevent LD bias in the N_e calculation considering the number of genome wide SNPs used.

3.3 Mitochondrial DNA sequencing and analyses

The control region (mtDNA) was amplified using Polymerase chain reaction (PCR) and GoTaq Flexi DNA polymerase (Promega). PCR primers were selected from Pardini *et al.* (2001): light strand ProL2 (5'-CTG CCC TTG GCT CCC AAA GC-3', and Keeney *et al.* (2003): heavy strand 282H (5'-AAG GCT AGG ACC AAA CCT-3'. These primers have been successfully tested on Galapagos sharks (Pazmiño et al., 2017; van Herwerden et al., 2008) . Reactions, PCR conditions and visualization were carried out following Pazmiño *et al*. (2017). Cleaned-up products were sent to Georgia Genomics Facility (http://dna.uga.edu, USA) for sequencing in forward and reverse directions. Forward and reverse sequences were assembled into contigs, trimmed to 945 bp, edited and aligned in GENEIOUS v5.4.7 (http://www.geneious.com, Kearse *et al.* 2012).

Genetic diversity of the mtDNA control region was assessed as number of haplotypes, haplotype (h), and nucleotide (π) diversity within each locality using ARLEQUIN v.3.5.1.2 (Laurent Excoffier & Lischer, 2010). An analysis of molecular variance (AMOVA) was also performed in ARLEQUIN. Pairwise Φ_{ST} was estimated after 10,000 permutations in order to detect population genetic partitioning between locations using ARLEQUIN. Correction for multiple testing was performed following the FDR procedure (Benjamini & Hochberg, 1995). Additionally, we tested for demographic population expansion and reduction by calculating Tajima's D (Tajima, 1989) in DNASP v4.10 (Rozas et al., 2003). All positions containing missing data were eliminated for this purpose. MtDNA control region sequences were used for phylogenetic reconstruction under the Maximum Likelihood (ML) method using default settings

of the software MEGA 6.06 (Tamura et al., 2007). The model of sequence evolution was estimated using Partition Finder v1.1.0 (Lanfear et al. 2012) and posterior parameter distributions were examined using Tracer v.1.6 (Rambaut et al. 2014). A total of 1,000 bootstrap replicates were performed. Finally, an haplotype network was calculated and drawn using NETWORK v4.2.0.1(Bandelt, Forster, & Röhl, 1999) with a Median-joining algorithm and based on Maximum Parsimony.

4. Results

4.1 Neutral and outlier SNPs variation

A total of 208 individuals, including two *C. obscurus* were successfully genotyped for SNPs. After the first quality check step, including call rate and Minor Allele Frequency filters, the number of loci was reduced from the initial 57,341 to 8,368 SNPs for 206 *C. galapagensis*. A total of 26 SNPs failed to conform to HWE across all populations and were removed from the data set. Ten pairs of loci were identified as linked (r^2>0.2); subsequently one locus from each pair was randomly selected and deleted.

The number of outliers identified by different approaches varied and differed between data subsets. The whole Indo-Pacific outlier data set contained 31 and 559 loci using ARLEQUIN and LOSITAN, respectively. All loci detected by ARLEQUIN were common between both approaches. All outliers detected by both methods were removed to ensure a purely neutral Pacific-wide data set of 7,274 SNPs in the first instance. At the regional scale, the central-west region (consisting of HAW, ELZ, MID, NOR, LHW, KER and SAF) contained 27 outliers common to both methods (LOSITAN and PCADAPT). All outliers, common and LOSITAN/PCADAPT-specific, were removed from the central-west Pacific data, with a total of 6,476 neutral SNPs remaining. Finally, the east Pacific group (consisting of MEX, EGAL and WGAL) contained 13 common outliers (LOSITAN and PCADAPT). The east Pacific neutral data set contained a total of 6,852 loci after removing these outliers. To define non-neutral data sets, loci were only considered as statistical outliers if detected by both analyses (Supplemental Table 3).

Heterozygosity values for neutral SNPs varied from 0.194 (±0.110) in South Africa (SAF) to 0.237 (±0.118) in Mexico (MEX) (Table 3.1). The maximum likelihood tree from neutral SNP data (Fig. 3.2) showed geographic structure and supported differentiation between east and central-west Pacific (including SAF) populations with strong support (99%) for a monophyletic clade containing all samples from SAF, HAW and the rest of west Pacific populations. East Pacific samples (WGAL, EGAL and MEX) did not form a single monophyletic sister clade to the SAF-central-west Pacific clade, but were distributed across several highly supported sister clades. Fine-scale structure of the global population using neutral SNPs, examined using NETVIEW P analysis at various k-NN thresholds ranging from 10 to 100, consistently identified two distinct genetic clusters at k-NN ranging from 35 to 40. The best clustering pattern was identified at k-NN=38 (Fig. 3.1C), as before: a SAF, HAW and west Pacific cluster and an east Pacific (MEX, EGAL and WGAL) admixed cluster. Broad-scale population structure of neutral SNPs was further tested independently using DAPC with prior group membership defined by locality, and this revealed a similar pattern seen based on both NETVIEW P and pairwise F_{ST} estimations (Fig. 3.3A). Galapagos shark population subdivision was also strongly supported by STRUCTURE analyses, which tests for the presence of distinct populations assuming a number of subpopulations (K) (between two and ten, Fig. 3.3B). The strongest and most likely substructure pattern corresponded to K=2 based on DeltaK statistics computed in STRUCTURE HARVESTER. All results consistently highlighted an east versus central-west Pacific genetic break for Galapagos shark. Neutral loci for the central-west Pacific within region cluster failed to identify further population structure (Fig. 3.3C), but SAF and HAW were differentiated from the west Pacific (Australian and New Zealand) populations using outlier SNPs (Fig. 3.3D). Similarly, within the eastern Pacific (where neutral SNPs failed to differentiate between the three sampling locations, Fig. 3.3E) outlier SNPs identified using either LOSITAN (n=234, Fig. 3.3F) or PCADAPT (n=346, data not shown) independently indicated differentiation between MEX and the Galápagos Islands (there were insufficient common loci detected by these methods (due to different assumptions of each method – Finite island model vs no assumptions about population demographics, respectively).

Table 3.1 Summary statistics averaged for 7,784 Neutral SNPs and 945 bp of mitochondrial control region: number of individuals sequenced (*n*), expected heterozygosity (*He*), number of haplotypes (*H*), haplotype diversity (*h*), and nucleotide diversity (π) for each sampling locality (west Galapagos, WGAL; east Galapagos, EGAL; Mexico, MEX; Lord Howe Island, LHW; Middleton Reef, MID; Norfolk Island, NOR; Elizabeth Reef, ELZ; Kermadec, KER; Hawaii, HAW; and South Africa, SAF)

mtDNA	WGAL	EGAL	MEX	LHW	MID	NOR	ELZ	KER	HAW	SAF
n	24	49	6	27	30	30	29	23	8	3
H	4	8	4	5	5	6	5	7	7	2
h	0.543	0.492	0.866	0.621	0.514	0.774	0.645	0.790	0.964	0.666
	± 0.084	± 0.077	± 0.129	± 0.056	± 0.088	± 0.052	± 0.065	± 0.055	± 0.077	± 0.314
π	0.00856	0.00826	0.02895	0.01479	0.01316	0.03529	0.01661	0.03968	0.13636	0.00673
	± 0.007	± 0.006	± 0.020	± 0.010	± 0.009	± 0.020	± 0.011	± 0.023	± 0.078	± 0.008
SNPs	WGAL	EGAL	MEX	LHW	MID	NOR	ELZ	KER	HAW	SAF
n	27	54	12	19	19	17	18	19	18	3
He	0.229	0.230	0.237	0.206	0.206	0.207	0.207	0.205	0.202	0.194
	± 0.110	± 0.109	± 0.118	± 0.099	± 0.099	± 0.100	± 0.100	± 0.099	± 0.098	± 0.110

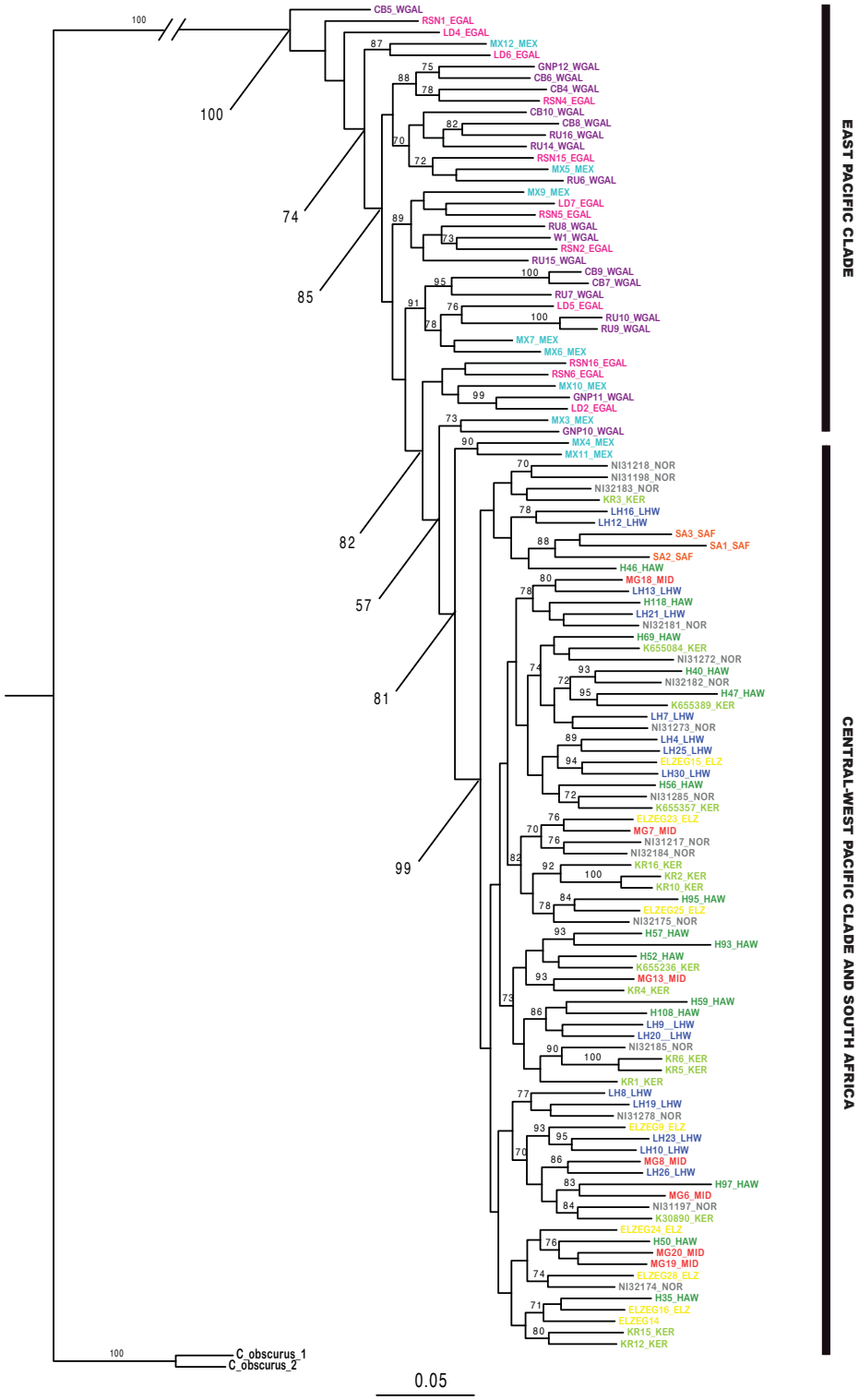

Fig. 3.2 Outgroup rooted Maximum Likelihood phylogram of *C. galapagensis* generated using SNPhylo software from Neutral SNPs and 1000 bootstrap replicates. Two *C. obscurus* individuals were used as out-group. Only bootstrap values > 50% are shown.

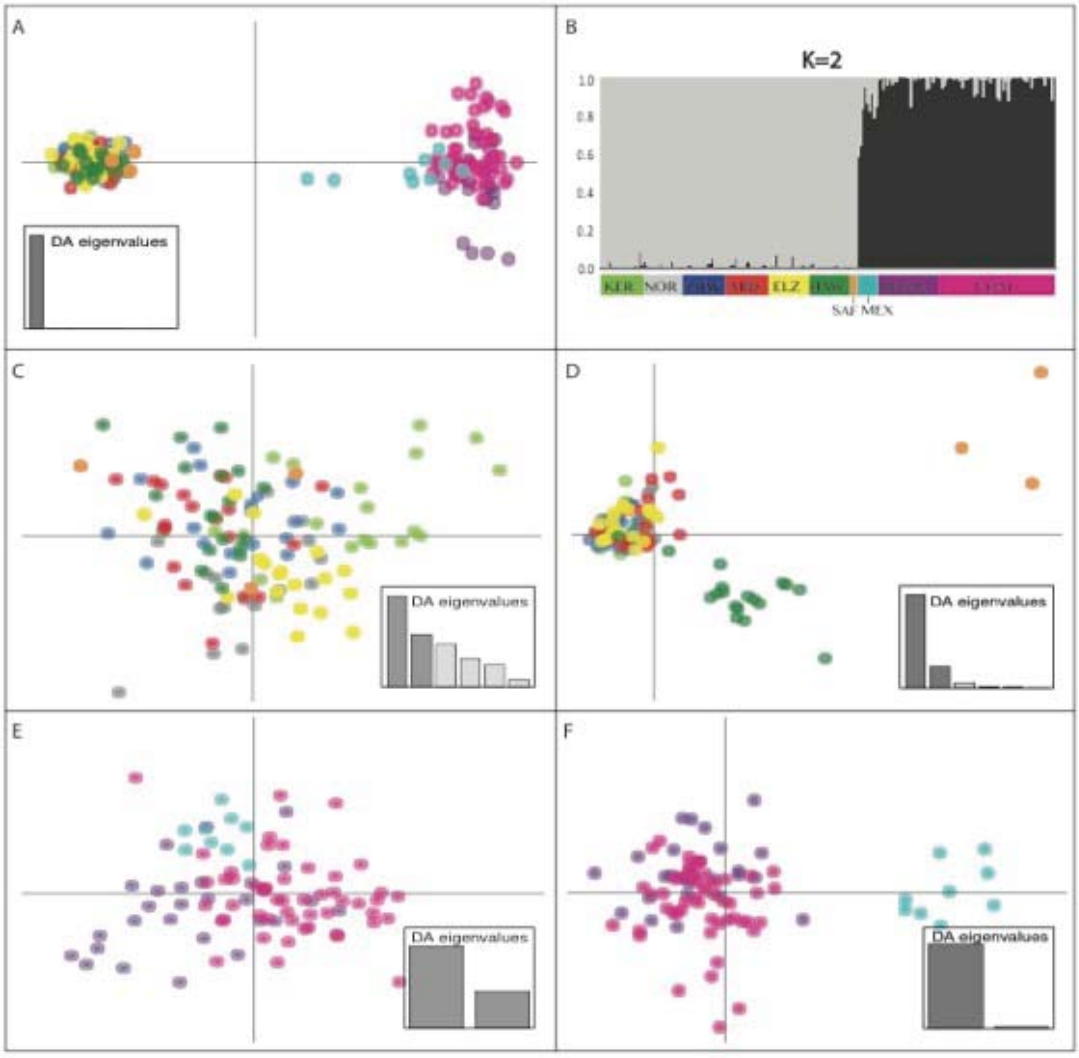

Fig. 3.3 Population genetic structuring of Galapagos sharks from across the Indo Pacific based on either neutral or outlier SNP data. Panels A-C and E are derived from neutral SNPs and panels D and F are derived from outlier SNPs at different spatial scales as detailed below. [A] Discriminant Analysis of Principal Components (DAPC) scatterplot of all locations sampled using 7,274 Neutral SNPs, drawn in the R package ADEGENET. Each dot represents an individual of *C. galapagensis*, and colors represent the population of origin: Elizabeth (ELZ, yellow) and Middleton (MID, red) Reefs, Lord Howe Island (LHW, dark blue), Norfolk Island (NOR, grey), Kermadec Island (KER, light green), Revillagigedo Islands in Mexico (MEX, pale blue), east and west Galapagos Islands (EGAL, pink and WGAL, purple), Hawaii (HAW, dark green), and Walters Shoals off Southern Africa (SAF, orange). Group membership was defined by sample locality and colours detailed here also apply to panels B - F. [B] Population assignment and clustering for K=2 calculated for 7,274 neutral SNPs from all locations sampled, using STRUCTURE software. [C] DAPC scatterplot at the within region level, using 6,476 neutral SNPs for the central-west Pacific genetic cluster (HAW, SAF, KER, NOR, LHW, ELZ, and MID). [D] DAPC scatterplot for the within region level, using 27 outlier SNPs from the central-west Pacific genetic cluster (HAW, SAF, KER, NOR, LHW, ELZ, and MID); [E] DAPC scatterplot from 6,852 neutral SNPs within the east Pacific cluster (MEX, WGAL and

EGAL). [F] DAPC scatterplot from 234 outlier SNPs within the east Pacific cluster (MEX, WGAL and EGAL).

Effective population size (N_{eLD}) estimates for the two - east and central-west Pacific - populations were consistently recovered from all analyses (including STRUCTURE). Using an r^2=0.20 threshold, the east Pacific population (n=87) N_{eLD} was estimated to be 820 (P_{Crit}=0.02) to 738 (P_{Crit}=0.05); while the central-west Pacific population (n=110) N_{eLD} ranged from 4618 (P_{Crit}=0.02) to 3421 (P_{Crit}=0.05). A more conservative threshold of r^2=0.10 was also tested with no significant changes of estimated N_e for either population (Table 3.2).

Table 3.2 N_{eLD} estimated using neutral SNPs for the genetic clusters recovered by structure analyses: east Pacific (n=87), and west Pacific (n=110). Values presented for Allele frequency below four Critical values (0.05, 0.02, 0.01, and 0)

		0.05	0.02	0.01	0
East Pacific	N_{eLD}	738.1	779.5	800.2	819.9
	95% CI	724.4-752.2	766.5-793.0	787.1-813.8	806.3-833.9
West Pacific	N_{eLD}	3420.9	4023.1	4229.2	4617.8
	95% CI	3172.8-3710.7	3743-4348.2	3939.3-4564.8	4298.3-4988.4

4.2 mtDNA genetic variation

A total of 229 *C. galapagensis* and three *C. obscurus* individuals were successfully sequenced for the mitochondrial control region (945 bp). Of the 945 base pairs (bp), 99 were polymorphic (10.4%), and 65% of these were parsimony informative. Summary statistics for mtDNA showed overall mtDNA haplotype (*h*) and nucleotide (π) diversity was 0.794 (±0.014) and 0.004 (±0.000) respectively (Table 3.1). Hawaii had the highest overall haplotype and nucleotide diversity (*h*=0.964 ±0.077; π=0.136 ±0.078). A total of 30 different mtDNA haplotypes were identified. Three common haplotypes: Hap1, Hap2 and Hap20 represent 79.4% of the individuals (Fig. 3.1B). The remaining 20.5% individuals either shared haplotypes with nine (or fewer) individuals or contained unique haplotypes. Haplotype 1 occurred across the entire Pacific and in SAF. Haplotype 2 occurred in individuals from the Galápagos Islands (east Pacific) exclusively and haplotype 20 was restricted to Australia, New Zealand (southwest

Pacific) and SAF. The AMOVA revealed significant differences between the east and central - west Pacific regions (Table 3.3). Additionally, variation among and within localities was also significant and explained 4.13% and 66.26% of the total variation, respectively. Estimates of population pairwise Φ_{ST} and F_{ST} indicated a pattern of broad scale phylogeographic structure (Table 3.4). The Φ_{ST} between the two Galápagos Islands populations (EGAL and WGAL) was low and non-significant. However, when comparing the Galápagos Islands with all remaining locations, both Galápagos populations were significantly different before and after FDR correction, with values ranging from 0.301 to 0.539 ($p<0.05$). Additionally, significant differentiation was detected when comparing MEX and LHW ($\Phi_{ST}=0.258$), and MEX and MID ($\Phi_{ST}=0.340$). A similar pattern of significant differentiation between the east Pacific (MEX, WGAL and EGAL) and central-west Pacific (LHW, MID, NOR, ELZ, KER and HAW) populations was observed with F_{ST} ranging from 0.024 between MEX and HAW, to 0.035 between WGAL and KER. Mitochondrial data also revealed significant differences between HAW and Australian populations LHW, MID, NOR and ELZ.

Table 3.3 Hierarchical AMOVA results based on mtDNA. Regions are east Pacific (west Galapagos, WGAL; east Galapagos, EGAL; Mexico, MEX); central -west Pacific – Indian Ocean (Lord Howe Island, LHW; Middleton Reef, MID; Norfolk Island, NOR; Elizabeth Reef, ELZ; Kermadec, KER; Hawaii, HAW; and South Africa, SAF).

Source of variation	d.f	Sum of squares	Variance components	Percentage of variation	P values
Among regions	2	61.249	0.49362	29.61	<0.001
Among localities within regions	8	20.585	0.06886	4.13	<0.001
Within localities	218	240.856	1.10484	66.26	<0.001

Results from the Maximum Likelihood analysis of mtDNA using the G+I (gamma distributed with invariant sites) evolutionary model, displayed a poorly supported phylogenetic tree (Fig. 3.4). High Bootstrap values were observed only in two clades: the first one including samples from HAW, and a second clade represented by samples from ELZ, KER, NOR, LHW and MID. Neutrality tests for population expansion showed significantly negative values for Tajima's D in the central-west Pacific populations ($D=-2.114$ $p<0.05$), while non-significant Tajima's D values were found for the east Pacific group members (MEX, EGAL and WGAL).

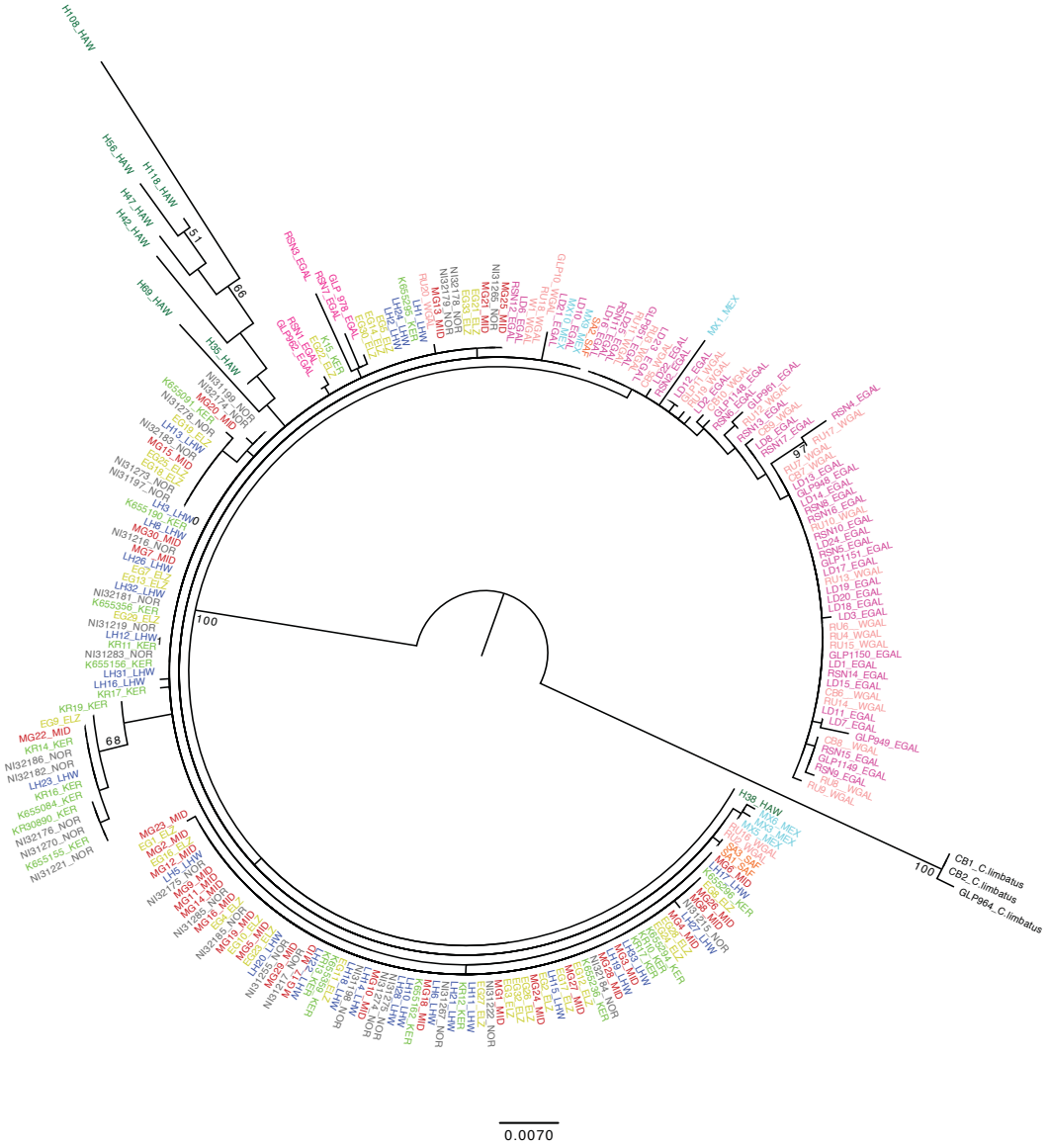

Fig. 3.4 Maximum Likelihood phylogenetic tree of the mitochondrial control region showing relationship between sampling locations: EGAL, WGAL, MEX, LOR, MID, NOR, HAW, SAF, ELZ and KER. Bootstrap support values higher than 50% are shown in the branches. Colors represent the population of origin: Elizabeth (ELZ, yellow) and Middleton (MID, red) Reefs, Lord Howe Island (LHW, dark blue), Norfolk Island (NOR, grey), Kermadec Island (KER, light green), Revillagigedo Islands in Mexico (MEX, pale blue), east and west Galapagos Islands (EGAL, pale pink and WGAL, pink), Hawaii (HAW, dark green), and Walters Shoals off Southern Africa (SAF, orange).

Table 3.4 Estimates of Φ_{ST} values for pairwise comparisons of sampling localities using mtDNA control region below diagonal, and F_{ST} values based on neutral SNPs above diagonal. Sampling localities as follows: Lord Howe Island, LHW; Middleton Reef, MID; Norfolk Island, NOR; Elizabeth Reef, ELZ; Kermadec, KER; Hawaii, HAW; and South Africa, SAF. Shading values correspond to significance at $P < 0.05$.

	WGAL	EGAL	MEX	LHW	MID	NOR	ELZ	KER	HAW	SAF
WGAL	-	0.002	0.002	0.033*	0.033*	0.032*	0.034*	0.035*	0.030*	0.015
EGAL	0	-	0.002	0.031*	0.031*	0.030*	0.032*	0.032*	0.028*	0.013
MEX	0.459*	0.539*	-	0.024*	0.024*	0.023*	0.025*	0.025*	0.024*	0.005
LHW	0.392*	0.464*	0.258*	-	0.001	0.001	0.002	0.001	0.000	0.000
MID	0.460*	0.519*	0.340*	0.000	-	0.002	0.003	0.003	0.002	0.000
NOR	0.301*	0.390*	0.049	0.062	0.089	-	0.001	0.002	0.000	0.000
ELZ	0.395*	0.467*	0.222	0.000	0.000	0.041	-	0.002	0.002	0.000
KER	0.318*	0.414*	0.056	0.104	0.140	0.000	0.098	-	0.002	0.001
HAW	0.376*	0.504*	0.135	0.325*	0.364*	0.282*	0.334*	0.258	-	0.008
SAF	0.489*	0.536*	0.190	0.000	0.000	0.000	0.000	0.000	0.000	-

* Significantly different values between populations at $p < 0.05$.

5. Discussion

Overall, global results are congruent with at least two Galapagos shark populations: one on either side of the Pacific Ocean and possibly three (east, central and west Pacific), four (Galápagos, Mexico, Hawaii and west Pacific) or more (the former and additional under- or un-sampled) Galapagos shark populations when taking more subtle marker-specific results into account. Specifically, we caution that the apparent lack of an Indian Ocean population is inconclusive and note that additional samples from southern Africa and elsewhere in the Indian Ocean are required to properly examine the Indo-Pacific wide population structure of Galapagos sharks. Geographic structure within the east and west Pacific *C. galapagensis* populations may also be further resolved with additional samples. Herein, neutral SNPs, outlier SNPs and mtDNA suggest a range of population structures within the Pacific (Figures 3.1 and 3.3). Similarly, in sandbar sharks (*Carcharhinus plumbeus*) mtDNA control region analysis identified divergence between Hawaii and the east coast of Australia ($\Phi_{ST} = 0.467$), whereas nuclear microsatellites did not (F_{ST}=0.062, Portnoy *et al.* 2010). Population structure and phylogeny based on neutral SNPs placed Hawaii (and Walters Shoals, southern Africa) within the west

Pacific population; Mexico and Galápagos within the East Tropical Pacific population. In contrast, outlier SNPs differentiated Hawaii (with under sampled Walters Shoals) from the west Pacific, and Mexico from the Galápagos population. A similar pattern showing isolation of Hawaiian populations was reported for the coral *Porites lobata* (Baums et al. 2012) and the fish *Acanthurus triostegus* (Lessios & Robertson 2006). A strongly supported phylogenetic link (based on the SNP phylogeny) between east and west Pacific lineages via two Mexican animals suggests *C. galapagensis* entered the central-west Pacific from the east Pacific via Mexico.

Population structure analyses are self-referential, and consequently the geographic scale of analysis influences results, with subtle differences in structure more likely to be significant in regional than global analyses. For example, a regionally focused neutral SNP analysis of Galapagos shark population structure within the Galapagos archipelago identified two distinct populations (EGAL and WGAL; Pazmiño *et al.* 2017), whereas results from our current Pacific-wide analysis indicate that the Galapagos Islands all belong to a single regional genetic group (using neutral and outlier SNPs), which forms a separate cluster from Mexico (using outlier SNPs only). Only ~12% of polymorphisms are shared between the dataset from Pazmiño *et al.* (2017) and our current dataset due to specific SNP filtering criteria. Hence, we highlight the importance of including both regional and global scale assessments to accurately inform conservation at different geographic scales. We did not find any regional population structure within the southwest Pacific (ELZ, MID, LHW, NOR, and KER) using either mtDNA, neutral or outlier SNPs (data not shown), despite local populations being separated by relatively large geographic distances.

Although neutral SNPs differentiate east from central and west Pacific Galapagos shark populations, mtDNA shows that (1) MEX is significantly different from LHW and MID but not from other central and west Pacific locations, hinting at connectivity between regions, possibly due to very few migrants per generation; (2) the HAW population is differentiated from most other locations, except KER and MEX, suggesting some level of connectivity between Mexico, Hawaii, and the Kermadec Islands (New Zealand); and (3) differentiation within the east

Pacific, separating MEX from the Galápagos Islands (EGAL and WGAL, Φ_{ST} = 0.459-0.539). This third pattern could be due to secondary barriers between MEX and the Galápagos Islands, generating historical geographic isolation. Alternatively low sample sizes (MEX n=6, HAW n=8) could be affecting such patterns. Importantly, an acoustic telemetry study of Galapagos shark movements within the East Tropical Pacific (n=76) indicated *C. galapagensis* is a highly resident species, with most migrations occurring within a range of 0-50 km (Lizardi *et al.* in prep). In addition, no movement was recorded between Revillagigedo and the Galápagos Islands, despite including intermediate locations (potential stepping-stones). Bonnethead sharks (*Sphyrna tiburo*) show similar asymmetry between neutral SNPs and mtDNA in the west Atlantic region, possibly due to sex-biased dispersal (Portnoy et al., 2015), and also exhibit strong population structure indicative of a species complex rather than a single species (Fields, Feldheim, Gelsleichter, Pfoertner, & Chapman, 2016).

Galapagos sharks showed high overall genetic diversity (a total of 30 mtDNA haplotypes), and mtDNA haplotype and nucleotide diversities (h= 0.794 ± 0.014; π= 0.004 ± 0.000) within the range of other oceanic shark species (h = 0.595 to 0.959; and π = 0.0013 to 0.013 (Camargo et al., 2016; Chabot & Allen, 2009; Clarke et al., 2015; Duncan et al., 2006; Keeney & Heist, 2006; Portnoy et al., 2010). Among all populations, Hawaii had the highest haplotype and nucleotide diversity. The presence of divergent haplotypes with many mutations in this population suggests multiple colonization events from neighboring locations, further supporting Hawaii as an important location linking east and west Pacific populations, likely via Mexico in the east and New Zealand in the west. Additional sampling, including intermediate South Pacific Islands, is needed to better determine structure and patterns of movement and colonization in the central-south Pacific. MtDNA of Galapagos sharks exhibited a greater (compared to neutral SNPs) or smaller (compared with outlier SNPs) magnitude of divergence. This phenomenon identifies differences in patterns of gene flow based on neutral vs statistical outlier nuclear markers, and based on mitochondrial (maternal only) vs nuclear (biparental) markers (Daly-Engel *et al.* 2012; Chabot 2015). Notably, other globally distributed live-bearing

shark species purportedly displayed evidence of female philopatry and male-mediated gene flow (Chapman, Feldheim, Papastamatiou, & Hueter, 2015), based on tagging and genetic (mitochondrial and putatively neutral microsatellite markers) data (e.g. *Carcharhinus limbatus*, Hueter *et al.* 2004; Keeney *et al.* 2005; *C. plumbeus*, Portnoy *et al.* 2010). Additionally, mark-recapture and genome-wide SNP data have detected philopatry in bonnethead sharks (*Sphyrna tiburo*, Driggers *et al.* 2014; Portnoy *et al.* 2015). However, the absence of genetic differentiation (mtDNA, neutral and outlier SNPs) within the west Pacific region suggests female Galapagos sharks are not philopatric, indicating that evidence for "natal philopatry" needs to be carefully examined prior to asserting sex-biased dispersal.

Galapagos sharks are capable of crossing extensive swathes of open ocean, evident from their broad geographic distribution (Compagno, 1984), and also by empirical observations of tagged individuals swimming across up to 2,000 km of ocean between remote Pacific islands (e.g. French Frigate Shoals, Hawaii and Palmyra Atoll, C. Meyer unpublished data). Despite this inherent capacity for long-distance movements and hence gene flow, we found clear evidence of at least two (east Pacific and central-west Pacific) and possibly four (west Pacific, Mexico, Galápagos Islands and Hawaii) Galapagos shark populations in the Pacific. Reliance on shelf habitats for crucial aspects of their ecology may ultimately explain the population structure seen in this potentially wide-ranging shark. Galapagos shark diet is composed largely of reef-associated organisms (Wetherbee et al., 1996), whilst juvenile Galapagos sharks form aggregations over reefs (Compagno, 1984), suggesting that both foraging and natal ecology are tied to shelf habitats. Results based on outlier SNPs support the biogeographic provinces defined by Glynn & Ault (2000), which separate mainland Ecuador, Costa Rica, the Galapagos Archipelago and Cocos Island (Equatorial province) from mainland Mexico and the Revillagigedo Islands (Northern province) based on reef building coral species. This is consistent with empirical tracking studies showing Galapagos sharks to be highly reliant on oceanic islands, with most individuals showing long-term (up to 9 years, C. Meyer unpublished data) fidelity to a single, or several closely-adjacent, islands, and repeated use of the same

insular shelf habitats (0-200m depth) (Papastamatiou et al., 2015). Thus oceanic islands apparently serve as important connecting steps for Galapagos shark dispersal within the Pacific Ocean. The EPB limits connectivity and gene flow for a range of tropical marine taxa (Rocha *et al.* 2007; Van Cise *et al.* 2016) including corals (*Pocillopora damicornis*, Combosch & Vollmer, 2011 and *Porites lobata*, Baums et al., 2012), fish (*Myripristis berndti*, Craig, Eble, Bowen, & Robertson, 2007); *Doryrhamphus excisus* and *Cirrhitichthys oxycephalus*, Lessios & Robertson, 2006), and lobsters (*Panulirus penicillatus*, Chow et al., 2011), and is a plausible explanation for the existence of at least two genetically distinct Galapagos shark populations within the Pacific. Furthermore, studies using mitochondrial control region and microsatellites also support the EPB as an important barrier defining phylogeographic structure for other globally distributed species, including tope sharks (*Galeorhinus galeus*, Chabot 2015; Chabot & Allen 2009) and silky sharks (*Carcharhinus falciformis*, Clarke *et al.* 2015). Notwithstanding, our results highlight the presence of migrants between the central-west and east Pacific regions. This pattern has been previously detected in sea urchins (Lessios, Kane, & Robertson, 2003) and fish (Lessios & Robertson 2006). The study by Lessios & Robertson (2006) reported examples of fish populations occurring on the two sides of the EPB with an extreme level of divergence (*Doryrhamphus excisus* and *Cirrhitichthys oxycephalus*), as well as transpacific species with populations that have recently, or continue to exchange genes (*Myripristis berndti*, *Stethojulis bandanensis* and *Zanclus cornutus*), demonstrating that the EPB is not completely impassable, but rather a barrier that is permeable for several marine species, including Galapagos sharks.

Our findings have important implications for management and conservation of Pacific Galapagos sharks. We found strong divergence between two Pacific populations, but also identified connections between the east Pacific (via Mexico) and the central Pacific (via Hawaii) to New Zealand in the southwest Pacific, indicating that effective management requires protecting both demographically (albeit restricted) interconnected stocks, and the associated intermediate locations. The intra-regional Galapagos shark population structure identified by

analysis of statistical outlier loci may indicate regional adaptive variation that may hinder or prevent effective replacement of extirpated sub-populations (Clarke et al., 2015). However, we acknowledge processes other than local adaptation may be responsible for significant structure based on outlier loci (Bierne et al., 2013; Cruickshank & Hahn, 2014) and further investigation is required to confirm their functional nature.

Our genetic effective population size (N_e) estimates suggest Pacific Galapagos sharks are currently genetically healthy overall, with the central-west Pacific Galapagos shark stock having almost five-fold more breeding individuals than the east Pacific population (Table 3.2). However, N_e estimates for the two Galápagos Islands sub-populations were low (Pazmiño et al., 2017), emphasizing the need for appropriate, regional management. Overall, our study highlights the importance and potential impacts of using genome-wide genetic data for applied Galapagos shark conservation. Using a dramatically increased number of variable genetic markers compared to previous studies (van Herwerden et al., 2008) has lead to a precise estimation of diversity and population demographic parameters, including effective population size, relevant for the species conservation.

We highlight the importance of using both neutral and outlier markers to better understand population structure and genetic diversity of the species at a global scale to efficiently delimitate conservation units, and ultimately to achieve effective conservation and management in the short and long term. Our understanding of population structure may be enhanced by studying additional material from under- (Mexico and Walters Shoals) and unsampled (e.g. the Indian and Atlantic Oceans) intermediate locations across the Galapagos shark distribution. In order to enhance long-term conservation efforts of Galapagos sharks we further recommend evaluating the stability of identified regional populations and regular monitoring of each identified stock in order to document temporal demographic changes within stocks. Informing for improved conservation management of this near threatened shark species across its Pacific Ocean distribution was relatively straightforward and necessary, but implementing such Pacific-wide management may be challenging.

Chapter 4 – Introgressive hybridisation between *Carcharhinus galapagensis* and *Carcharhinus obscurus* in the east Pacific

Contribution:

Diana Pazmiño and Claudia Junge: data collection

Mauricio Hoyos-Padilla, Stephen Donnellan, Clinton A.J. Duffy, Charlie Huveneers, and Bronwyn Gillanders collaborated with tissue samples and editing of the manuscript

Diana Pazmiño: data analysis, writing and editing chapter.

Lynne van Herwerden, Gregory Maes, Colin Simpfendorfer: Supervision and editing.

This chapter has been prepared as a publication entitled "**Introgressive hybridisation between *Carcharhinus galapagensis* and *Carcharhinus obscurus* in the east Pacific**" and will be submitted to *Molecular Ecology* with the following authors: Diana A. Pazmiño, Lynne vanHerwerden, Colin Simpfendorfer, Claudia Junge, Stephen C. Donnellan, E. Mauricio Hoyos-Padilla, Clinton A. J. Duffy, Charlie Huveneers, Bronwyn Gillanders, Gregory E. Maes.

1. Abstract

With a few documented cases of ongoing hybridisation in cartilaginous fish to date, shark hybridisation remains poorly investigated. Historical admixture between *Carcharhinus galapagensis* and *Carcharhinus obscurus* has been hypothesised. I sampled many individuals from contact zones where both species co-occur across the Pacific Ocean, and used a combination of mitochondrial and nuclear genome-wide single nucleotide polymorphisms (SNP) markers to examine genetic admixture and introgression between Galapagos and dusky sharks. Using empirical analytical approaches and simulations, I identified a set of 1873 highly informative, reliable SNPs that distinguish these two species. Overall, my results suggest high discriminatory power of SNPs (F_{ST}=0.47, p<0.05) between the two species, unlike mitochondrial DNA, which fails to differentiate the two species (Φ_{ST} = 0.00 p>0.05). We identified four hybrid individuals (~1% of samples) based on allele frequencies of these SNPs and detected bi-directional introgression between *C. galapagensis* and *C. obscurus* in the Gulf of California and along the east Pacific coast of the Americas. Importantly, four cases of misidentification were also detected between our target species and two other *Carcharhinus* species (*C. falciformis* and *C. brachyurus*), using mitochondrial control region. Given the morphological similarities between these four species, we emphasize the importance of including a combination of mtDNA and genome-wide diagnostic markers to assess taxonomic identification, detect patterns of hybridisation, and better inform management and conservation of these sharks.

2. Introduction

Natural hybridisation challenges the biological concept of species. It refers to two different populations or species successfully interbreeding and producing viable “hybrid” offspring (Arnold, 1997; Mayr, 1982). Both hybridisation and introgression, which is the incorporation of alleles from one species into another (Anderson, 1949; Harrison & Larson, 2014), are important factors for evolutionary diversification (Mallet, 2005; Seehausen, 2004, 2006). Molecular genetic techniques have been used commonly to assess species delimitation

and for studying hybridisation and introgression in freshwater species, and have been helpful to demonstrate the evolutionary consequences associated with these processes (Hemmer-Hansen *et al.*, 2014). While hybridisation processes have been well-documented in terrestrial and freshwater organisms, particularly plants (Mallet, 2005; Hemmer-Hansen *et al.*, 2014; Pujolar *et al.*, 2014; Kelley *et al.*, 2016), emerging evidence of this process in coral reef bony fish is leading the way towards a better understanding of processes such as adaptation and speciation in the marine environment, especially due to hybrids' potential to adapt to changing environments and occupy unexploited ecological niches (van Herwerden *et al.* 2006; Montanari *et al.* 2012, 2016; Hobbs & Allen 2014; DiBattista *et al.* 2015). In contrast to the bony fishes, hybridisation in cartilaginous fishes remains largely unstudied. Morgan *et al*. (2012) described the first case of ongoing interspecific hybridisation between two shark species in eastern Australia - *Carcharhinus tilstoni* and *C. limbatus* (blacktip shark). The authors suggest the sparseness of hybridisation records might be the result of the difficulties of observing hybrids between species that show slight phenotypic differences (Morgan et al., 2012). Additionally, Cruz *et al*. (2015) identified for the first time interspecific hybridisation between the freshwater stingrays *Potamotrygon motoro* and *P. falkneri* in the Parana River (South America) using morphological and genetic (mtDNA, microsatellites and SNP) data.

Furthermore, in comparison with bony fishes' external fertilization reproductive strategy, Chondrichthyans have internal fertilization involving mate choice, which adds an additional pre-zygotic barrier to hybridisation within this taxonomic group (Last & Stevens, 2009). Despite their important role in maintaining ecosystem dynamics and health (Heupel et al., 2014), many shark species are under threat from fishing and habitat loss (Dulvy et al., 2014). Studying and understanding the role of hybridisation (if occurring) in commercially important species with taxonomy issues, such as dusky and Galapagos sharks, is essential to achieve appropriate species identification, and therefore to monitor catches and produce accurate estimations of population productivity to ensure long-term sustainable fisheries

(Ovenden et al., 2010; Morgan et al., 2012; Portnoy & Heist, 2012; Simpfendorfer & Dulvy, 2017; Tillett et al., 2012), which requires healthy populations.

Although efforts to resolve phylogenetic relationships within elasmobranchs are continuously increasing, molecular genetic techniques to discriminate sharks at the species level can have limited resolution as they mostly rely on short mitochondrial sequences or DNA-barcoding (Fields et al., 2015; Holmes et al., 2009; Naylor et al., 2012; Naylor et al., 2016; White & Last, 2012). Among the most common markers used for phylogenetic studies are the fast-evolving mtDNA control region and protein-coding genes NADH dehydrogenase subunit 2 (NADH2), NADH dehydrogenase subunit 4 (NADH4), Cytochrome oxidase 1(COI), Cytochrome b (Cyt-b) (López, Ryburn, Fedrigo, & Naylor, 2006; Naylor et al., 2012; Straube et al., 2013). Species delimitation based on this approach is particularly problematic for species that have diverged recently or that have been or still are hybridising, and which might still share ecological, morphological, and reproductive compatibilities (Choleva et al., 2014; Montanari, Hobbs, Pratchett, Bay, & Van Herwerden, 2014).

Galapagos (*Carcharhinus galapagensis*, Snodgrass and Heller, 1905) and dusky (*C. obscurus*, Lesueur 1818) sharks are morphologically similar closely-related species (Garrick, 1982). Precaudal vertebral counts (PVC) and dorsal fin heights are the main morphological characters used to distinguish them (Garrick, 1982). They also differ in habitat preference. The Galapagos shark has a circumglobal distribution in tropical and warm temperate regions, inhabiting mostly isolated oceanic islands, and has a PVC ranging from 103-109 (Compagno, 1984; Garrick, 1982; Wetherbee et al., 1996). Despite a few recorded cases of *C. galapagensis* migrating distances of over 2000 km, acoustic telemetry studies from Mexico and Hawaii indicate their preference to remain within 30-50 km of their home range, after being tagged (Lizardi et al., in preparation; Kohler *et al.* 1998; Lowe *et al.* 2006; Meyer *et al.* 2010; Papastamatiou *et al.* 2015). The dusky shark also has a circumglobal tropical to warm temperate distribution, but it usually inhabits continental shelves and near-shelf waters and has a PVC ranging from 86–97 (Camhi, Pikitch, & Babcock, 2008; Compagno, 1984; Garrick, 1982; Last

& Stevens, 1994; Rogers, Huveneers, Goldsworthy, Mitchell, & Seuront, 2013). Tagging studies of *C. obscurus* indicate that long seasonal migrations are common (Davies and Joubert, 1967; Bass *et al.*, 1973; Kohler *et al.*, 1998). Importantly, the distribution of Galapagos and dusky sharks only overlaps in a few regions, namely Cabo Pulmo National Park, inside the Gulf of California, northeast Pacific Ocean (Lizardi et al., in preparation; Last and Stevens, 1994); Revillagigedo Islands on the west coast of Mexico, Eastern Pacific (Garrick, 1982); and Norfolk Island off the east coast of Australia, southwest Pacific (Duffy, 2015). Additionally, occasionally Galapagos and dusky sharks have been reported to co-occur along the Ecuadorian mainland coast, east Pacific Ocean (Bearez, 2015).

Morphological similarities between these and other *Carcharhinus* species means that misidentification is not uncommon (Duffy, 2016; Garrick, 1982; Naylor, 1992; Ovenden et al., 2010; Portnoy & Heist, 2012; Tillett et al., 2012). Previous genetic studies using mtDNA have failed to distinguish Galapagos and dusky sharks. For example, Naylor *et al.* (2012) questioned the validity of Galapagos and dusky sharks after the mitochondrial gene NADH2 (~1044 bp) failed to distinguish between them. However, Corrigan *et al.* (2017) recently confirmed that *C. galapagensis* and *C. obscurus* are differentiated lineages using a combination of mtDNA and nuclear Single Nucleotide Polymorphisms (SNPs), thereby demonstrating the importance of including different marker classes to define species relationships within *Carcharhinus*, and the relevance of genome-wide data to achieve accurate taxonomic identification in recently diverged systems with limited genomic resources. Corrigan et al. (2017) concluded historic hybridisation had occurred between the two species, since a small amount of admixture was observed in Galapagos sharks from the Indo-Pacific and dusky sharks from the Atlantic. However, the authors were unable to detect signals of contemporary ongoing hybridisation. We hypothesized that the reported apparent lack of ongoing hybridisation may be the result of limited sampling from a single contact zone (Norfolk Island). Under this premise, we predict that genetic exchange may be occurring between Galapagos and dusky sharks in other contact zones (e.g. along the western Mexican coast), and that it should be possible to detect this by sampling a large number of individuals and using genetic markers capable of detecting low

levels of admixture, such as SNPs. SNPs, generated by next generation sequencing (NGS) techniques are genome-wide distributed markers that possess sufficient discriminatory power to investigate admixture levels between divergent taxa (Allendorf et al., 2010; Hohenlohe et al., 2013; Wiley, Qvarnström, Andersson, Borge, & Sætre, 2009). They have been successfully used to confirm hybrid classes and introgression levels between freshwater stingrays (Cruz et al., 2017), and more importantly, between two morphologically indistinguishable *Carcharhinus* species: *C. limbatus* and *C. tilstoni* (Maes et al., in prep). We aimed to: 1) evaluate the divergence between Galapagos and dusky sharks using a combination of mtDNA and SNPs; 2) develop a panel of diagnostic SNPs to investigate the extent of introgression between *C. galapagensis* and *C. obscurus*; and 3) assess the power and resolution of the mitochondrial control region to detect cases of misidentification within the genus *Carcharhinus.*

1. Methods

1.1 Sample collection and DNA extraction

A total of 208 *C. galapagensis* and 209 *C. obscurus* sharks were sampled from across the Indo-Pacific Ocean. Species ID of both Galapagos and dusky sharks was genetically validated from previous analyses (Pazmiño et al., 2017; C. Junge pers Comm). SNPs for all the individuals from both species were generated as detailed in Pazmino et al. (2017). MtDNA control region sequences for 23 Galapagos and 23 dusky sharks were sequenced as per Pazmino et al. (2017) (Table 4.1, Figure 4.1A). Additional *C. obscurus*, *C. brachyurus*, *C. falciformis*, and *Sphyrna lewini* mtDNA control region sequences were obtained from GenBank to assess species misidentification within *Carcharhinus* (Table 4.2). DNA was extracted from fin clips following a modified salting out protocol from Sunnucks and Hales (1996) as detailed in (Pazmiño *et al.*, 2017). DNA quality and concentration were determined spectrophotometrically using a NanoDrop 1000 (Thermo Scientific) instrument and agarose gel electrophoresis (0.8% in 1 X TBE containing gel green).

1.2 mtDNA amplification and sequencing

I amplified the mitochondrial control region using a PROMEGA GoTaq Flexi DNA polymerase kit and control region primers (light strand ProL2 5'-CTG CCC TTG GCT CCC AAA GC-3' and heavy strand 282H 5'-AAG GCT AGG ACC AAA CCT-3') (Pardini *et al.,* 2001; Keeney *et al.,* 2003). PCR reactions were carried out in 25 µl volumes containing 5.0 µl PCR buffer [5X], 1.5 µl $MgCl_2$ [2.5mM], 0.5 µl [2mM] deoxynucleotide triphosphates (dNTPs), 0.5 µl each of the forward and reverse primers, each at [10 pmol], 0.125 µl Taq DNA polymerase (5 Units, PROMEGA) and 1 µl of diluted DNA [at a concentration of 10-25 ng/µl]. PCR cycling conditions included an initial denaturation at 95^{o}C for 2 minutes, 30 cycles at 95^{o}C for 30 seconds, 53^{o}C for 30 seconds, and 72^{o}C for 1.5 minutes, and a final extension of 72^{o}C for 10 minutes. Pre-stained Biotium Gel-Green agarose gels (1.5%) were used to visualize PCR products. I cleaned PCR products using Sephadex G50 spin columns and sent cleaned-up products to Georgia Genomic Facility (USA) for sequencing.

Table 4.1 Locations and sample sizes of the Pacific Galapagos shark (*C. galapagensis*) and dusky shark (*C. obscurus*) populations genotyped for hybridisation and introgression assessment using neutral genome-wide SNPs, including four individuals detected as hybrids.

Species	Location	Country	n	Diversity indices		
				$H_{n.b}$ (±SD)	H_o(±SD)	p-value
C. galapagensis	Kermadec Island	New Zealand	19	0.109 (±0.159)	0.109 (±0.167)	0.348
	Middleton Reef	East Australia	19	0.107 (±0.156)	0.108 (±0.167)	0.349
	Elizabeth Reef	East Australia	18	0.110 (±0.161)	0.111 (±0.171)	0.344
	Norfolk Island	East Australia	17	0.106 (±0.160)	0.106 (±0.168)	0.322
	Lord Howe Island	East Australia	23	0.109 (±0.156)	0.109 (±0.165)	0.312
	Galápagos Islands	Ecuador	82	0.131 (±0.151)	0.130 (±0.155)	0.416
	Revillagigedo	Mexico	9	0.137 (±0.167)	0.140 (±0.186)	0.530
	Hawaii	U.S.A	18	0.107 (± 0.160)	0.104 (±0.162)	0.338
	Walters Shoals	South Africa	3	0.104 (±0.195)	0.101 (±0.214)	0.236
Total			208			
C. obscurus	Coffs Harbour	New South Whales	15	0.175 (±0.168)	0.178 (±0.181)	0.566
	Moreton Bay	Queensland, Australia	16	0.151 (±0.169)	0.152 (±0.178)	0.480
	Thirroul	New South Whales	6	0.149 (±0.188)	0.146 (±0.205)	0.461
	Indonesia	Indonesia	7	0.160 (±0.183)	0.161 (±0.201)	0.537
	Kingscote Jetty	Norfolk Island, Australia	23	0.143 (±0.169)	0.141 (±0.174)	0.416
	Northern Territory	North Australia	21	0.147 (±0.168)	0.144 (±0.172)	0.418
	South Australia	South Australia	6	0.148 (±0.186)	0.146 (±0.202)	0.464
	St. Vincent Gulf	South Australia	9	0.165 (±0.176)	0.168 (±0.198)	0.594
	Spencer Gulf	South Australia	28	0.160 (±0.161)	0.162 (±0.171)	0.562
	Blythedale	South Africa	13	0.138 (±0.177)	0.132 (±0.180)	0.386
	Cascade Jetty	Norfolk Island, Australia	6	0.148 (±0.186)	0.148 (±0.207)	0.466
	East Cheyne Inlet	Western Australia	15	0.150 (±0.171)	0.154 (±0.188)	0.468
	West Cheyne Inlet	Western Australia	10	0.147 (±0.179)	0.148 (±0.195)	0.529
	Cape Inscription	Western Australia	26	0.158 (±0.164)	0.158 (±0.169)	0.522
	Perth	Western Australia	8	0.155 (±0.176)	0.149 (±0.185)	0.552
Total			209			
Hybrids	Cabo Pulmo	Mexico	2	-	-	-
	Clipperton	France	1	-	-	-
	Galápagos Islands	Ecuador	1	-	-	-
Total			**421**			

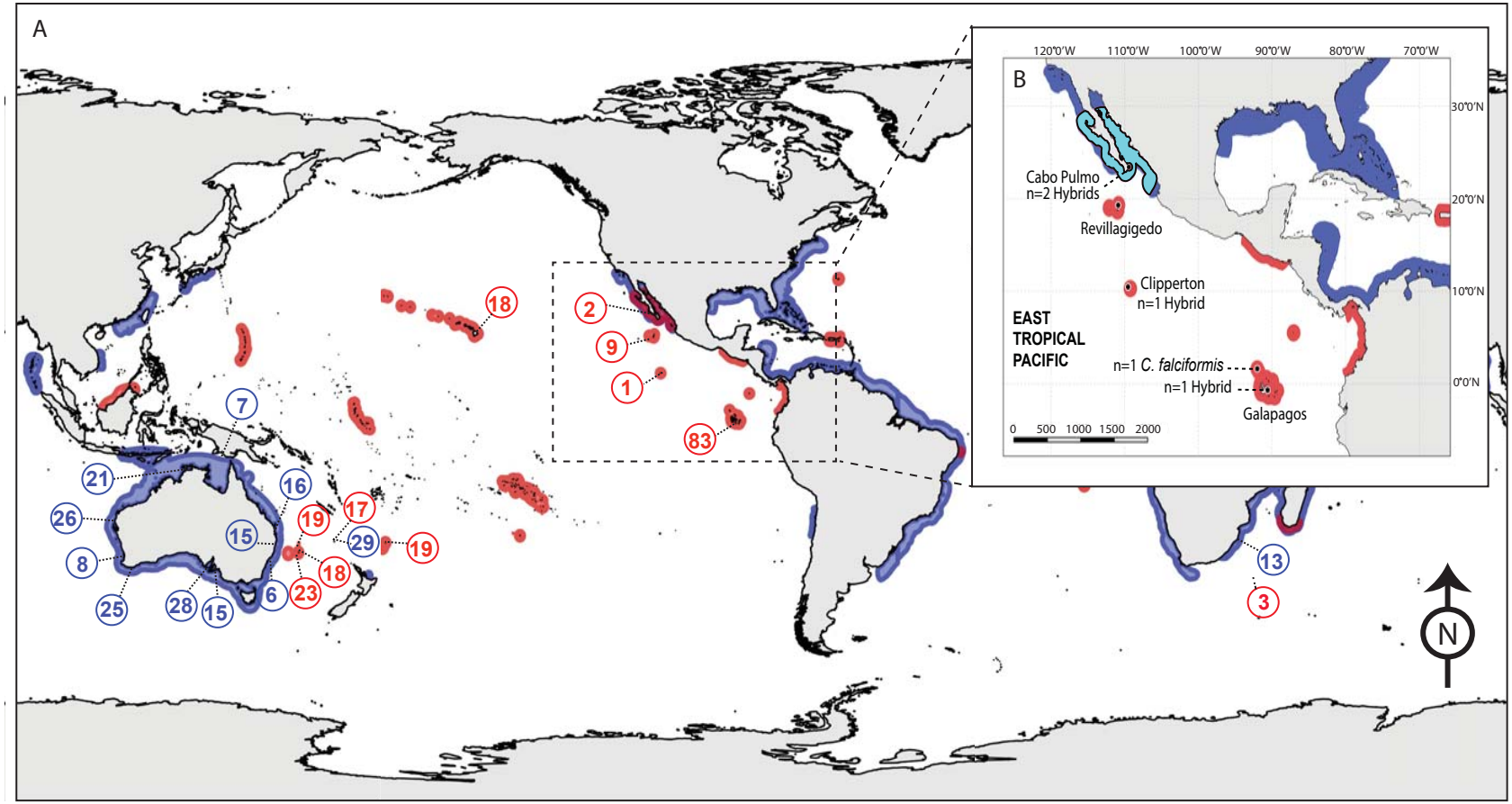

Fig. 4.1 Map of species distribution, in blue for *C. obscurus*, red for *C. galapagensis*, and light blue for the area where both species occur (off Mexico). (A) Sampling locations across the Indo-Pacific, circles indicate the number of *Carcharhinus galapagensis* and *C. obscurus* (red and blue, respectively) within the study area; (B) east Pacific collection sites and location of hybrids detected.

Table 4.2 Sample sizes used for misidentification assessment using the control region mtDNA (549 bp) for four *Carcharhinus* species and a hammerhead (*Sphyrna lewini*) as outgroup.

Species	n	Diversity indices		GenBank accession numbers	Source
		h	π		
C. galapagensis	23	0.679 ± 0.066	0.005 ± 0.004	-	Pazmiño et al. (in rev); Pazmiño et al. (in prep)
C. obscurus	23	0.960 ± 0.025	0.018 ± 0.011	HQ853257-853274	Benavides et al. 2011 South Australian Museum
C. falciformis	21	0.928 ± 0.039	0.032 ± 0.018	KM267565-267579	Clarke et al. 2015
C. brachyurus	19	0.964 ± 0.027	0.039 ± 0.021	HQ711308-711322	Benavides et al. 2011
Putative hybrids	4	0.500 ± 0.265	0.009 ± 0.008	-	Present study
Sphyrna lewini	1	-	-	DQ438148	Duncan et al. 2006
Total	**90**				

1.3 mtDNA-based species delimitation

This study investigated the potential of mitochondrial Control Region (CR) to differentiate between several *Carcharhinus* species, including *C. galapagensis*, *C. obscurus*, *C. falciformis* and *C. brachyurus* using phylogenetic approaches. I visualized, manually assembled, trimmed and aligned the CR sequences using GENEIOUS v5.4.7 (http://www.geneious.com, Kearse *et al.*, 2012). Two phylogenetic analyses were performed to identify the evolutionary relationships between the species of interest, after including an *S. lewini* individual as an outgroup. The best model of sequence evolution for the CR sequences examined was determined using PARTITION FINDER v.1.1.0 (Lanfear et al., 2012) and posterior parameter distributions were examined using TRACER v.1.6 (Rambaut et al., 2014). The first Maximum Likelihood (ML) analysis used default settings in MEGA v.6.06 (Tamura et al., 2007). The second Bayesian Inference analysis used 20,000,000 Markov Chain Monte Carlo (MCMC) simulations under the Bayesian information criterion (Bandelt et al., 1999) in MRBAYES v.4.0 (Huelsenbeck et al., 2001). Overall genetic diversity of CR sequences for each species was assessed by calculating haplotype (*h*), and nucleotide (π) diversities using ARLEQUIN v.3.5.1.2 (Laurent Excoffier & Lischer, 2010). Pairwise differentiation between species (pairwise Φ_{ST}) was also estimated to detect genetic partitioning using ARLEQUIN with 100,000 permutations.

Finally, I drew a Minimum Spanning Tree using POPART (available at: http://popart.otago.ac.nz; Bandelt *et al.*, 1999).

1.4 SNP sequencing and species-specific diagnostic SNPs selection

I assessed DNA extraction quality as per Pazmiño *et al.* (2017). SNP sequencing and characterization were performed by Diversity Array Technology Pty Ltd (DArT, Canberra Australia) as per Sansaloni *et al*. (2010) and Kilian *et al*. (2012). DArT SNP calling and quality filtering procedures followed Pazmiño *et al.* (2017). In order to reduce low-quality and uninformative data (Larson, Seeb, Everett, et al., 2014) from the initial data set of 57,341 SNPs, I used the custom *dartqc* pipeline (available at: https://github.com/esteinig/dartQC) to filter SNPs according to: 1) call rate (CR=100%); 2) Minor Allele Frequency (MAF>0.02); and 3) duplicate SNPs (with identical cloneID), keeping the best SNPs based on MAF score only. The output file was manually converted into a GENEPOP format file and transformed into different formats as required using PGDSpider v2.0.6.0 (Lischer & Excoffier, 2012). Our filtered dataset comprised 1,873 genome-wide SNPs.

Measures of genetic diversity, including observed (H_o), and unbiased expected (H_{nb}) heterozygosity (corrected for population sample size) were calculated at the intraspecific level using GENETIX v.4.05.2 (Belkhir et al., 2004) and ARLEQUIN v.3.5.1.2 (Laurent Excoffier & Lischer, 2010). Pairwise F_{ST} between species was calculated according to θ of Weir and Cockerham (1984) and Nei's minimum distance (Nei, 1978). Following SNP quality checks and filtering, I evaluated relatedness among individuals within and between species, and defined individuals with mixed ancestry (putative hybrids) using STRUCTURE v2.3.4 (Pritchard et al., 2000) and NETVIEW P R package (Neuditschko et al., 2012; Steinig et al., 2016). The latter analysis consists of three components: (1) calculation of an Identity-by-Similarity (IBS) distance matrix reconstructed in PLINK, which relies on allele-sharing distance (ASD); (2) a minimum spanning tree reconstruction; and (3) a network construction using nearest neighbor thresholds (k-NN) ranging from 10 to 100 (Purcell et al., 2007; Neuditschko et al., 2012). This

high resolution NETVIEW P network was reconstructed for the entire data set. Individuals that did not either fall within the two parental clusters or were of mixed ancestry, were removed before selecting pure parental data sets.

An initial Bayesian assignment was performed on the parental species data set using STRUCTURE to evaluate the discreteness and level of admixture between 208 *C. galapagensis* and 209 *C. obscurus* individuals. The analysis comprised 10 independent runs performed with a burn-in of 100,000 steps, followed by 1,000,000 additional Markov Chain Monte Carlo (MCMC) iterations. An admixture ancestry model was assumed with independent allele frequencies and no population priors. A total of 100 individuals (50 from each species) resulting from this run were then selected as pure parental individuals (q>99.5%) for further simulations and analysis. In order to select diagnostic markers from the total set of SNPs, F statistics were calculated for each locus using the PEGAS R Package (Paradis, 2010). Two different subsets of diagnostic unlinked markers were selected based on genetic differentiation (F_{ST}) values of either $F_{ST} > 0.90$ or $F_{ST} > 0.95$. STRUCTURE and NETVIEW P analyses were run with the full data set and with both diagnostic marker data sets.

1.5 SNP validation and hybrid identification

To test the power of the selected SNPs to assign/classify individuals, I used a simulation approach using HYBRIDLAB v.1.1 (Einar Eg Nielsen, Bach, & Kotlicki, 2006). The fifty purest individuals of each species from the initial STRUCTURE run were selected as pure parents to simulate the hybrid classes. Both parental classes, plus eight hybrid class categories were generated with 50 random simulated genotypes each: 1) Pure *C. galapagensis* (Gal), 2) Pure *C. obscurus* (Obs), 3) first generation (F_1) hybrids, 4) second generation (F_2) hybrids, 5) first-generation backcrosses between Gal and F_1 hybrids (bGal), 6) first-generation backcrosses between Obs and F_1 hybrids (bObs), 7) second-generation backcrosses between Gal and bGal, 8) second-generation backcrosses between Obs and bGal, 9) second-generation backcrosses between Obs and bObs, and 10) second-generation backcrosses between Gal and bObs.

Simulated data were then reassigned to their most likely hybrid class using NEWHYBRIDS and STRUCTURE. Parameters used were as per empirical data. With the SNP data from the pure parental individuals plus the putative hybrids (n=104), I performed a Bayesian assignment method using NEWHYBRIDS v.1.1 (E. C. Anderson & Thompson, 2002), to determine the posterior probability that each putative hybrid belongs specifically to one of the ten categories previously simulated. The run used uniform priors for a burn-in of 100,000 sweeps, followed by 1,000,000 MCMC iterations in each analysis. The frequency classes with the respective expected proportions are summarized in Table 4.3.

Table 4.3 Expected proportions (*Q*) of the 10 genotype classes (*g*) assumed by the NEWHYBRIDS software. Assignment criteria for *C. galapagensis* (Gal) and *C. obscurus* (Obs) and each hybrids category

Class (*g*)	*Q*	(Gal, Gal)	(Gal, Obs)	(Obs, Gal)	(Obs, Obs)
Pure Gal	1.000	1.000	0.000	0.000	0.000
bGal x Gal	0.875	0.750	0.125	0.125	0.000
bGal (Gal x F_1)	0.750	0.500	0.250	0.250	0.000
bObs x Gal	0.625	0.250	0.375	0.375	0.000
F_1	0.500	0.000	0.500	0.500	0.000
F_2	0.500	0.250	0.250	0.250	0.250
bGal x Obs	0.375	0.000	0.375	0.375	0.250
bObs (Obs x F_1)	0.250	0.000	0.250	0.250	0.500
bObs x Obs	0.125	0.000	0.125	0.125	0.750
Pure Obs	0.000	0.000	0.000	0.000	1.000

2. Results

2.1 Pure parental and hybrid identification based on SNPs

The initial data set was reduced from 57,341 to 2,105 SNP loci following the first filtering steps based on call rate (CR=100%), Minor Allele Frequency (MAF>0.02), and

cloneID duplicates. Monomorphic loci were also removed, resulting in a final data set of 1,873 SNPs. Overall, high genetic differentiation was observed between *C. galapagensis* and *C. obscurus* (F_{ST}=0.47, p<0.05) using nuclear SNPs. Overall genetic diversity in *C. galapagensis* (H_o=0.118; $H_{n.b}$=0.121) was lower than in *C. obscurus* (H_o=0.153; $H_{n.b}$=0.153). NETVIEW P network visualization from all individuals based on 1,873 SNPs (KNN=190) clearly showed close relatedness of conspecifics. Four individuals fell outside of the two discrete species clusters and were considered as putative hybrids: one from the southern Galápagos Islands (RSN3), two from Cabo Pulmo (MX1, MX2) and one from Clipperton Island (France) off the west coast of Mexico (MX13) (Figure 1B). Three of these individuals (RSN3, MX1, and MX13) connected to both clusters. Although MX2 appeared to be more related to the *C. obscurus* group when all SNP loci were used, it separated from the core *C. obscurus* cluster (Figure 4.2A, Table 4.1). A similar pattern was detected by the initial STRUCTURE analysis including all 421 samples, with the same four individuals showing high levels of admixture between the two species (Figure 4.3). Additionally, two individuals from Norfolk Island (Cgal_N1321_77 and Cgal_N1321_80) originally misidentified and labeled as *C. galapagensis,,* clustered within the dusky group and were therefore relabeled as *C. obscurus.*

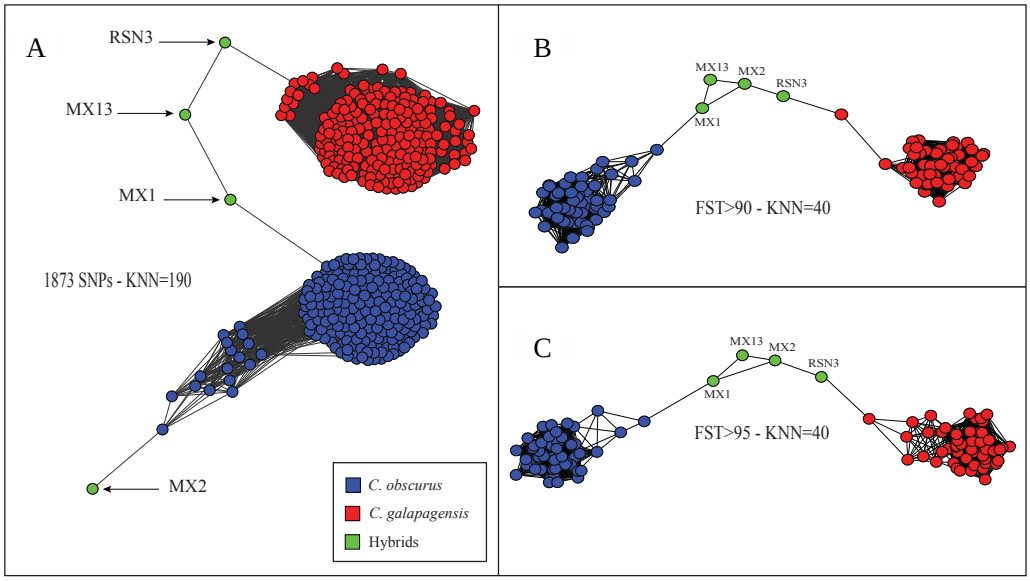

Fig. 4.2 Network reconstruction with Netview P v.0.4.2.5 to evaluate relatedness among individuals of *C. galapagensis* (red), n=208 and *C. obscurus* (blue), n=209, using: a) all individuals (n=421) and all filtered SNPs (1,873 loci); b) pure parental individuals only (n=100, 50 per species) and SNPs with F_{ST}>0.90 (117 loci); and c) pure parental individuals only (n=100, 50 per species) and SNPs with F_{ST}>0.95 (69 loci). Interspecies hybrids are shown in green.

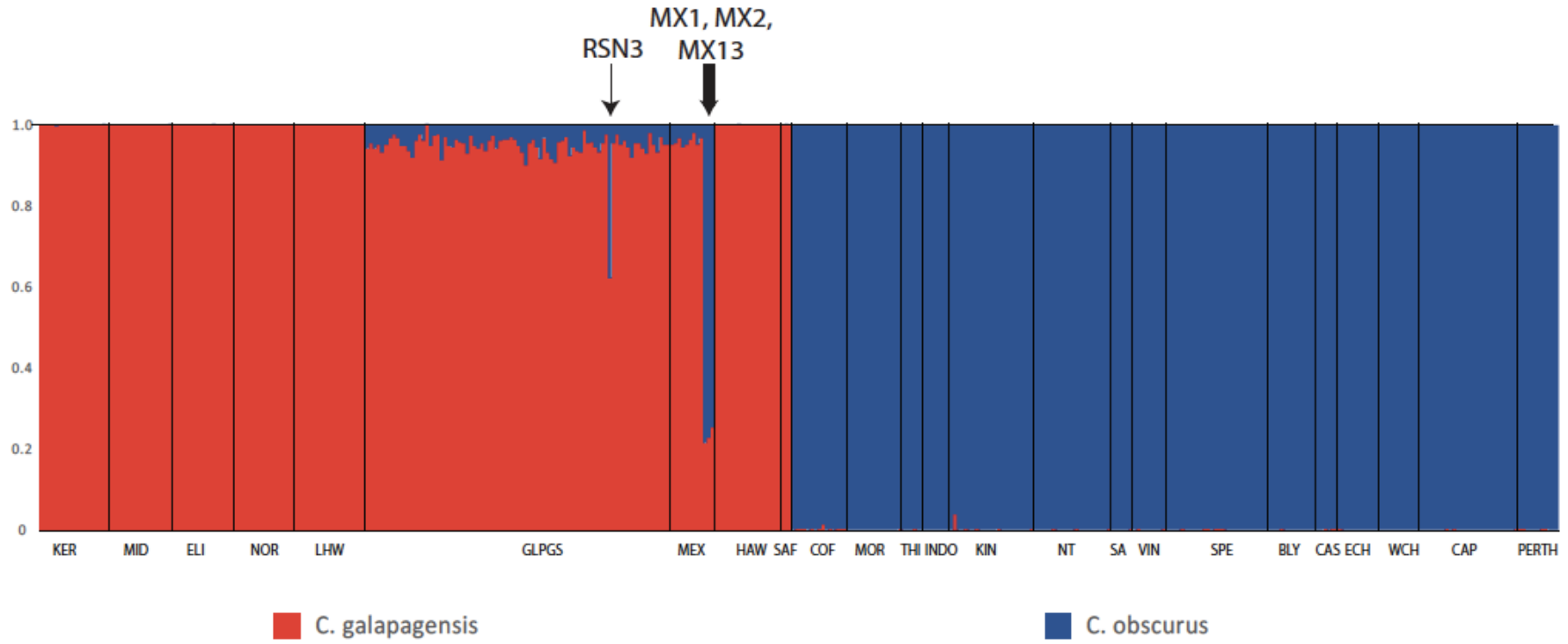

Fig. 4.3 Initial species assignment and clustering (k=2 along X-axis) using STRUCTURE, based on 1,873 SNPs in 421 individuals. Each individual is represented by a colored bar according to species (*C. galapagensis* red, *C. obscurus* blue). Mixed color bars indicate proportional SNP representations of each species for that individual (Y-axis). Localities are shown below the bar plot: Kermadec Island (KER), Middleton Reef (MID), Elizabeth Reef (ELI), Norfolk Island (NOR), Galapagos Islands (GLPGS), Mexico (MEX), Hawaii (HAW), South Africa (SAF), Coffs Harbour (COF), Moreton Bay (MOR), Thirroul (THI), Indonesia (INDO), Kingstone Jetty, Norfolk Island (KIN), Northern Territory (NT), South Australia (SA), St. Vincent Gulf (VIN), Spencer Gulf (SPE), Btlythedale (BLY), Cascade Jetty, Norfolk Island (CAS), east Cheyne Inlet (ECH), west Cheyne Inlet (WCH), Cape Inscription (CAP), and Perth (PERTH). Considerable admixture levels were observed in four putative hybrid individuals (three in MEX and one in GLPGS).

A second STRUCTURE run including a total of 417 individuals (putative hybrids removed) was consistent with the presence of two clusters (k=2) based on DeltaK statistics computed in STRUCTURE HARVESTER: the first one corresponding to *C. galapagensis* and the second one to *C. obscurus*. Importantly, this analysis provided information regarding the level of admixture of each individual, which led to the selection of individuals with the highest probability of assignment to one or the other cluster, with alternatively fixed alleles. We subsequently defined these individuals as pure parental *C. galapagensis* or *C. obscurus* (Supplemental Table 4).

2.2 Simulated data

Following the pure parental selection, individual locus F_{ST}s calculated in PEGAS were used to filter and select two discriminant sets of loci at two F_{ST} thresholds: a) 117 loci with F_{ST}>0.90 and b) 69 loci with F_{ST}>0.95 (Supplemental Table 5). These subsets where then used to simulate both pure parental, and eight hybrid classes (including, F_1, F_2, first and second generation backcrosses in both directions). A total of 500 individuals were simulated for each data set, fifty per hybrid class, to test the power of the selected markers. STRUCTURE runs for the first simulated data set (117 SNPs; F_{ST}>90) showed a clear differentiation among pure parental and all hybrid classes, except for F_1 and F_2 hybrids. While the difference among the other hybrid categories was about 12%, F_1 and F_2 showed a similar proportion of admixture (0.499) and could not be distinguished from one another (Figure 4.4A). The second simulated data set (69 SNPs; F_{ST}>95) showed a similar trend. However, the accuracy around *Q* was lower in general when using fewer SNPs, and more variable in later generation backcrosses (Figure 4.4B). When testing the power of the first SNP panel (117 SNPs; F_{ST}>90), all simulated individuals corresponding to parental classes (pure *C. galapagensis* and *C. obscurus*), F_1, and F_2 Hybrids were correctly assigned by NEWHYBRIDS to their corresponding class. For first and second-generation backcrosses, accuracy ranged from 98-99 per cent (Figure 4.5A). For the second and smaller data set (69 SNPs; F_{ST}>95), NEWHYBRIDS correctly classified all the individuals from the parental *C. galapagensis* class, F_1, and F_2 hybrid classes. Accuracy for the

parental *C. obscurus* class was 99 per cent, and ranged from 93-99 for first and second-generation backcrosses (Figure 4.5B).

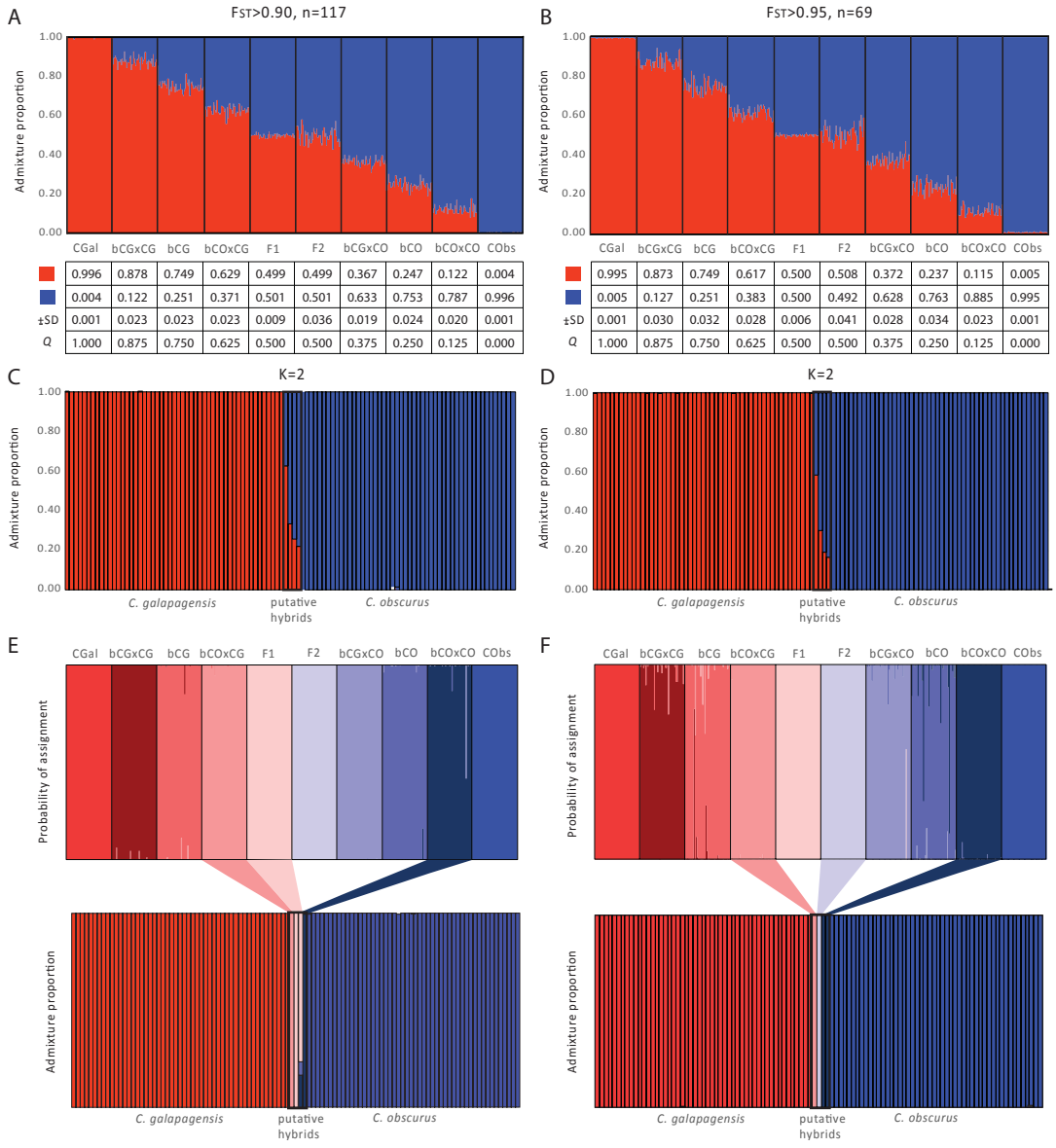

Fig. 4.4 Bayesian admixture analysis in STRUCTURE of (A) Simulated individuals and 117 SNPs with F_{ST}>0.90; (B) simulated individuals and 69 SNPs with F_{ST}>0.95; (C) Empirical data and 117 SNPs with K=2; (D) Empirical data and 69 SNPs with K=2; assignment of putative hybrids and pure parental individuals to the corresponding simulated category using (E) 117 SNPs and (F) 69 SNPs. Simulated categories include: pure *C. galapagensis* (Gal), pure *C. obscurus* (Obs), F_1 hybrids, F_2 hybrids, first-generation backcross – Gal x F_1 (bG), first generation backcross – Obs x F_1 (bO) and second generation backcrosses (bGxG, bOxG, bGxO, bOxO). Observed admixture proportion values and their standard deviation (±SD) and expected proportion values (Q) are included for each simulated category.

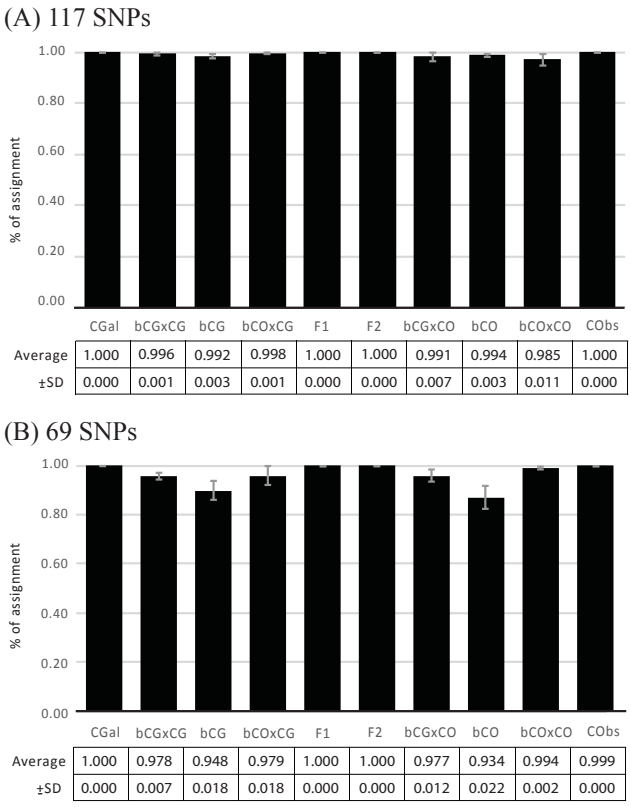

Fig. 4.5 Confidence of assignment probability of simulated data in NEWHYBRIDS using subsets of a) 117 SNPs and b) 69 SNPs, to identify 10 genotype classes including parentals and hybrids between *Carcharhinus galapagensis* and *C. obscurus*.

2.3 Empirical data

Two clusters (k=2), one per parental species were identified as the best scenario using STRUCTURE HARVESTER. From a total of 104 individuals used for this analysis, 50 were assigned as *C. galapagensis*, 50 as *C. obscurus*, and four individuals were identified as putative hybrids by the initial NETVIEW P analysis, which showed different levels of admixture between the two clusters. Putative hybrids had similar levels of admixture in both SNP subsets (Figure 4.4C-D). Results from the NEWHYBRIDS assignment were congruent with those from STRUCTURE (Figure 4.4E-F). The same four individuals (RSN3, MX1, MX2, and MX13) were assigned to non-parental classes in the NEWHYBRIDS analysis. In the analysis based on 117 SNPs, hybrids were assigned to three classes: (1) RSN3 was assigned as a second generation backcross between a *C. galapagensis* and a first generation backcross *C. obscurus* with a posterior probability of 0.99; (2) MX2 and MX13 were assigned as F_1 Hybrids with

posterior probabilities of 1.00 and 0.76 respectively; and (3) MX1 was assigned as a second generation backcross between a *C. obscurus* and a first generation backcross of the same species with a posterior probability of 0.99 (Figure 4.4E, Supplemental Table 6A). In the analysis based on 69 SNPs three hybrid classes were also detected. However, not all the individuals were assigned to the same class as with the previous data set: (1) RSN3 was ratified as second generation backcross class (bObs x Gal); (2) MX2 was assigned as an F_2 hybrid with posterior probability of 1.00; (3) MX1 and MX13 were assigned to the second generation backcross between a *C. obscurus* and a first generation backcross of the same species, both with a posterior probability of 0.99 (Figure 4.4F, Supplemental Table 6B). Network visualizations from the two subsets of SNPs (F_{ST}>90 and F_{ST}>95) at KNN=40 showed a similar topology (Figure 4.2B-C). The presence of two well-defined clusters, one for each species, is congruent with STRUCTURE clustering results. All individuals, except the four putative hybrids, were assigned to either the *C. galapagensis* or the *C. obscurus* cluster. Hybrid individuals formed an intermediate admixed cluster linking the two main clusters (Figure 4.2B-C).

2.4 Misidentification assessment using mtDNA

Forty-six unique control region haplotypes were detected among 90 individuals of four species of *Carcharhinus*: *C. galapagensis* (n=23), *C. obscurus* (n=23), *C. falciformis* (n=21), *C. brachyurus* (n=19), four individuals previously defined as putative hybrids between *C. galapagensis* and *C. obscurus* based on the above mentioned SNP analyses and one hammerhead shark (*S. lewini*) used as outgroup (Table 4.2). Overall haplotype and nucleotide diversities ranged from h=0.679 ± 0.066 and π= 0.005 ± 0.004 for *C. galapagensis* to h=0.964 ± 0.027 and π=0.039 ± 0.008 for *C. brachyurus*. A total of 166 polymorphic sites were detected, 46 of which were parsimony informative. While control region pairwise Φ_{ST}s were high and significant between the other species, *C. galapagensis* and *C. obscurus* were indistinguishable (pairwise Φ_{ST} = 0.00 p>0.05, Supplemental Table 7). The mitochondrial control region minimum spanning tree showed closely related haplotypes within *Carcharhinus* species and 17

to 19 mutation steps between species, except for *C. galapagensis* and *C. obscurus* which fell into a single cluster and shared three haplotypes (H6, H32, and H31, Figure 4.6).

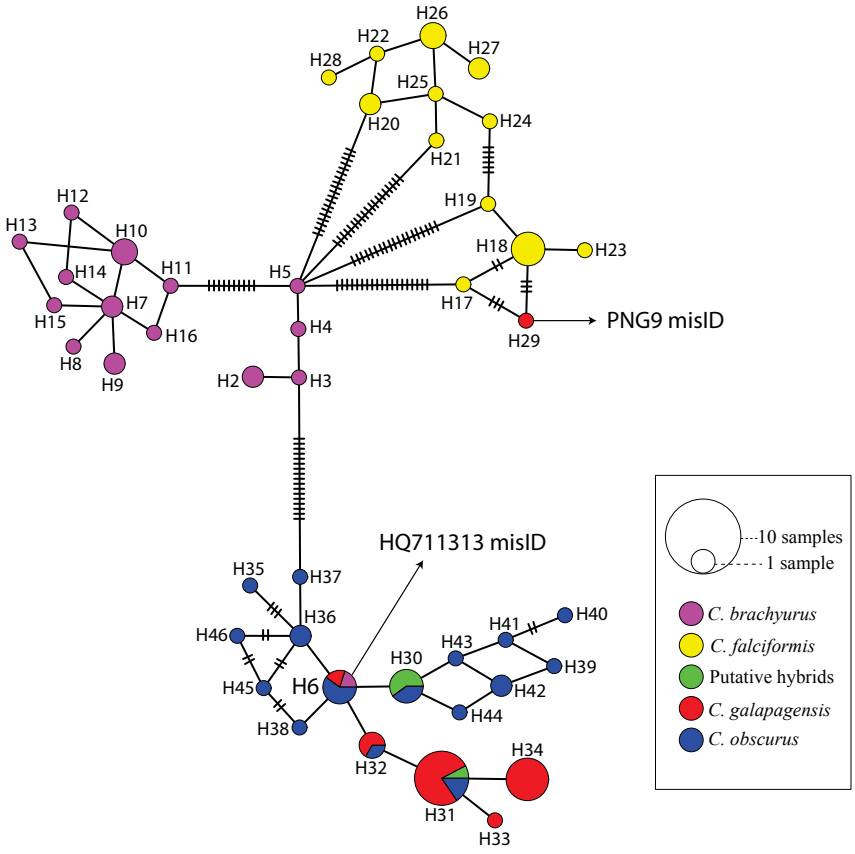

Fig. 4.6 Minimum Spanning Tree based on mitochondrial control region (549 bp) haplotypes of four species of *Carcharhinus* (*C. brachyurus*, *C. falciformis*, *C. galapagensis* and *C. obscurus*). Relative circle size represents haplotype frequency and each dash represents a mutation step between haplotypes (one, if no dash). Black arrows identify haplotypes of misidentified individuals (H29 and HQ711313).

Phylogenetic reconstructions based on ML and Bayesian approaches were consistent (Figure 4.7 - 4.8). Trees showed highly supported clades for *C. falciformis* and *C. brachyurus* but *C. obscurus* and *C. galapagensis* formed a single highly supported clade with low internal resolution between haplotypes. Hybrids fell within the Galapagos/dusky shark clade. Two apparently misidentified individuals were also detected: one sample originally identified as *C. galapagensis* (PNG9 from the Galápagos Islands) fell within the *C. falciformis* mtDNA clade, and a second individual labeled in GenBank as *C. brachyurus* (HQ711313 from Benavides *et al.*, 2011) was assigned to the *C. obscurus/C. galapagensis* mtDNA clade (Figure 4.7 – 4.8,

Table 4.4). PNG9 (misidentified individual) was removed from the *C. obscurus/C. galapagensis* dataset used to investigate hybridisation.

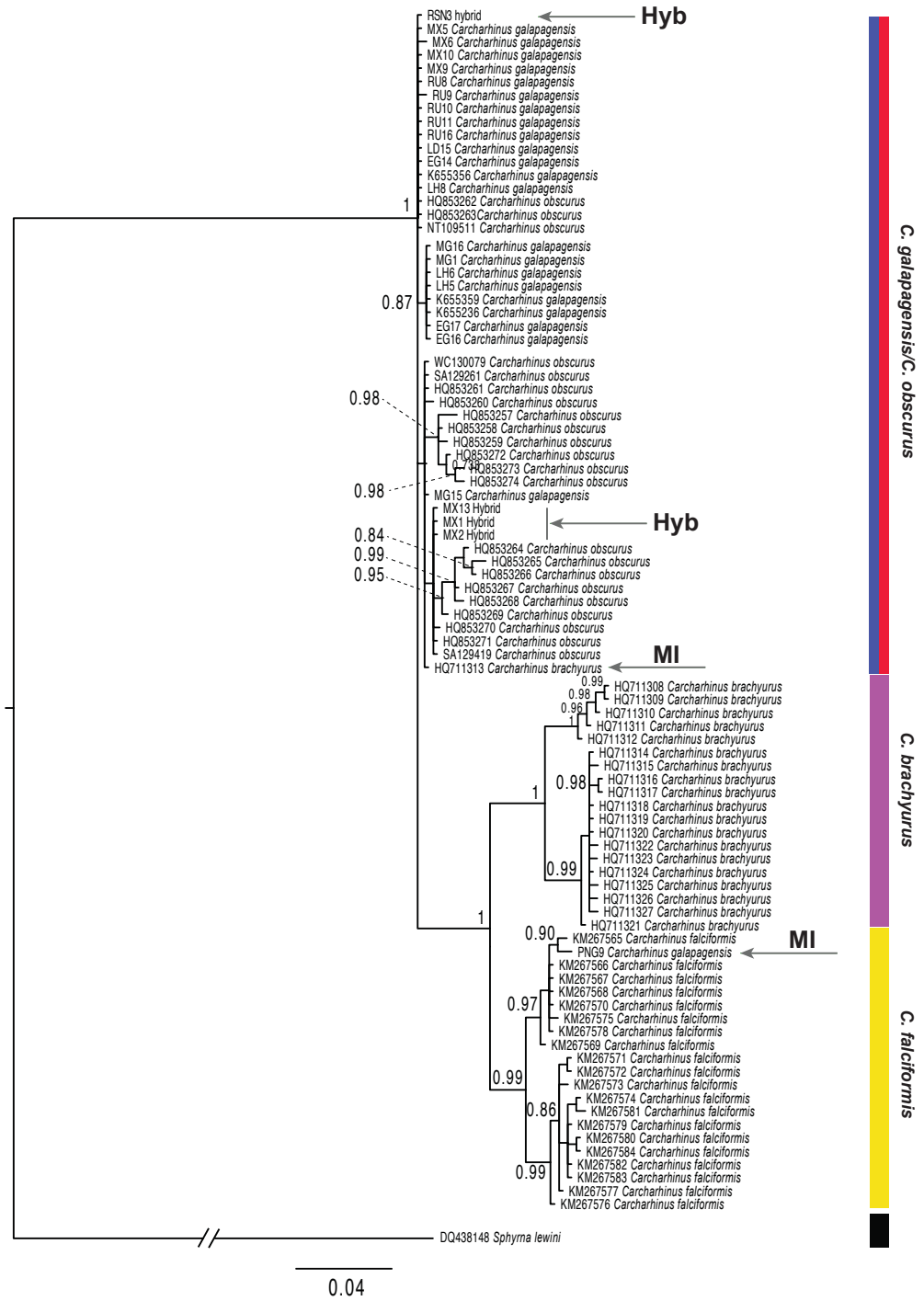

Fig. 4.7 Outgroup rooted Bayesian Inference phylogram of mitochondrial control region haplotypes of four species of *Carcharhinus* (*C. brachyurus, C. falciformis, C. galapagensis* and *C. obscurus*) generated using MrBayes software. One *Sphyrna lewini* individual was used as outgroup. Posterior probability values > 75 are shown. Arrows indicate misidentified (MI) and hybrid (Hyb) individuals.

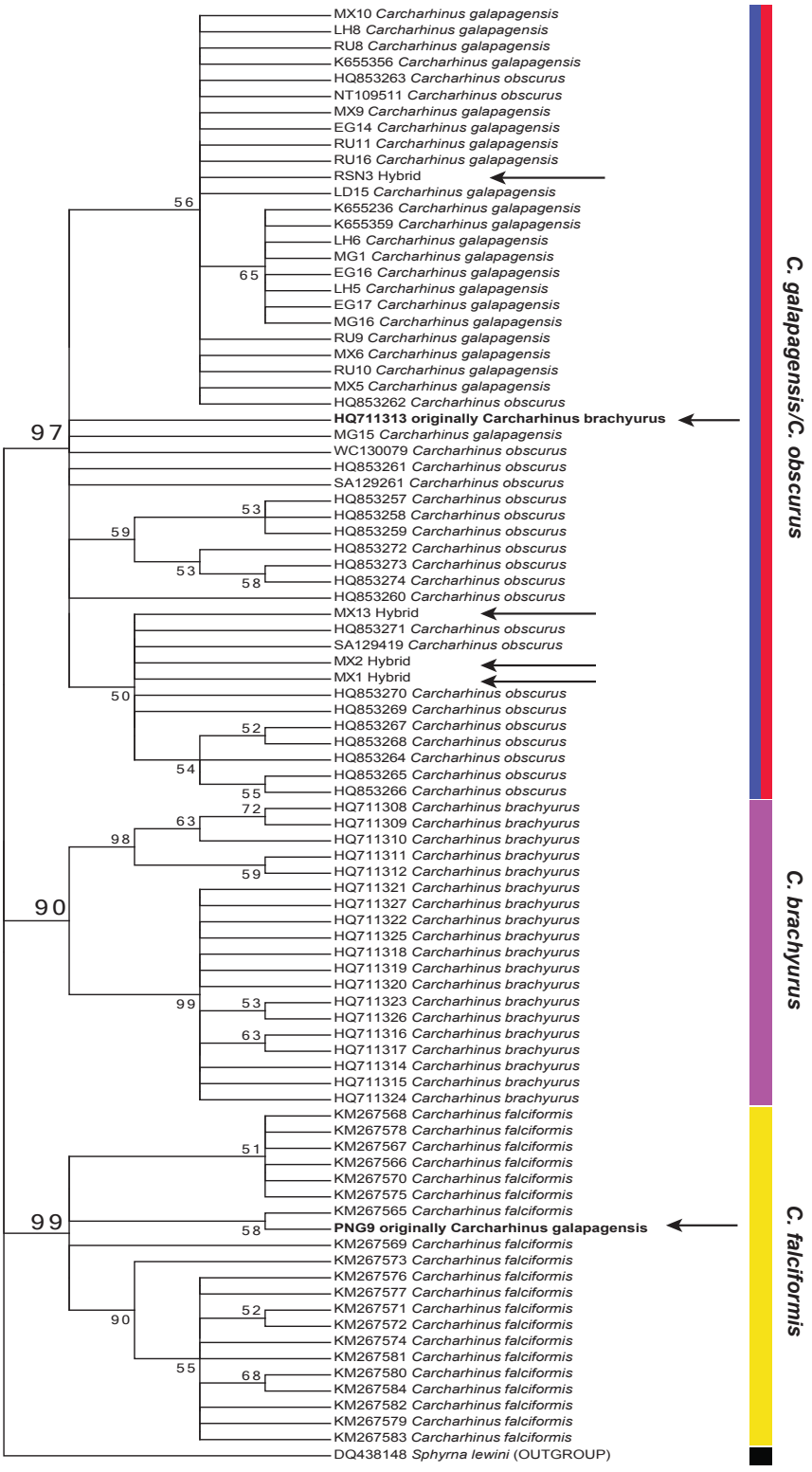

Fig. 4.8 Outgroup rooted Maximum Likelihood consensus tree of four *Carcharhinus* spp. (*C. brachyurus, C. falciformis, C. obscurus* and *C. galapagensis*) generated using MEGA software for the control region mtDNA (549 bp) and 1000 bootstrap replicates. One *Sphyrna lewini* individual was used as outgroup. Bootstrap support values > 50% are shown. Arrows indicate misidentified and hybrid individuals.

Table 4.4 List of misidentified individuals and new assignments, either as a different species or as hybrids from *Carcarhinus galapagensis* and *C. obscurus* mating.

Individual ID	Location	First species assignment	Old Criteria	New assignment	New Criteria
PNG9	Darwin & Wolf (Galápagos Islands)	*C. galapagensis*	Morphology	*C. falciformis*	MtDNA control region
RSN3	Darwin & Wolf (Galápagos Islands)	*C. galapagensis*	Morphology	Hybrid *C. obscurus/C. galapagensis*	Nuclear SNPs
Cgal_NI321_77	Norfolk Island (Australia)	*C. galapagensis*	Morphology and control region	*C. obscurus*	Nuclear SNPs
Cgal_NI321_80	Norfolk Island (Australia)	*C. galapagensis*	Morphology and control region	*C. obscurus*	Nuclear SNPs
MX1	Cabo Pulmo (Mexico)	*C. galapagensis*	Morphology	Hybrid *C. obscurus/C. galapagensis*	Nuclear SNPs
MX2	Clipperton (France)	*C. galapagensis*	Morphology	Hybrid *C. obscurus/C. galapagensis*	Nuclear SNPs
MX13	Cabo Pulmo (Mexico)	*C. obscurus*	Morphology	Hybrid *C. obscurus/C. galapagensis*	Nuclear SNPs
HQ711313	Mexico	*C. brachyurus*	Control region	*C. obscurus/C. galapagensis*	MtDNA control region

3. Discussion

3.1 SNP validation and hybrid detection

This is the first study to document evidence for contemporary hybridisation between *C. galapagensis* and *C. obscurus*. I found different levels of admixture between individuals from three distant east Pacific locations (more than 1000 km apart): the Galápagos Islands (Ecuador), Cabo Pulmo (Mexico), and Clipperton Island (France, Eastern Tropical Pacific). I identified the level, frequency, and direction of hybridisation and introgression between these two species by developing and genotyping 1,873 genome-wide SNPs. SNPs are considered effective markers to address hybridisation and introgression questions, given their biallelic nature, low mutation rate, and low homoplasy (F. Balloux & Goudet, 2002; Pujolar, Jacobsen, Als, Frydenberg, Magnussen, et al., 2014). I also highlight the importance of a robust and broad sampling strategy across the Pacific distribution of both species, especially from contact zones, together with a comprehensive quality assessment and data filtering process to accurately define species relationships and detect rare hybridisation and introgression. Overall, results highlighted the discriminatory power of SNPs compared to mtDNA (F_{ST}=0.47, $p<0.05$; Φ_{ST} = 0.00 $p>0.05$). While challenging, correct selection of pure parental individuals is crucial to identify unbiased diagnostic markers (Nussberger et al., 2013). We defined 50 individuals (reference individuals) from each parental species based on a STRUCTURE clustering analysis to identify pure parental representatives of both species. Thus, retaining only samples without any sign of admixture (probability of assignment > 99%) across 1,873 loci to define diagnostic SNP panels for each species. Individuals from the Galapagos Islands showed a low level of admixture (lower than the putative hybrids RSN3, MX1, MX2 and MX13). Such a level of admixture might indicate a further backcross category is present within the archipelago, or could reflect natural differentiation within this population (between the E and W Galapagos populations, Chapter 2). Ten categories including pure parental species, F_1, F_2, first and second-generation backcrosses were successfully simulated with a posterior probability higher than 0.98. A previous study raised concern regarding the overestimation of SNP power given the initial

selection of pure parental individuals (Nussberger et al., 2013). However, given the high level of SNP differentiation between the two parental shark species I do not expect significant bias in the results. Additionally, selection of loci for diagnostic purposes is a complex matter, and must be carefully balanced to retain an appropriate number of loci with discriminatory power. Selection based on high F_{ST} values only, might lead to reduced data sets if the F_{ST} threshold is set too high, which may therefore lead to reduced accuracy. Both subsets of diagnostic unlinked SNPs (F_{ST}>0.90 and F_{ST}>0.95) consistently identified the same four hybrid individuals from different hybrid classes (~1% of the total sample examined). However, the subset of 117 F_{ST}>0.90 diagnostic SNPs with call rate of 1.00, Minor Allele Frequency of 0.02, had better accuracy in identifying hybrids up to the third generation compared to the 69 F_{ST}>0.95 diagnostic SNP markers

Findings are consistent with those from Corrigan *et al.* (2017), who used NADH2 mtDNA and 2,152 nuclear SNPs to investigate the relationship between Galapagos and dusky sharks. The authors from this latter study suggested historical hybridisation prior to species isolation, and acknowledged a need to obtain a larger number of samples from both species to further evaluate this matter. The current study sampled threefold more samples than Corrigan *et al.* (2017), with approximately evenly distributed sampling between species, and included three contact zones: 1) Norfolk Island, from which I characterised SNPs for 17 Galapagos and 29 dusky sharks, compared to Corrigan et al. (2017) who produced genomic SNP data for one Galapagos and five dusky sharks; 2) Cabo Pulmo in the Gulf of California, from which I included two samples originally labelled as dusky sharks, but reassigned these as hybrids; and 3) the Revillagigedos archipelago off the western coast of Mexico, from which I included six Galapagos shark samples.

Evidence of hybridization also included one hybrid from the southern Galápagos Islands (RNS3), corresponding to a second-generation hybrid backcross between a pure *C. galapagensis* (Gal) and a first generation *C. obscurus* backcross (Obs x F_1 hybrid). Given the lack of mtDNA differentiation between *C. galapagensis* and *C. obscurus* (Fig 4.6 this study), it

was not possible to define mtDNA (maternal) based directionality of hybridization. However, differential patterns of abundance of these two shark species may be an important factor driving hybridisation in other locations in the east Pacific. While Galapagos sharks are common in the Galápagos Islands, the closest dusky sharks have been reported to the Galápagos Islands is 1000 km east in Ecuadorian continental shelf waters, where Galapagos sharks have been occasionally reported as well (Bearez, 2015). The presence of unreported dusky sharks in the Galápagos islands is unlikely, given the extensive sampling effort in the archipelago and continuous monitoring by the Galápagos National Park authorities. Both species are more likely to make contact along the continental shelf where they could be reproducing. Mexican hybrid individuals MX1 and MX13, both from Cabo Pulmo contact zone in the Gulf of California, correspond to a second-generation *C. obscurus* backcross (F_2 hybrid x Obs) and an F_1 hybrid, respectively. Dusky sharks occur throughout the west coast of Mexico (Garrick, 1982; Musick, Grubbs, Baum, & Cortés, 2009), which explains the presence of hybrids with more dusky than Galapagos shark genetic material at Cabo Pulmo. The fourth individual, MX2, is an F_1 hybrid collected from Clipperton Atoll, 965 km west of Mexico, at the edge of the Eastern Pacific Barrier (Snodgrass & Heller, 1905). While Galapagos sharks are common at Clipperton Atoll, dusky sharks are not. A review of the growing literature on reef fish hybridisation highlights that hybridisation is most prevalent between closely-related species, particularly when one of the species is rare in the contact zone, resulting in a lack of conspecific partners (Montanari et al., 2014, 2016). Differences in abundance between Galapagos and dusky sharks have also been reported in the Revillagigedo Islands, where dusky sharks have only been found occasionally (Garrick, 1982). Despite significant sampling of both Galapagos and dusky sharks at Norfolk Island, I was not able to detect hybridisation in this particular contact zone, probably because both species are common here (Duffy, 2015). Nevertheless, two Norfolk Island sharks originally classified as Galapagos sharks based on phenotype, were reclassified as dusky sharks based on the diagnostic SNP panel developed in this study. Extended sampling from locations where I detected hybridisation, and across the west coast of Central and South America is needed to evaluate shark populations in other areas where the species may co-occur and hybridize (e.g.

Cocos and Malpelo Islands), to establish the presence/absence and extent of hybridisation in these locations. Furthermore, re-classification of both individuals from Norfolk Island (Cgal_N1321_77 and Cgal_N1321_80) could not be confirmed with PVC and are based solely on SNPs.

The presence of an F_1 hybrid at Clipperton and a second-generation backcross (with more *C. galapagensis* genetic material) in the Galápagos Islands suggests movement of female Galapagos sharks (potentially hybrid mothers) from the primary area of contact (Gulf of California) towards the Galápagos Islands potentially using Clipperton atoll as a stepping-stone. The possibility of these F_1 and second-generation backcross hybrids having migrated from the area of contact is low since these hybrids from Clipperton and the Galápagos Islands were both juveniles and therefore were most likely born locally, as juveniles are less likely to travel long distances than adults (Meyer *et al.*, 2010; Lizardi et al., in preparation). The identification of backcrossed hybrids up to the second generation suggests that F_1 hybrids are reproductively viable. However, knowledge of hybrid fitness in sharks is limited and further investigation is required to better understand the dynamics of mating in these two closely-related shark species, particularly during hybridisation. Based on these findings, I posit that both species predominantly maintain their habitat and mate preference, evident from the rarity of hybridisation (only ~1%) compared to the two previous cases of hybridisation in elasmobranchs described by Morgan *et al*. (2012) and Cruz *et al*. (2015). Finally, I agree with Morgan *et al.* (2012) on the importance of considering inter-species hybridisation when using mtDNA for species identification in sharks, as hybrids may either be missed or mis-assigned. Therefore, combining molecular (both nuclear and mitochondrial), morphological and ecological information is crucial to elucidate historical and contemporary factors that promote genetic exchange among species as has been extensively documented in other hybridizing marine taxa such as fishes (reviewed by Montanari et al., 2016), corals (reviewed by Willis et al., 2006), plants, crustaceans, echinoderms, gastropods, turtles, and fur seals (reviewed by Arnold & Fogarty, 2009).

3.2 Species delimitation and misidentification

Species identification of whaler sharks (*Carcharhinus*) is a challenge for researchers, who often rely on tissue samples collected in the field without sacrificing the animals, or from dead specimens collected for other purposes to study shark populations. This means that most of the time researchers do not have access to important diagnostic traits such as precaudal vertebral counts (the most reliable morphological trait to discriminate the shark species examined here). Species misidentification post-capture can also be problematic as standard processing of carcasses involves the removal of the head and fins, making it difficult or impossible to identify species (Dudgeon et al., 2012; Tillett et al., 2012). Ultimately, this contributes to erroneous data on landings and poorly informed conservation efforts (Domingues, de Amorim, & Hilsdorf, 2013). Misidentifications between *Carcharhinus* species, whose congeners present subtle morphological differences (Garrick, 1982) is a major ongoing problem for conservation and has been presented and discussed elsewhere (e.g. Fischer *et al.*, 2012; Tillett *et al.*, 2012; Domingues *et al.*, 2013; Duffy, 2016).

Mitochondrial DNA has provided important insights to improve management and conservation of wild populations by informing on both population structure within a species range and evolutionary relationships between species (Holmes et al., 2009; Naylor et al., 2012). While in most cases, mitochondrial markers are useful, in some cases they aren't and more powerful tools are required. When divergence is recent or if there is hybridisation, mtDNA may not discriminate between species. We obtained discordant results from mitochondrial control region and nuclear SNPs in Galapagos and dusky sharks: nuclear SNPs provided a strong signal of differentiation between species, but mtDNA did not. This discordant pattern between mitochondrial and nuclear markers might reflect a lack of selection acting on the mitochondrial control region compared to high F_{ST} SNP markers (potentially under adaptive pressure), or could be the result of mitochondrial introgression following a range expansion as suggested by Corrigan *et al*. (2017), and/or incomplete lineage sorting of the mitochondrial genome. These results support Corrigan *et al.*'s (2017) conclusion that Galapagos and dusky sharks are valid

species. By using a combination of mtDNA and genome-wide SNPs, I detected eight species misidentifications: I found four dusky - Galapagos shark hybrid individuals that had been defined as pure *C. galapagensis* prior to this study; two Norfolk Island sharks originally identified as *C. galapagensis* were reassigned as pure *C. obscurus* after genetic admixture analysis using SNPs only as mtDNA was uninformative. Further, using phylogenetic and mtDNA haplotype network analysis we re-assigned an apparent Galapagos shark from the Galápagos Islands (individual PNG9) to *C. falciformis*, as the mtDNA sequence nested within the *C. falciformis* mtDNA clade. Finally, the control region sequence from an individual labelled as *C. brachyurus* in Genbank (from Benavides, Feldheim, et al., 2011) clustered within the *C. galapagensis/C. obscurus* rather than the *C. brachyurus* clade, suggesting either mislabelling (as noted before for PNG9) or evidence of hitherto undocumented historic introgressive hybridization between each of these two species and Galapagos/dusky sharks. Discriminating between mislabelling and hybridization alternatives requires a re-examination and resequencing of the questionable samples to validate the original species identification and an extended SNP phylogenomic analysis along with the *C. obscurus*-*C. galapagensis* SNPs developed in this study. I highlight the importance of identifying misidentified / mislabelled individuals in widely used databases such as GenBank, so that these can be validated, otherwise either mislabelling or unrecognized hybridization may go unnoticed and confound findings, which in turn would have detrimental conservation management implications. This is particularly important, when investigating signals of hybridisation between Galapagos and dusky sharks using SNPs, as the presence of one or a few misidentified individuals can erroneously suggest the presence of hybrids in the absence of SNP data for such mislabelled individuals.

Results from mitochondrial control region sequences in the present study are congruent with those from Corrigan et al. (2017), who also failed to differentiate between Galapagos and dusky sharks using the NADH2 gene. From a practical point of view, improving the training of observers or staff collecting samples may help solve some misidentification problems.

However, when identification relies on body parts, the use of non-expensive techniques such as mtDNA sequencing must be considered as an initial step to assess taxonomic identification for most *Carcharhinus* species. Furthermore, when dealing with closely related, recently diverged species, such as Galapagos/dusky sharks, more powerful tools (e.g. nuclear genome-wide SNPs) are needed to accurately assess species delimitation.

Chapter 5 - General discussion

1. Main findings and conservation implications

The genomics revolution has had a positive impact on the field of conservation, especially for non-model organisms (NMOs) with limited genomic resources (da Fonseca et al., 2016). Most shark and ray species are NMOs, which suffer from overexploitation and habitat loss pressure, as well as having a limited recovery capability due to species-specific life-history traits (Dulvy et al., 2014; Simpfendorfer & Dulvy, 2017). Applying genomics tools, this thesis aimed at better informing shark conservation management efforts, by producing and analysing extensive genome-wide Single Nucleotide Polymorphisms (SNPs) for the Galapagos shark (*Carcharhinus galapagensis*), which is considered Near-Threatened by the IUCN Red List. High-resolution genome-wide SNP analyses provided useful information to better guide management and conservation of this species across the Pacific Ocean, by identifying a total of five Galapagos shark conservation units: two at a local scale, within the east Pacific – the west and east Galapagos islands; the third also in the east Pacific (Mexico); the fourth in the central Pacific (Hawaii) and the fifth in the west Pacific, which includes New Zealand and several offshore Australian locations (Norfolk and Lord Howe Islands; Elizabeth and Middleton reefs). The five conservation units identified may be locally adapted, but additional work is required to validate this in this NMO. This thesis also identified, for the first time, hybridisation between Galapagos and dusky sharks, which produces viable, relatively rare hybrids within the east Pacific.

Various genetic parameters, including effective population size; genetic diversity and differentiation; gene-flow, were estimated from the SNPs generated, which permitted an accurate stock delimitation and identification of the most vulnerable stocks. Such information is required to develop effective management plans that may ensure the preservation of the species in the short and long term. It was clear that a combination of mitochondrial and nuclear data analyses is required to develop adequate conservation strategies. While mitochondrial markers have been widely used in the past in combination with conventional nuclear markers such as microsatellites, the low number of available markers has limited the resolution and accuracy of

analyses (Garner et al., 2016; Shafer et al., 2015). My results highlighted the importance of genome-wide markers (e.g. SNPs) as powerful nuclear genomic tools to estimate population structure at various geographic scales, with high accuracy and statistical support (Morin, Martien, & Taylor, 2009). Although SNPs have been widely used to investigate wild populations in other marine systems (e.g. Greenland halibut in the North Atlantic, Westgaard et al., 2016; Chinook salmon along the Pacific coast of North America, Narum et al., 2008; Narum, Buerkle, Davey, Miller, & Hohenlohe, 2013), these markers have not yet been widely used in elasmobranchs, and are limited to few shark species and locations (e.g. *Sphynra tiburo* along the north-eastern coast of the Gulf of Mexico, Portnoy et al., 2015; *Carcharhinus amblyrhynchos* in the Indian and Pacific Ocean, Momigliano et al., 2017). Results from the present and other shark studies using genomic tools have allowed a better understanding of phylogeographic patterns and of evolutionary processes affecting their populations (Momigliano et al., 2017), highlighting the need to integrate genomic tools along with other approaches to inform shark conservation, while addressing one of the most pressing problems for nearly 50 per cent of shark and ray species: data deficiency.

Considering informed conservation management changes required at the small geographic scale, I would consider the within jurisdiction case of Galapagos sharks in the Galápagos Islands first. This study represents the first effort to use genomic tools to inform shark conservation within the archipelago. Data presented here complements previous acoustic and satellite-tagging efforts carried out on other shark species (*Sphyrna lewini*, Ketchum, Hearn, Klimley, Peñaherrera, et al., 2014) to better understand current and historic patterns of connectivity in the southern Galápagos Islands, which can be implemented in management plans to ensure the protection of this and other shark species. Information provided herein is of utmost importance for *C. galapagensis* within the Galápagos Marine Reserve, where it is not only considered an iconic, but also an ecologically important species with conservation priority. Marine Protected Areas often have specific management plans for cartilaginous fishes and the Galápagos Marine Reserve (GMR) is no exception (Danulat & Edgar, 2002). However, the

information used to develop such plans can be biased towards economically important, or even charismatic species. This is the case in the Galápagos Marine Reserve, where most studies have focused on movement patterns of the scalloped hammerhead (*Sphyrna lewini*) using satellite and acoustic tagging methodologies (Hearn et al., 2014; Ketchum, Hearn, Klimley, Espinoza, et al., 2014; Ketchum, Hearn, Klimley, Peñaherrera, et al., 2014). Based on the hammerhead study, a Marine Sanctuary was created in the northern Galapagos Islands in 2016, which aims to protect a large biomass of specific shark species inhabiting this region (Salinas-de-León et al., 2016). Although this represents a significant effort and investment towards shark conservation, it does not represent all thirty-three species inhabiting the archipelago. Under this premise, studies using genetics/genomics approaches provide crucial complimentary data to ecological studies; thereby better informing conservation across more of the archipelago.

Results from Chapter 2 revealed the presence of at least two discrete shark populations with low effective population sizes and demographic independence (less than 10% gene flow) between them, indicating differential/targeted management is required to sustain these populations in the long term. In particular, one of the stocks (western, Isabela) was identified as more vulnerable than the other, eastern (Santa Cruz and San Cristóbal) stock. Furthermore, I caution against inferences about population structure of Galapagos sharks from the northern islands (Darwin and Wolf) in the present study, as very few samples were obtained from the far north. Therefore, additional samples are required to determine if Galapagos sharks from these northern islands may represent a separate stock. Understanding the threats and risks of more species of more shark populations across the Galapagos Islands is crucial to better inform policy makers so that they can properly regulate fishing and other human activities that threaten marine diversity (Reyes et al., 2014). Additionally, investigating the potential of non-extractive activities (e.g. dive tourism), capable of generating income for local economies is an important step to gain legitimacy for shark conservation in the archipelago (Hearn et al., 2014; Vianna, Meekan, Pannell, Marsh, & Meeuwig, 2012). Research focused on the economic dynamics of single-day diving industry in Santa Cruz Island demonstrated the importance of sharks for the

economy in the most tourist-focused of the Galápagos Islands (total gross income of more than 1.9 million US dollars per year for dive companies) (Peñaherrera, Llerena, & Keith, 2013). Although the latter study does not include the major income source of diving tourism (live-aboard diving tours), it provides important information for future studies and highlights the potential of well-managed tourism to produce high incomes, which may in fact outweigh the revenue generated from shark fishing as reported for Palau (Vianna et al., 2012).

Extending the study more broadly, to the Ocean basin scale, better resolved Galapagos shark population structure and genetic diversity across the Pacific, is clearly important for improved conservation management given the genetic structure detected within the Galápagos Marine Reserve. The phylogeographic assessment presented in Chapter 3 is key to understanding historical demographic patterns of *C. galapagensis*. I identified the presence of two Evolutionary Significant Units (ESUs) - one on either side of the Pacific Ocean – using neutral SNPs, indicating the Eastern Pacific Barrier (EPB) is a significant biogeographic break for Galapagos sharks. At the evolutionary level, understanding the divergence and connectivity of a species can provide valuable clues about their migration and colonization patterns. Additionally, I was able to identify and highlight the presence and importance of two connecting populations between the far eastern and west Pacific populations - based on putatively adaptive loci: Mexico in the east and Hawaii in the central Pacific. This extends our ability to enhance conservation outcomes for widespread shark species like Galapagos sharks by identifying local diversity that should be protected, including intermediate populations across the Pacific.

There is a further lesson in this - when doing assessments at different geographic scales apparently contradictory population structures may become apparent. Specifically, the two Galápagos Islands populations previously identified (Chapter 2), were no longer discernable when doing the trans-Pacific analyses of all Galapagos shark populations sampled (chapter 3), because when analysing amongst Galápagos Islands population structure separately, two genetic stocks were identified. However, analyses including samples from across the Pacific collapsed

the amongst Galápagos Islands genetic structure into a single stock, because the SNP loci retained after filtering for the combined analysis represented only 12% of the loci available for the independently analysed data sets, post-filtering. This indicates that informative SNPs at local scale are "lost" or swamped at global scales, due to the overriding evolutionary signal at the larger spatial scale. Ultimately, this emphasizes the importance of performing both local and regional scale assessments independently to properly understand population structure and to identify concrete actions to protect all identified stocks, regardless of the spatial scale at which analyses were performed. Importantly, the use of markers putatively under selection (outlier loci), provided the greatest resolution, suggesting footprints of local adaptation at both the within region level and across the Pacific. Taken together, this suggests that there are at least five Adaptive Units (AUs) across the Pacific (two within the Galápagos Islands, Mexico, Hawaii and the west Pacific). Although using outlier SNPs is not yet common practice in conservation genomics of elasmobranchs, mainly due to debate around the correct approach to define specific loci as outliers, these loci (likely associated with adaptive divergence) have been suggested to be important tools to identify and protect adaptive diversity in marine organisms such as the Atlantic cod (*Gadus morhua,* Bradbury et al., 2013). By analysing outlier loci, the cod study was able to detect further levels of differentiation (when none were detected by neutral markers) within both the eastern and western Atlantic, as well as successfully assigning individuals to the region of origin, emphasising their importance for informing conservation decisions. Local adaptation plays an important role in responses to environmental change (Savolainen et al., 2013). Thus identifying AUs in Galapagos sharks, and protecting adaptive variation will help ensure population persistence in the long term. Overall, these results indicate that regional assessments should be developed in some areas of the species distribution, particularly the Galápagos Islands, where effective population size estimates are low and populations are largely demographically isolated.

Finally, I highlight the importance of using neutral nuclear genome-wide markers in combination with mitochondrial DNA to identify whether hybridisation occurs between the two

closely related species – *Carcharhinus galapagensis* and *C. obscurus* - which co-occur in parts of their distribution ranges in both the east and west Pacific. As per other marine systems where hybridisation has been detected, ecological and behavioural factors related to this process have not yet been well studied in sharks. Montanari et al. (2016) provided a summary of the most common factors (ecological and behavioural) associated with marine fish hybridisation and highlights the importance of combining this data with genetic assessments in order to elucidate the causes of hybridisation. I hypothesise that rarity of parental species, indicated as the primary factor facilitating hybridisation in about 80% of marine fish hybrid reports reviewed by Montanari et al. (2016), may be facilitating hybridisation between *C. galapagensis* and *C. obscurus*. Identifying hybridisation is important in terms of conservation management, but requires confirming species identities first, because misidentification is not uncommon within the genus *Carcharhinus* (Duffy, 2016). Misidentification can be particularly problematic and undermines conservation efforts, especially when several species are traded under a single designation (Domingues et al., 2013). Exploitation of sharks and rays has increased in recent decades (Dulvy et al., 2014) and includes practices like “finning”, that often involve discarding the animal bodies and keeping only the fins or gills, which are of greatest economic interest (Shivji et al., 2002). Therefore, appropriate techniques that discriminate between species (particularly those more susceptible to “finning”) and that are capable to detect low levels of introgression, will help ensure conservation efforts are effectively protecting and prioritising such species. Although mitochondrial DNA provides a good genetic tool to define most species relationships, this is not true for *C. galapagensis* and *C. obscurus*, possibly due to historic mitochondrial introgression (Corrigan et al., 2017) or incomplete lineage sorting between these closely related species.

In Chapter 4 I found SNPs to be highly informative and able to discriminate between *C. galapagensis* and *C. obscurus*, despite evidence of ongoing hybridisation in east Pacific, but not west Pacific waters. Although the proportion of hybrids was relatively low (1%), carefully selected species-specific (diagnostic) SNP panels could detect signals of bi-directional

introgression between the two species. These findings support growing evidence that SNPs are powerful, reliable tools to investigate hybridisation processes (Nussberger et al., 2013). Four hybrid individuals from different hybrid classes (first generation hybrids and second generation backcrosses) confirmed the viability of hybrids. However, the extent of the influence on parental species and hybrid fitness is still to be investigated. This corresponds to the second record of interspecific ongoing hybridisation in sharks, the first case being of hybridisation between *Carcharhinus limbatus* and *C. tilstoni* along the east coast of Australia (Morgan et al. 2012). Previous studies aiming to investigate fish hybridisation have successfully used highly discriminatory SNP panels to assess levels of genetic admixture and hybridisation in bony fishes, such as the rainbow and cutthroat trout (*Oncorhynchus mykiss* and *O. clarkii*, Amish et al., 2012). However, my study is the first that used SNPs to successfully detect and measure the levels of genetic admixture and contemporary introgression in sharks. Importantly, the diagnostic (species-specific) loci detected here will provide a new tool for fisheries management, given their potential to accurately define both parental species and their hybrids. Furthermore, using this SNP approach has demonstrated its utility to improve genetic stock identification (GSI) in sharks, an important tool for fisheries management. For example, by using 96 diagnostic SNPs Larson et al. (2014) were able to identify three differentiated genetic stocks of the Chinook salmon (*Oncorhynchus tshawytscha*) in western Alaska, previously thought to be a single stock. Methods employed herein will serve as a guide for future work that examines more elasmobranch species.

2. Future directions

Integrative work including diverse tools and analytical approaches, such as movement tracking, morphological data and molecular markers is essential to better understand species conservation status, to identify current threats and therefore, to develop management plans that maximise the protection of sharks and help them recover from past and current pressures (Cutter, 2013; Dudgeon et al., 2012). The field of conservation genomics of highly mobile and widespread organisms such as sharks can be challenging, particularly considering the difficulty

of obtaining well-preserved tissue samples, especially when research relies on fisheries data and landed shark body parts (Dudgeon et al., 2012; Shivji et al., 2002). Available and commonly used methods such as DNA barcoding are useful most of the time. However, as Chapter 4 demonstrated, mitochondrial DNA can lack sufficient power to differentiate species in some cases, but this is only the first of many challenges associated with shark conservation (Helyar et al., 2011; Hemmer-Hansen et al., 2014). After correct species identification, a common major challenge is the need for additional information to enforce conservation actions and enhance protection. We need tools capable of accurately assessing taxonomy, along with a proper understanding of species evolution and behaviour, to assess the role and impact of current management practices in places with various levels of fisheries pressure. Identifying philopatric behaviour and/or nursery areas, for example, can help to define and prioritize areas for conservation (Hueter et al., 2004; Portnoy et al., 2015). Importantly, genome-wide sequencing techniques have become a time and money efficient method to obtain enormous amounts of data with a wide range of conservation applications (Allendorf et al., 2010; da Fonseca et al., 2016), many of which have not yet been fully explored. For example, the use of genomics approaches within forensic fisheries to provide information of provenance of carcasses and unlabelled body parts is still in its infancy (Dudgeon et al., 2012; Ogden, 2011). Species and population specific diagnostic markers, such as SNPs, promise a whole new perspective and increased resolution to assist fisheries management, but are still to be developed and validated for most shark species (Corrigan et al., 2017; Krück, Innes, & Ovenden, 2013; Ogden, 2011).

With technology moving forward in the field of conservation genetics and genomics, and accessibility to genome resources continuously growing for non-model organisms, the opportunity to produce and manipulate genome-wide data capable of accurately responding to the conservation questions has exponentially increased. It is now possible to understand population structure and connectivity patterns in a very efficient manner, as well as investigating the ecological and evolutionary mechanisms behind it. Thus allowing accurate stock assessment as demonstrated in Chapters 2 and 3. Results from this thesis provide evidence

of this improvement, illustrates the multiple advantages of genomic tools for conservation applications, and emphasises the importance of comparing assessments at different geographic scales in order to achieve a comprehensive understanding of conservation needs. Future studies aiming to develop baseline genomic resources to address data-deficiency problems, and to inform conservation status of elasmobranchs can use the Galapagos shark study case as an example to expand the potential of genome-wide data applications (e.g. to investigate local adaptation as an important source of variation driving population structure patterns). Sequencing large portions or the full genome of *C. galapagensis* (or a closely related congener) is no longer an unthinkable/unachievable task. Whilst sequencing and annotating a whole genome still requires a significant investment of economic and computational resources, the advantages of having a reference genome for the Carcharhinidae family (the more speciose family of sharks) will be a significant advance that informs shark conservation. A reference genome will enable accurate annotations and identification of functional genome-wide loci, and therefore, the delimitation of conservation units to preserve both neutral and adaptive variation of wild populations (de Guia & Saitoh, 2007).

I highlight the importance of an appropriate sampling strategy in order to accurately infer connectivity patterns and population genetic structure, to ultimately inform conservation of a species. Although the sampling strategy used here was sufficient to provide first insights into *C. galapagensis* demographic patterns within the Galápagos Islands, sampling intermediate (central) locations and increasing the number of samples from the northern Islands (Darwin and Wolf), will allow a more comprehensive understanding of the management and conservation needs of this species locally. Similarly, increasing sampling locations across the Pacific Ocean: e.g. Malpelo, Cocos and other eastern Pacific Islands along with western Pacific islands, including intermediate islands and atolls between New Zealand and Hawaii, will provide useful information to identify colonization and migratory (stepping stone) routes, and potentially new conservation units. Likewise, sampling gaps is important to consider when studying hybridisation/introgression processes. This study included samples from three contact zones of

C. galapagensis and *C. obscurus* (east coast of Australia, the Revillagigedo Islands, and Gulf of California), and allowed us to detect rare cases of hybridisation in the Eastern Tropical Pacific. However, increasing the number of samples of both species from these, and other potential contact zones, together with addressing the ecological and behavioral mechanisms that facilitate this hybridisation will enhance our understanding of this process and the consequences thereof for species diversity and conservation. Furthermore, a phylogenomic assessment combining mtDNA and nuclear SNPs, and including more species of the genus *Carcharhinus* is required to resolve species relationships and to unveil potential undetected hybridisation and introgression cases between closely related species, impossible to detect by using a mitochondrial DNA approach only.

I emphasise the importance of using genome-wide data to analyse signals of population structure accurately and estimate genetic differentiation, even at small geographic scales, as demonstrated in this thesis. Overall, this work sets a precedent for *C. galapagensis* research, and for shark conservation efforts in general. Understanding population structure, genetic diversity, and resilience of widely distributed species at different geographic scales will enable an update of the Galapagos shark conservation status on the IUCN list of threatened species, based on up to date accurate information. Additionally, this research will provide guidance for further studies using genome-wide SNPs as a tool to ultimately inform management decisions based on the existence of discrete conservation units that require management as independent populations, rather than being considered as a single widespread population that is inappropriately managed as such. Such a change in management may be challenging, considering that the species range crosses many different national boundaries, each with their own rules and regulations, along with being difficult to enforce at such large spatial scales. Therefore, updated IUCN listings may be crucial to reflect better informed conservation requirements, once identified.

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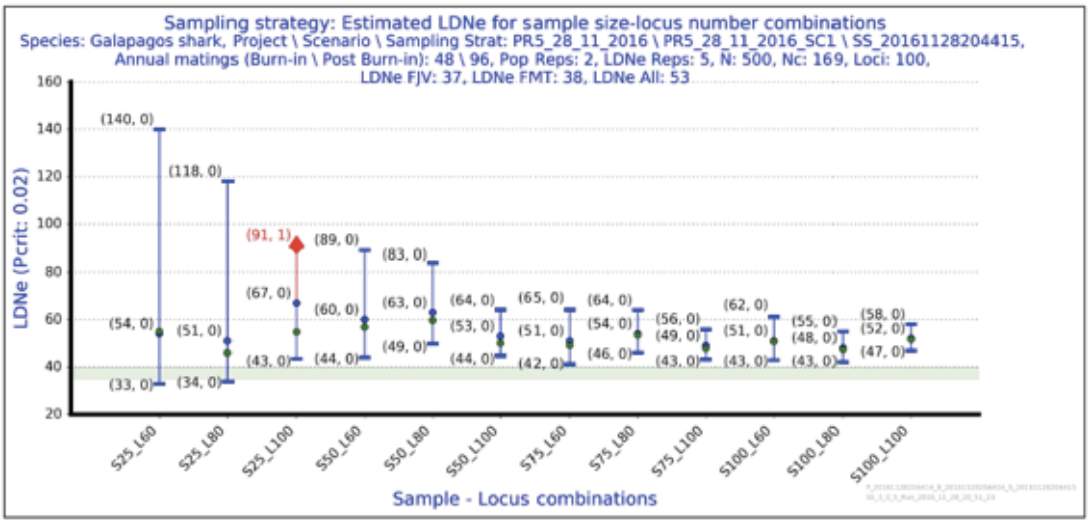
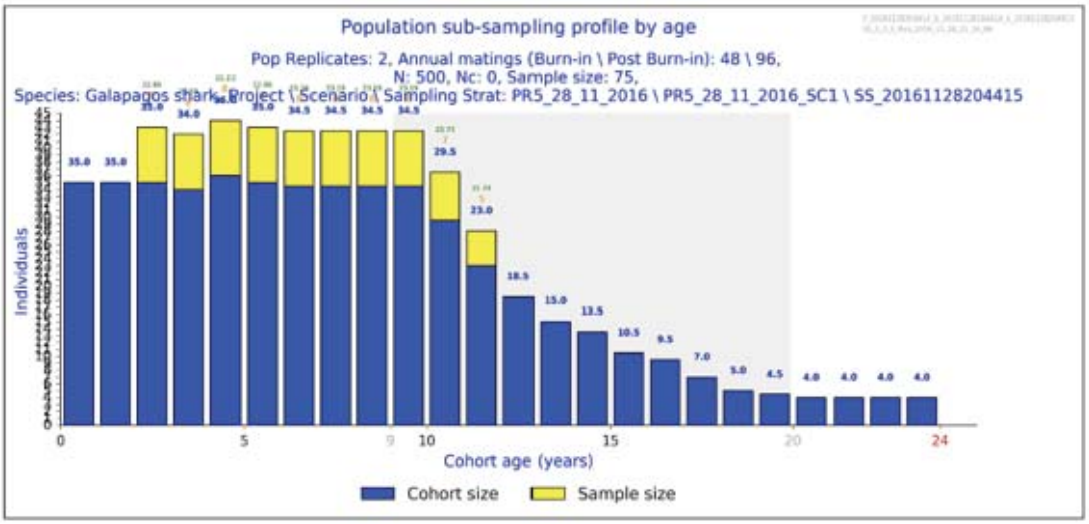
Appendix

Supplemental Figure 1 Sampling strategy demographic plot (A) and sampling strategy LDN_e analysis plot (B) showing the accuracy of each sample size (25-100) and locus number (60-100) simulated in the NeOGen software.

A



B



Supplemental Table 1 List of shark mitogenomes published to date, arranged by date of publication.

Shark species	Common name	Family	Sequencing platform	Reference	GenBank ID	size
Mustelus manazo	Smooth-hound shark	Triakidae	Not specified	Cao et al. 1998	AB015962	16707
Scyliorhinus canicula	Small-spotted catshark	Scyliorhinidae	Not specified	Delarbre et al. 1998	Y16067	16697
Chiloscyllium griseum	Grey bamboo shark	Hemiscylliidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2013	NC017882	16755
Carcharhinus obscurus	Dusky shark	Carcharhinidae	454 shotgun pyrosequencing	Blower et al. 2013	KC470543	16706
Pseudotriakis microdon	False catshark	Pseudotriakidae	3130xl Genetic Analyzer	Tanaka et al. 2013	AB560493	16700
Galeocerdo cuvier	Tiger shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2014	KF111728	16703
Rhincodon typus	Whale shark	Rhincodontidae	Hiseq 2000 (Illumina) and GS-FLX (454 Life Sciences)	Alam et al. 2014	KF679782	16875
Glyphis glyphis	Speartooth shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al 2014	KF006312	16702
Alopias superciliosus	Big-eye thresher shark	Alopiidae	ABI 3730 DNA Analyzer (Applied Biosystems)	Chang et al. 2014	KC757415	16719
Carcharodon carcharias	Great white shark	Lamnidae	Not specified	Chang et al. 2014	KC914387	16744
Megachasma pelagios	Megamouth shark	Megachasmidae	ABI 3730 automated sequencer (Applied Biosystems)	Chang et al. 2014	KC702506	16694
Scoliodon macrorhynchos	Spadenose shark	Carcharhinidae	Not specified	Chen et al. 2014	JQ693102	16693
Chiloscyllium punctatum	Brownbanded bamboo shark	Hemiscylliidae	Not specified	Chen et al. 2014	JQ082337	16703
Carcharias taurus	Grey nurse shark	Odontaspididae	HiSeq 2000 (Illumina)	Bowden et al. 2015	KT337317	16715
Carcharias taurus	Sand tiger shark	Odontaspididae	Not specified	Chang et al. 2015	KF569943	16773
Cetorhinus maximus	Basking shark	Cetorhinidae	ABI 3130 genetic analyzer (Applied Biosystems)	Hester et al. 2015	KF597303	16670
Alopias pelagicus	Pelagic thresher	Alopiidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2015	KF412639	16692
Prionace glauca	Blue shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2015	KF356249	16705
Carcharhinus leucas	Bull shark	Carcharhinidae	Not specified	Chen et al. 2015	NC023522	16704
Carcharhinus sorrah	Spot-tail shark	Carcharhinidae	Not specified	Chen et al. 2015	KF612341	16707
Orectolobus japonicus	Japanese wobbegong	Orectolobidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2015	KF111729	16706
Isurus oxyrinchus	Shortfin mako	Lamnidae	Not specified	Chang et al. 2015	KF361861	16701
Sphyrna lewini	Scalloped hammerhead	Sphyrnidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al 2015	JX827259	16726
Carcharhinus acronotus	Blacknose shark	Carcharhinidae	Not specified	Yang et al. 2016	KF728380	16719
Carcharhinus plumbeus	Sandbar shark	Carcharhinidae	454 shotgun pyrosequencing	Blower & Ovenden 2016	KJ740750	16706
Pseudocarcharias kamoharai	Crocodile shark	Pseudocarchariidae	ABI 3730 automated sequencer (Applied Biosystems)	Li et al. 2016	KM575726	16694
Isurus paucus	Longfin mako shark	Lamnidae	Not specified	Chang et al. 2016	KJ616742	16704
Sphyrna zygaena	Hammerhead shark	Sphyrnidae	MiSeq v3 600 cycles kit (Illumina)	Bolaño-Martínez et al. 2016	KM489157	16731

Supplemental Table 1 Continue.

Carcharhinus melanopterus	Blacktip reef shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2016	KJ720818	16706
Carcharhinus falciformis	Silky shark	Carcharhinidae	MiSeq v2 500 cycle kit (Illumina)	Galván-Tirado et al. 2016	KF801102	17774
Heterodontus zebra	Zebra bullhead shark	Heterodontidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2016	KC845548	16720
Carcharhinus macloti	Hardnose shark	Carcharhinidae	Not specified	Chen et al. 2016	KJ865755	16701
Carcharhinus longimanus	Oceanic whitetip shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Li et al. 2016	KM434158	16908
Scoliodon laticaudus	Spadenose shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Periasamy et al. 2016	KP336547	16695
Carcharhinus brevipinna	Spinner shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2016	KM244770	16706
Triaenodon obesus	Whitetip reef shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2016	KJ748376	16700
Pseudocarcharias kamoharai	Crocodile shark	Pseudocarchariidae	Not specified	Chang et al. 2016	KM597489	16688
Lamna ditropis	Salmon sharks	Lamnidae	Not specified	Chang et al. 2016	KF562053	16699
Carcharhinus tjutjot	Indonesian whaler shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2016	KP091436	16705
Cephaloscyllium umbratile	Blotchy swell shark	Scyliorhinidae	Not specified	Chen et al. 2016	KT003686	16698
Carcharhinus leucas	Bull shark	Carcharhinidae	MiSeq v2 500 cycle kit (Illumina)	Diaz-Jaimes et al. 2016	KJ210595	16100
Sphyrna tiburo	Bonnethead shark	Sphyrnidae	MiSeq v3 600 cycle kit (Illumina)	Diaz-Jaimes et al. 2016	KM453976	16723
Carcharhinus amboinensis	Pigeye Shark	Carcharhinidae	As per grey bamboo shark	Feutry et al. 2016	KM921745	16704
Ginglymostoma cirratum	Nurse shark	Ginglymostomatidae	MiSeq version 2 (Illumina)	Kashiwagi et al. 2016	KU904394	16692
Lamna nasus	Porbeagle shark	Lamnidae	Illumina NextSeq	Diaz-Jaimes et al. 2016	KX610464	16697
Eusphyra blochii	Winghead Shark	Sphyrnidae	Not specified	Feutry et al. 2016	KU892590	16727
Carcharhinus amblyrhynchoides	Graceful Shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Feutry et al. 2016	KF956523	16705
Glyphis garricki	Northern River shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Feutry et al. 2016	KF646786	16702
Stegostoma fasciatum	Zebra shark	Stegostomatidae	Not specified	Chen et al. 2016	KU057952	16658
Hemipristis elongata	Snaggletooth shark	Hemigaleidae	Not specified	Huang et al. 2016	KU508621	16691
Loxodon macrorhinus	Sliteye shark	Carcharhinidae	ABI 3730 automated sequencer (Applied Biosystems)	Wang et al. 2016	KT347599	16702
Hemigaleus microstoma	Sicklefin weasel shark	Hemigaleidae	ABI 3730 automated sequencer (Applied Biosystems)	Mai et al. 2016	KT003687	16701
Proscyllium habereri	Catshark	Proscylliidae	Not specified	Chen et al. 2016	KU721838	16708
Halaelurus burgeri	Blackspotted catshark	Scyliorhinidae	Not specified	Chen et al. 2016	KU892589	19100
Squalus formosus	Taiwan spurdog shark	Squalidae	Not specified	Chen et al. 2016	KU951280	16735
Etmopterus pusillus	Smooth lanternshark	Etmopteridae	Not specified	Chen et al. 2016	KU892588	16729
Squaliolus aliae	Small eye pygmy shark	Dalatiidae	Not specified	Chen et al. 2016	KU873080	16717
Mustelus griseus	Spotless smooth-hound	Triakidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2016	KF889325	16754
Chlamydoselachus anguineus	Frilled shark	Chlamydoselachida	HiSeq 2000 (Illumina)	Bustamante et al. 2016	KU159431	17313

Supplemental Table 2 List of ray mitogenomes published to date, arranged by date of publication.

Rays and skates	**Common name**	**Family**	**Sequencing method**	**Reference**	**GenBank ID**	**size**
Dasyatis zugei	Pale-edged stingray	Dasyatidae	Not specified	Chen et al. 2013	JX524174	18264
Taeniura meyeni	Blotched fantail ray	Dasyatidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2013	JX827260	17638
Mobula japonica	Spine tail devil ray	Mobulidae	ABI 3730 automated sequencer (Applied Biosystems)	Poortvliet & Hoarau 2013	JX392983	18880
Dasyatis bennetti	Frilltailed stingray	Dasyatidae	Not specified	Yang et al. 2013	KC196067	17668
Zearaja chilensis	Yellow nose skate	Rajidae	Ion Torrent sequencing	Vargas-Caro et al. 2014	KJ913073	16909
Dasyatis akajei	Red stingray	Dasyatidae		Chen et al. 2014	KC526959	17658
Pastinachus atrus	Cow tail ray	Dasyatidae	HiSeq system (Illumina)	Austin et al. 2014	NC023808	18162
Taeniura lymma	Blue spotted ribbontail ray	Dasyatidae	HiSeq system (Illumina)	Austin et al. 2015	KM881715	17652
Dipturus trachyderma	Rough skin skate	Rajidae	Miseq 600v3 (Illumina)	Vargas-Caro et al. 2015	KR152643	16907
Mobula mobular	Giant devil ray	Myliobatidae	MiSeq 600v3 (Illumina)	Bustamante et al. 2015	KT203434	18913
Pristis clavata	Dwarf sawfish	Pristidae	ABI 3730 automated sequencer (Applied Biosystems)	Feutry et al 2015	KF381507	16804
Pristis pristis	Large tooth sawfish	Pristidae	Miseq (Illumina)	Feutry et al. 2015	SRR1732112-203	
Pristis pectinata	Smalltooth Sawfish	Pristidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2015	KP400584	16802
Rhinobatos hynnicephalus	Ringstraked guitarfish	Rhinobatidae	Not specified	Chen et al. 2015	KF534708	16776
Manta birostris	Giant Manta ray	Myliobatidae	HiSeq (Illumina)	Hinojosa-Alvarez et al. 2015	KF413894	18075
Potamotrygon motoro	Ocellate river stingray	Potamotrygonidae	ABI 3730 Genetic Analyzer (Applied Biosystems)	Song et al. 2015	KF709642	17448
Aetobatus flagellum	Longheaded eagle ray	Myliobatidae	Not specified	Zhang et al. 2015	KF482070	20201
Himantura granulata	Mangrove whipray	Dasyatidae	Not specified	Chen et al. 2016	KF751650	17657
Anoxypristis cuspidata	Narrow Sawfish	Pristidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2016	KP233202	17243
Rhinobatos schlegelii	Brown guitarfish	Rhinobatidae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2016	KJ140136	16780
Rhynchobatus australiae	White-spotted guitarfish	Rhinobatidae	Not specified	Si et al. 2016	KU746824	16804
Sinobatis borneensis	Borneo leg skate	Anacanthobatidae	Not specified	Si et al. 2016	KX014715	16701
Notoraja tobitukai	Leadhued skate	Arhynchobatidae	Not specified	Si et al. 2016	KX150853	16799
Himantura jenkinsii	Jenkins whipray	Dasyatidae	Not specified	Si et al. 2016	KU873081	16670
Rhina ancylostoma	Bowmouth guitarfish	Rhinobatidae	ABI 3730 automated sequencer (Applied Biosystems)	Si et al. 2016	KU721837	17217
Himantura leoparda	Leopard whipray	Dasyatidae	Miseq (Illumina)	Shen et al. 2016	KR019776	17690
Neotrygon kuhlii	Blue-spotted stingray	Dasyatidae	Miseq (Illumina)	Shen et al. 2016	KR019777	17974
Himantura microphthalma	smalleye whip ray	Dasyatidae	ABI 3730 automated sequencer (Applied Biosystems)	Wang et al. 2016	KF840390.1	17636
Pteroplatytrygon violacea	Pelagic stingray	Dasyatidae	Not specified	Yang et al. 2016	KJ641617	17665
Gymnura poecilura	Longtail butterfly ray	Gymnuridae	ABI 3730 automated sequencer (Applied Biosystems)	Chen et al. 2016	KJ617038	17874

Supplemental Table 3 Outlier SNPs identified by LOSITAN, PCAdapt and ARLEQUIN, respectively.

LOSITAN GLOBAL SCALE			**LOSITAN REGIONAL SCALE**			**PCADAPT REGIONAL SCALE**	**ARLEQUIN GLOBAL SCALE**		
SNP ID	p-val	F_{ST}	SNP ID	p-val	F_{ST}	SNP ID	SNP ID	p-val	F_{ST}
SNP0011	0.1811	1.0000	SNP0046	1	0.949848	SNP0012	SNP0051	0.0000	0.621692
SNP0049	0.1411	0.9982	SNP0069	0.999599	0.150316	SNP0036	SNP0816	0.0061	0.528149
SNP0051	0.6671	1.0000	SNP0123	0.993988	0.104669	SNP0078	SNP1030	0.0000	0.583178
SNP0067	0.1203	0.9939	SNP0127	0.996505	0.11296	SNP0269	SNP1135	0.0000	0.347255
SNP0070	0.1296	0.9975	SNP0140	0.999983	0.156575	SNP0291	SNP2404	0.0000	0.345121
SNP0106	0.1514	0.9998	SNP0168	0.998338	0.109798	SNP0303	SNP2577	0.0000	0.454097
SNP0109	0.1170	0.9998	SNP0204	1	0.580941	SNP0307	SNP3146	0.0241	0.306902
SNP0141	0.1299	0.9989	SNP0229	0.997168	0.098861	SNP0327	SNP3226	0.0000	0.564139
SNP0150	0.1824	1.0000	SNP0300	0.999878	0.176603	SNP0348	SNP3514	0.0000	0.361589
SNP0179	0.1640	0.9997	SNP0325	0.999126	0.137096	SNP0369	SNP3577	0.0000	0.328882
SNP0183	0.1275	0.9954	SNP0327	1	0.454106	SNP0394	SNP3943	0.0378	0.298656
SNP0184	0.1220	0.9990	SNP0332	0.997262	0.099307	SNP0407	SNP4075	0.0007	0.322342
SNP0222	0.1133	0.9979	SNP0346	0.999376	0.143722	SNP0449	SNP4668	0.0000	0.551482
SNP0231	0.3782	1.0000	SNP0378	0.999999	0.177608	SNP0474	SNP5049	0.0050	0.324312
SNP0245	0.2237	1.0000	SNP0406	0.99488	0.09933	SNP0486	SNP5244	0.0000	0.495713
SNP0256	0.1260	0.9987	SNP0407	0.999989	0.222433	SNP0526	SNP5587	0.0015	0.45407
SNP0269	0.1162	0.9985	SNP0409	0.994906	0.099424	SNP0530	SNP5681	0.0000	0.447763
SNP0278	0.1136	0.9983	SNP0417	0.998916	0.106869	SNP0560	SNP5720	0.0000	0.567686
SNP0285	0.1704	1.0000	SNP0430	0.999999	0.28289	SNP0584	SNP6101	0.0000	0.469672
SNP0318	0.1326	0.9995	SNP0443	0.999429	0.134823	SNP0687	SNP6126	0.0010	0.320871
SNP0330	0.1810	1.0000	SNP0448	0.996746	0.107902	SNP0709	SNP6298	0.0000	0.335147
SNP0339	0.1385	0.9996	SNP0462	0.999995	0.196428	SNP0727	SNP6384	0.0000	0.486227
SNP0346	0.1906	1.0000	SNP0482	0.99648	0.112802	SNP0767	SNP6865	0.0011	0.316004
SNP0351	0.0976	0.9946	SNP0492	1	0.189122	SNP0852	SNP7274	0.0000	0.563527
SNP0360	0.0960	0.9979	SNP0504	0.999932	0.144816	SNP0976	SNP7397	0.0000	0.584989
SNP0379	0.1233	0.9992	SNP0512	0.999848	0.171015	SNP0987	SNP7655	0.0000	0.581046
SNP0413	0.1179	0.9994	SNP0526	0.998092	0.118057	SNP0997	SNP7835	0.0000	0.524177
SNP0426	0.1209	0.9982	SNP0550	0.999946	0.14695	SNP1029	SNP8150	0.0000	0.429055
SNP0436	0.1130	0.9991	SNP0553	1	0.308974	SNP1046	SNP8160	0.0000	0.342693
SNP0442	0.1162	0.9972	SNP0587	0.999458	0.146517	SNP1064	SNP8172	0.0017	0.482339
SNP0444	0.1230	0.9991	SNP0601	0.997795	0.097101	SNP1065	SNP8274	0.0000	0.369978
SNP0453	0.1120	0.9979	SNP0616	0.999647	0.124968	SNP1123			
SNP0467	0.2201	1.0000	SNP0619	0.996597	0.096413	SNP1152			
SNP0486	0.1914	1.0000	SNP0621	0.999667	0.156184	SNP1248			
SNP0494	0.1440	1.0000	SNP0624	0.999331	0.139749	SNP1378			
SNP0496	0.1358	1.0000	SNP0642	0.999312	0.109455	SNP1652			
SNP0515	0.1917	1.0000	SNP0655	0.999957	0.148967	SNP1769			
SNP0526	0.1760	1.0000	SNP0687	1	0.522921	SNP1818			
SNP0535	0.1615	1.0000	SNP0692	0.999816	0.152764	SNP1850			
SNP0549	0.2457	1.0000	SNP0713	0.995256	0.10616	SNP1994			
SNP0553	0.1723	0.9995	SNP0728	0.99967	0.169386	SNP2017			
SNP0557	0.1762	1.0000	SNP0732	0.999852	0.172551	SNP2054			
SNP0568	0.1320	0.9996	SNP0736	0.999839	0.169761	SNP2057			
SNP0590	0.1283	0.9995	SNP0750	0.997235	0.124107	SNP2118			
SNP0602	0.2695	1.0000	SNP0769	0.999917	0.127878	SNP2141			
SNP0612	0.1653	0.9998	SNP0806	0.996885	0.10873	SNP2157			
SNP0630	0.1129	0.9966	SNP0815	0.999565	0.121797	SNP2194			

Supplemental Table 3 Continue.

SNP0631	0.1009	0.9940	SNP0820	0.999395	0.165179	SNP2204			
SNP0639	0.1645	1.0000	SNP0828	0.9986	0.125834	SNP2272			
SNP0648	0.1083	0.9974	SNP0843	0.993342	0.094623	SNP2374			
SNP0677	0.1862	0.9998	SNP0852	0.999998	0.327996	SNP2419			
SNP0691	0.1317	0.9973	SNP0861	0.998601	0.124773	SNP2463			
SNP0706	0.1295	0.9995	SNP0879	0.999004	0.134541	SNP2501			
SNP0707	0.1374	0.9979	SNP0959	0.999999	0.30113	SNP2520			
SNP0732	0.1292	1.0000	SNP0963	0.998254	0.120346	SNP2527			
SNP0736	0.1502	0.9997	SNP0976	0.995435	0.100944	SNP2539			
SNP0748	0.4349	1.0000	SNP0984	0.999923	0.166237	SNP2563			
SNP0754	0.1639	0.9999	SNP0997	0.999999	0.213477	SNP2583			
SNP0757	0.1091	0.9975	SNP1017	0.993128	0.097313	SNP2584			
SNP0760	0.1112	0.9990	SNP1078	0.992403	0.091886	SNP2624			
SNP0782	0.1086	0.9963	SNP1092	1	0.18129	SNP2885			
SNP0790	0.1193	0.9976	SNP1104	0.999927	0.166898	SNP2896			
SNP0798	0.1701	0.9999	SNP1133	0.996812	0.101139	SNP2901			
SNP0802	0.1437	0.9993	SNP1169	0.99925	0.116635	SNP3100			
SNP0813	0.1751	0.9999	SNP1183	0.995154	0.103985	SNP3165			
SNP0818	0.3550	1.0000	SNP1187	0.998311	0.124266	SNP3201			
SNP0821	0.1898	1.0000	SNP1222	0.997912	0.116338	SNP3280			
SNP0838	0.2345	1.0000	SNP1224	0.999911	0.127349	SNP3313			
SNP0862	0.1122	0.9965	SNP1225	1	0.425597	SNP3457			
SNP0864	0.1315	0.9973	SNP1228	0.999315	0.161661	SNP3490			
SNP0924	0.2055	1.0000	SNP1239	0.997974	0.116917	SNP3590			
SNP0961	0.1528	0.9990	SNP1249	0.999435	0.14156	SNP3613			
SNP1019	0.1123	0.9981	SNP1250	1	0.220005	SNP3618			
SNP1030	0.0667	0.9632	SNP1323	1	0.211844	SNP3627			
SNP1032	0.3656	1.0000	SNP1330	0.999996	0.167721	SNP3628			
SNP1044	0.1739	1.0000	SNP1362	0.999837	0.122503	SNP3653			
SNP1049	0.1120	0.9990	SNP1379	0.999949	0.14074	SNP3665			
SNP1069	0.1026	0.9957	SNP1418	0.99877	0.127364	SNP3705			
SNP1077	0.0956	0.9978	SNP1461	0.999294	0.138629	SNP3738			
SNP1078	0.1067	0.9990	SNP1482	0.998646	0.125432	SNP3896			
SNP1124	0.1400	0.9996	SNP1485	1	0.191385	SNP3942			
SNP1127	0.1479	0.9979	SNP1504	0.999341	0.152079	SNP4001			
SNP1137	0.2304	1.0000	SNP1505	0.99748	0.120362	SNP4073			
SNP1157	0.1474	0.9996	SNP1517	0.997911	0.116327	SNP4095			
SNP1160	0.1499	0.9998	SNP1527	0.999686	0.15742	SNP4119			
SNP1189	0.1031	0.9991	SNP1528	0.996658	0.1074	SNP4275			
SNP1201	0.1952	1.0000	SNP1543	0.999922	0.143551	SNP4356			
SNP1218	0.1152	0.9970	SNP1553	0.994821	0.102713	SNP4522			
SNP1272	0.1214	0.9962	SNP1558	0.996398	0.106069	SNP4582			
SNP1277	0.1467	0.9994	SNP1563	0.999998	0.171494	SNP4636			
SNP1279	0.1215	0.9999	SNP1574	1	0.197662	SNP4714			
SNP1289	0.1411	0.9995	SNP1579	0.999531	0.14519	SNP4743			
SNP1296	0.1028	0.9978	SNP1610	0.999879	0.195798	SNP4869			
SNP1332	0.1339	0.9990	SNP1647	0.994324	0.097383	SNP4891			
SNP1333	0.4088	1.0000	SNP1666	0.999999	0.174545	SNP4986			
SNP1358	0.1099	0.9996	SNP1673	0.997591	0.113968	SNP5004			

Supplemental Table 3 Continue.

SNP1359	0.1045	0.9965	SNP1700	0.996431	0.106246	SNP5057			
SNP1426	0.1332	0.9994	SNP1755	1	0.338618	SNP5070			
SNP1436	0.1320	0.9999	SNP1772	0.996626	0.105382	SNP5121			
SNP1444	0.1181	0.9988	SNP1776	0.999995	0.165689	SNP5135			
SNP1462	0.1024	0.9959	SNP1784	0.995086	0.100103	SNP5146			
SNP1468	0.1407	0.9981	SNP1787	0.995552	0.096481	SNP5149			
SNP1472	0.2196	1.0000	SNP1800	1	0.184979	SNP5159			
SNP1480	0.0959	0.9969	SNP1820	0.997027	0.094021	SNP5160			
SNP1486	0.1474	0.9987	SNP1835	0.99971	0.122382	SNP5213			
SNP1499	0.1314	0.9973	SNP1860	0.997253	0.118399	SNP5231			
SNP1508	0.1515	0.9991	SNP1883	1	0.212698	SNP5240			
SNP1535	0.1176	0.9979	SNP1902	0.998332	0.120633	SNP5253			
SNP1550	0.1709	0.9996	SNP1913	0.999081	0.106547	SNP5352			
SNP1558	0.1012	0.9975	SNP1919	0.997906	0.116739	SNP5423			
SNP1568	0.1477	0.9999	SNP1928	1	0.234681	SNP5434			
SNP1569	0.1666	1.0000	SNP1948	0.999276	0.140793	SNP5467			
SNP1572	0.1167	0.9973	SNP1958	0.999912	0.182986	SNP5473			
SNP1588	0.1094	0.9976	SNP1960	0.999976	0.203526	SNP5504			
SNP1589	0.1258	0.9985	SNP1976	0.999488	0.158309	SNP5573			
SNP1611	0.1665	1.0000	SNP1983	0.999995	0.253697	SNP5578			
SNP1620	0.1213	0.9991	SNP1986	0.999801	0.151543	SNP5592			
SNP1632	0.1123	0.9997	SNP2016	0.999015	0.151522	SNP5596			
SNP1654	0.1888	1.0000	SNP2019	0.999434	0.143196	SNP5603			
SNP1657	0.1020	0.9958	SNP2023	1	0.33412	SNP5634			
SNP1660	0.1733	0.9998	SNP2036	0.99289	0.100595	SNP5639			
SNP1673	0.1133	0.9943	SNP2043	0.999794	0.129975	SNP5676			
SNP1675	0.1385	0.9998	SNP2056	0.999989	0.19487	SNP5724			
SNP1694	0.1180	0.9988	SNP2058	1	0.236343	SNP5745			
SNP1808	0.1433	0.9984	SNP2078	0.998073	0.117875	SNP5971			
SNP1813	0.1010	0.9954	SNP2090	0.999966	0.15103	SNP5982			
SNP1844	0.1852	1.0000	SNP2105	0.99574	0.108543	SNP6065			
SNP1871	0.0982	0.9984	SNP2113	0.999405	0.144658	SNP6078			
SNP1874	0.1039	0.9962	SNP2115	0.99846	0.131733	SNP6084			
SNP1899	0.1404	0.9998	SNP2118	1	0.352058	SNP6085			
SNP1901	0.1788	1.0000	SNP2119	1	0.182243	SNP6092			
SNP1910	0.1467	0.9999	SNP2126	1	0.329299	SNP6101			
SNP1924	0.1033	0.9962	SNP2129	0.999993	0.238241	SNP6111			
SNP1934	0.1301	0.9995	SNP2131	1	0.576685	SNP6184			
SNP1953	0.1881	1.0000	SNP2158	1	0.38238	SNP6205			
SNP1970	0.1203	0.9978	SNP2164	0.999401	0.110813	SNP6239			
SNP1990	0.1098	0.9996	SNP2170	0.999991	0.161223	SNP6326			
SNP2016	0.1519	0.9997	SNP2174	0.999779	0.149928	SNP6342			
SNP2047	0.1716	1.0000	SNP2181	0.999517	0.148806	SNP6345			
SNP2094	0.1413	0.9998	SNP2183	0.998393	0.125227	SNP6400			
SNP2096	0.1603	1.0000	SNP2199	0.997412	0.119752	SNP6404			
SNP2117	0.1470	0.9999	SNP2209	0.999538	0.136694	SNP6448			
SNP2127	0.1085	0.9972	SNP2233	0.999976	0.20346	SNP6454			
SNP2129	0.1118	0.9964	SNP2236	0.999955	0.174449	SNP6511			
SNP2146	0.1394	0.9971	SNP2241	1	0.221095	SNP6523			

Supplemental Table 3 Continue.

SNP2148	0.0980	0.9967	SNP2253	0.999591	0.122491	SNP6552			
SNP2155	0.1234	0.9983	SNP2278	0.999999	0.364136	SNP6583			
SNP2198	0.1949	1.0000	SNP2286	0.999689	0.155667	SNP6598			
SNP2201	0.1900	1.0000	SNP2288	1	0.20293	SNP6643			
SNP2203	0.1213	0.9988	SNP2293	0.999465	0.144363	SNP6669			
SNP2204	0.2756	1.0000	SNP2297	0.997445	0.09541	SNP6680			
SNP2211	0.1303	0.9995	SNP2301	0.999996	0.167732	SNP6685			
SNP2226	0.1602	0.9998	SNP2303	1	0.307354	SNP6692			
SNP2243	0.1748	0.9999	SNP2304	0.999262	0.137723	SNP6718			
SNP2245	0.1461	0.9999	SNP2307	0.995949	0.109664	SNP6737			
SNP2246	0.1028	0.9960	SNP2335	0.99509	0.103733	SNP6847			
SNP2255	0.1538	0.9997	SNP2337	0.996153	0.110817	SNP6886			
SNP2256	0.1151	0.9993	SNP2359	0.998795	0.142232	SNP6904			
SNP2279	0.1779	1.0000	SNP2366	0.994865	0.104418	SNP6928			
SNP2318	0.1452	0.9999	SNP2379	0.999185	0.138475	SNP6939			
SNP2323	0.3159	1.0000	SNP2391	0.998225	0.119441	SNP6991			
SNP2331	0.3074	1.0000	SNP2395	0.9995	0.116142	SNP7014			
SNP2334	0.1675	1.0000	SNP2397	0.999833	0.122281	SNP7017			
SNP2336	0.5197	1.0000	SNP2408	0.996409	0.112357	SNP7095			
SNP2343	0.1276	0.9988	SNP2419	0.999973	0.212542				
SNP2366	0.3241	1.0000	SNP2432	0.998461	0.101103				
SNP2369	0.1320	0.9995	SNP2443	0.996409	0.091386				
SNP2397	0.1121	0.9995	SNP2450	0.995449	0.101554				
SNP2406	0.1264	0.9991	SNP2456	0.998181	0.117026				
SNP2409	0.2162	1.0000	SNP2462	0.997854	0.09741				
SNP2411	0.2746	1.0000	SNP2474	0.995983	0.107575				
SNP2416	0.1026	0.9957	SNP2480	0.995903	0.097644				
SNP2435	0.0983	0.9944	SNP2483	0.999984	0.157034				
SNP2440	0.2390	1.0000	SNP2484	0.996611	0.110571				
SNP2449	0.1372	0.9994	SNP2493	0.998498	0.123348				
SNP2453	0.1569	0.9998	SNP2504	1	0.409806				
SNP2459	0.1578	1.0000	SNP2513	0.999999	0.17455				
SNP2464	0.1660	0.9998	SNP2523	0.999591	0.149931				
SNP2465	0.1034	0.9941	SNP2551	0.998488	0.102731				
SNP2476	0.1290	0.9994	SNP2556	0.999998	0.170778				
SNP2497	0.1913	1.0000	SNP2569	0.999937	0.138667				
SNP2506	0.1159	0.9971	SNP2616	1	0.338527				
SNP2508	0.1381	0.9998	SNP2622	0.992287	0.09511				
SNP2524	0.2096	1.0000	SNP2662	0.998767	0.141638				
SNP2546	0.1836	0.9997	SNP2678	0.999783	0.163299				
SNP2551	0.0979	0.9947	SNP2709	0.999764	0.148871				
SNP2582	0.3181	1.0000	SNP2712	0.99248	0.095592				
SNP2643	0.2922	1.0000	SNP2745	0.99634	0.10936				
SNP2661	0.1038	0.9961	SNP2747	0.998173	0.118894				
SNP2673	0.1504	0.9988	SNP2766	0.994317	0.089363				
SNP2704	0.1370	0.9997	SNP2771	0.996765	0.111727				
SNP2716	0.2170	0.9999	SNP2772	0.999785	0.16347				
SNP2739	0.2265	1.0000	SNP2817	0.999029	0.135034				
SNP2752	0.3982	1.0000	SNP2819	0.997836	0.097315				

Supplemental Table 3 Continue.

SNP2761	0.2579	1.0000	SNP2826	1	0.190899				
SNP2762	0.1419	0.9998	SNP2855	0.996877	0.11201				
SNP2795	0.1165	0.9986	SNP2867	1	0.186371				
SNP2810	0.1024	0.9959	SNP2878	0.999335	0.132373				
SNP2830	0.1369	0.9998	SNP2880	0.999999	0.176605				
SNP2831	0.2049	0.9999	SNP2887	1	0.191085				
SNP2843	0.1445	0.9988	SNP2909	0.999073	0.133083				
SNP2851	0.1038	0.9946	SNP2932	0.999769	0.11954				
SNP2870	0.1191	0.9995	SNP2949	0.996122	0.11552				
SNP2879	0.0963	0.9980	SNP2955	0.998654	0.102547				
SNP2885	0.3579	1.0000	SNP2963	0.999748	0.127814				
SNP2892	0.1088	0.9975	SNP2967	0.996934	0.112762				
SNP2894	0.1190	0.9949	SNP2981	1	0.466039				
SNP2913	0.1295	0.9957	SNP2989	0.997095	0.094325				
SNP2922	0.1507	0.9997	SNP3030	1	0.244389				
SNP2949	0.1062	0.9990	SNP3033	0.99837	0.105998				
SNP2951	0.1432	0.9997	SNP3037	1	0.192575				
SNP2995	0.2184	1.0000	SNP3046	0.99991	0.182406				
SNP3017	0.2090	1.0000	SNP3054	0.999575	0.122058				
SNP3019	0.2866	1.0000	SNP3080	0.999532	0.116916				
SNP3033	0.1048	0.9967	SNP3082	0.999292	0.11204				
SNP3059	0.1236	0.9997	SNP3084	0.999936	0.169109				
SNP3063	0.1105	0.9978	SNP3090	0.997152	0.110671				
SNP3120	0.1248	0.9993	SNP3127	0.999419	0.111099				
SNP3126	0.1912	1.0000	SNP3129	0.99684	0.10863				
SNP3138	0.1041	0.9951	SNP3153	1	0.306233				
SNP3153	0.2090	1.0000	SNP3155	0.997705	0.096646				
SNP3163	0.1267	0.9994	SNP3162	0.999978	0.154488				
SNP3174	0.1183	0.9987	SNP3164	0.997256	0.094582				
SNP3176	0.1389	0.9998	SNP3169	0.999987	0.219506				
SNP3183	0.2095	1.0000	SNP3177	0.998435	0.122533				
SNP3185	0.1127	0.9966	SNP3186	0.999718	0.12661				
SNP3207	0.1251	0.9982	SNP3187	0.999979	0.178832				
SNP3209	0.1653	1.0000	SNP3206	0.999879	0.159274				
SNP3230	0.1140	0.9996	SNP3218	0.997984	0.117491				
SNP3233	0.4361	1.0000	SNP3226	0.995729	0.108489				
SNP3245	0.1542	0.9998	SNP3228	1	0.21743				
SNP3255	0.1115	0.9977	SNP3237	0.998877	0.104466				
SNP3258	0.3237	1.0000	SNP3246	1	0.239127				
SNP3272	0.0990	0.9951	SNP3252	0.998299	0.101248				
SNP3284	0.2881	1.0000	SNP3307	1	0.276497				
SNP3293	0.1232	0.9960	SNP3355	0.993991	0.099875				
SNP3335	0.1341	0.9997	SNP3413	0.992769	0.09634				
SNP3362	0.1153	0.9971	SNP3428	0.9945	0.0915				
SNP3380	0.2228	0.9999	SNP3457	1	0.285945				
SNP3390	0.1534	0.9990	SNP3500	0.995918	0.103628				
SNP3392	0.1126	0.9935	SNP3519	0.992148	0.090983				
SNP3402	0.1118	0.9990	SNP3541	1	0.224596				
SNP3404	0.1417	0.9999	SNP3544	0.997039	0.107835				

Supplemental Table 3 Continue.

SNP3405	0.1407	0.9972	SNP3573	0.99955	0.161536				
SNP3418	0.1211	0.9989	SNP3600	0.999995	0.166104				
SNP3419	0.2379	1.0000	SNP3619	0.999163	0.137947				
SNP3468	0.3664	1.0000	SNP3627	0.999409	0.165818				
SNP3479	0.1506	0.9999	SNP3632	0.998304	0.120921				
SNP3487	0.1779	1.0000	SNP3661	0.999999	0.361282				
SNP3506	0.1599	0.9998	SNP3698	0.996516	0.097695				
SNP3507	0.1078	0.9956	SNP3763	0.995459	0.107129				
SNP3514	0.0141	0.4377	SNP3788	0.9991	0.113309				
SNP3521	0.2259	1.0000	SNP3795	0.999545	0.149985				
SNP3537	0.1315	0.9971	SNP3816	0.999074	0.143801				
SNP3552	0.1379	0.9997	SNP3823	0.999201	0.114739				
SNP3560	0.1465	0.9994	SNP3838	0.998081	0.133587				
SNP3572	0.2096	1.0000	SNP3840	0.999937	0.141948				
SNP3583	0.1073	0.9994	SNP3841	1	0.206365				
SNP3584	0.3562	1.0000	SNP3844	1	0.194642				
SNP3591	0.1229	0.9991	SNP3869	0.994868	0.099285				
SNP3635	0.1626	1.0000	SNP3872	0.999552	0.161662				
SNP3675	0.1034	0.9949	SNP3887	0.999026	0.134972				
SNP3683	0.1145	0.9992	SNP3915	0.999275	0.108941				
SNP3703	0.2390	1.0000	SNP3919	0.99985	0.133349				
SNP3729	0.1099	0.9996	SNP3921	0.995219	0.099536				
SNP3747	0.1053	0.9965	SNP3978	0.997933	0.120359				
SNP3767	0.2063	1.0000	SNP3982	1	0.25866				
SNP3783	0.1859	1.0000	SNP4007	0.998858	0.10429				
SNP3795	0.1800	0.9998	SNP4058	0.999892	0.178487				
SNP3799	0.1506	1.0000	SNP4061	0.999026	0.108183				
SNP3827	0.1307	0.9995	SNP4080	0.999847	0.123008				
SNP3838	0.2176	1.0000	SNP4099	0.999563	0.121739				
SNP3839	0.1777	0.9996	SNP4110	1	0.224584				
SNP3842	0.1049	0.9966	SNP4114	0.996451	0.104434				
SNP3860	0.1261	0.9993	SNP4138	0.99344	0.094652				
SNP3872	0.1907	1.0000	SNP4139	1	0.212949				
SNP3874	0.1695	0.9999	SNP4161	0.999999	0.178371				
SNP3884	0.1293	1.0000	SNP4167	0.998086	0.118521				
SNP3889	0.1240	0.9947	SNP4174	0.995567	0.102025				
SNP3898	0.1280	0.9968	SNP4180	0.996818	0.10833				
SNP3943	0.0588	0.9134	SNP4192	0.999127	0.137126				
SNP3945	0.1784	1.0000	SNP4238	0.998995	0.150974				
SNP3951	0.2327	1.0000	SNP4259	0.998774	0.127429				
SNP3976	0.1298	0.9971	SNP4293	0.999593	0.122541				
SNP3981	0.1725	1.0000	SNP4297	0.998853	0.147359				
SNP4002	0.1377	0.9983	SNP4341	0.997495	0.116646				
SNP4011	0.1812	0.9999	SNP4349	0.997815	0.123626				
SNP4012	0.2808	1.0000	SNP4350	1	0.240951				
SNP4047	0.1215	0.9988	SNP4381	0.996931	0.109016				
SNP4049	0.1078	0.9973	SNP4401	0.999569	0.151054				
SNP4083	0.3207	1.0000	SNP4422	0.995377	0.088289				
SNP4084	0.1011	0.9957	SNP4430	0.997202	0.120126				

Supplemental Table 3 Continue.

SNP4144	0.0935	0.9973	SNP4446	0.998219	0.123231				
SNP4151	0.1833	1.0000	SNP4456	0.997808	0.115831				
SNP4163	0.1407	0.9981	SNP4492	0.99983	0.122141				
SNP4181	0.3061	1.0000	SNP4508	0.998457	0.126015				
SNP4199	0.0959	0.9969	SNP4528	0.999305	0.138943				
SNP4200	0.1169	0.9997	SNP4533	0.999816	0.131166				
SNP4203	0.1190	0.9989	SNP4597	0.999984	0.214021				
SNP4214	0.1082	0.9962	SNP4623	0.999948	0.131371				
SNP4234	0.1594	1.0000	SNP4633	0.993778	0.095768				
SNP4242	0.1860	0.9994	SNP4646	0.9966	0.110774				
SNP4248	0.0994	0.9949	SNP4650	0.999973	0.15277				
SNP4266	0.1231	0.9997	SNP4665	1	0.200322				
SNP4277	0.0975	0.9945	SNP4688	0.999878	0.135408				
SNP4283	0.0964	0.9971	SNP4701	0.997827	0.097269				
SNP4289	0.1015	0.9958	SNP4748	0.999985	0.212979				
SNP4330	0.1697	0.9995	SNP4769	0.999989	0.221966				
SNP4346	0.2025	1.0000	SNP4771	0.997974	0.117392				
SNP4365	0.1531	0.9998	SNP4901	0.997977	0.108108				
SNP4424	0.1050	0.9967	SNP4938	0.999223	0.110929				
SNP4426	0.1676	1.0000	SNP4980	0.998644	0.139175				
SNP4427	0.1458	0.9996	SNP5012	1	0.22437				
SNP4470	0.1162	0.9972	SNP5042	0.999899	0.137305				
SNP4481	0.1568	1.0000	SNP5072	0.997956	0.119387				
SNP4483	0.1810	1.0000	SNP5107	1	0.445374				
SNP4484	0.0905	0.9963	SNP5125	0.995964	0.120445				
SNP4486	0.1009	0.9936	SNP5142	1	0.417135				
SNP4489	0.1237	0.9966	SNP5149	0.999999	0.294972				
SNP4495	0.1055	0.9948	SNP5157	0.999947	0.186662				
SNP4510	0.1340	0.9997	SNP5180	0.999948	0.166936				
SNP4512	0.1411	0.9999	SNP5240	1	0.723125				
SNP4535	0.1849	1.0000	SNP5289	0.999996	0.254688				
SNP4571	0.1604	0.9997	SNP5333	0.997963	0.128893				
SNP4602	0.1769	0.9999	SNP5362	0.999998	0.205244				
SNP4630	0.1369	0.9968	SNP5390	0.998526	0.137049				
SNP4631	0.1024	0.9937	SNP5423	0.996207	0.108574				
SNP4650	0.1009	0.9951	SNP5467	1	0.503349				
SNP4657	0.1707	1.0000	SNP5473	0.997929	0.128481				
SNP4668	0.0487	0.8705	SNP5476	1	0.198716				
SNP4679	0.3159	1.0000	SNP5517	0.997605	0.096158				
SNP4686	0.1092	0.9976	SNP5548	1	0.332467				
SNP4723	0.0909	0.9964	SNP5557	0.999999	0.212931				
SNP4724	0.1097	0.9959	SNP5564	0.999846	0.13306				
SNP4726	0.1358	0.9991	SNP5592	0.999888	0.156787				
SNP4742	0.1314	0.9996	SNP5609	0.999982	0.221138				
SNP4743	0.1171	0.9994	SNP5610	0.997892	0.128043				
SNP4748	0.2335	1.0000	SNP5634	0.999967	0.208147				
SNP4770	0.1214	0.9942	SNP5639	0.999996	0.199079				
SNP4785	0.1016	0.9983	SNP5695	0.998712	0.140506				
SNP4789	0.1015	0.9989	SNP5704	0.999974	0.176055				

Supplemental Table 3 Continue.

SNP4791	0.1366	0.9978	SNP5709	0.999786	0.129572				
SNP4794	0.1064	0.9950	SNP5794	0.998836	0.110186				
SNP4809	0.1208	0.9990	SNP6049	0.997997	0.132474				
SNP4817	0.1111	0.9990	SNP6068	0.998644	0.139175				
SNP4830	0.1058	0.9969	SNP6078	0.999497	0.170435				
SNP4838	0.1097	0.9959	SNP6084	0.999996	0.259712				
SNP4843	0.1246	0.9982	SNP6092	1	0.247394				
SNP4886	0.1356	0.9997	SNP6107	0.999167	0.156176				
SNP4893	0.0978	0.9946	SNP6119	0.997883	0.127944				
SNP4953	0.1048	0.9988	SNP6226	0.998488	0.122512				
SNP4963	0.1030	0.9959	SNP6227	0.994105	0.100641				
SNP4981	0.1664	1.0000	SNP6250	0.999751	0.145605				
SNP5000	0.1704	1.0000	SNP6327	1	0.230225				
SNP5009	0.1435	1.0000	SNP6336	0.999983	0.181508				
SNP5023	0.1413	0.9998	SNP6344	0.996775	0.111442				
SNP5028	0.1371	0.9997	SNP6364	0.999234	0.158509				
SNP5038	0.1419	0.9998	SNP6391	1	0.239887				
SNP5049	0.0529	0.8937	SNP6400	1	0.220444				
SNP5055	0.1082	0.9955	SNP6445	1	0.227563				
SNP5060	0.2000	0.9999	SNP6505	0.999981	0.179802				
SNP5063	0.1178	0.9985	SNP6523	0.999703	0.117329				
SNP5073	0.2121	1.0000	SNP6527	0.999971	0.174668				
SNP5080	0.1104	0.9994	SNP6553	0.999981	0.179911				
SNP5082	0.1236	0.9992	SNP6562	0.999158	0.137833				
SNP5092	0.2282	1.0000	SNP6588	0.998873	0.147846				
SNP5130	0.1103	0.9975	SNP6598	1	0.535224				
SNP5141	0.1506	1.0000	SNP6616	0.999092	0.109033				
SNP5170	0.1373	0.9998	SNP6664	0.998493	0.136489				
SNP5175	0.1768	0.9999	SNP6670	0.992776	0.092835				
SNP5194	0.2130	1.0000	SNP6685	0.997783	0.126789				
SNP5255	0.2661	1.0000	SNP6691	0.99999	0.187818				
SNP5298	0.1289	0.9970	SNP6715	0.999444	0.114891				
SNP5310	0.1210	0.9941	SNP6722	1	0.194985				
SNP5327	0.1546	0.9992	SNP6752	0.996125	0.108194				
SNP5389	0.1171	0.9947	SNP6772	0.994128	0.085265				
SNP5409	0.1046	0.9945	SNP6802	0.995819	0.106809				
SNP5416	0.2970	1.0000	SNP6806	0.99382	0.099781				
SNP5423	0.2440	0.9999	SNP6840	0.999618	0.165637				
SNP5449	0.1980	0.9998	SNP6859	0.998593	0.102074				
SNP5502	0.1263	0.9966	SNP6921	0.999071	0.153152				
SNP5509	0.1528	0.9990	SNP6926	1	1				
SNP5515	0.1903	1.0000	SNP6939	1	0.745347				
SNP5532	0.0977	0.9938	SNP6971	0.999985	0.213223				
SNP5587	0.1225	0.9944	SNP6976	0.998398	0.130813				
SNP5601	0.2858	1.0000	SNP6990	0.999999	0.37735				
SNP5606	0.1080	0.9954	SNP7030	1	0.248237				
SNP5633	0.1199	0.9951	SNP7065	0.995435	0.096112				
SNP5636	0.3816	1.0000	SNP7112	0.996195	0.108615				
SNP5650	0.1281	0.9986	SNP7129	0.999996	0.146956				

Supplemental Table 3 Continue.

SNP5657	0.1677	0.9996							
SNP5660	0.1841	1.0000							
SNP5664	0.1607	0.9992							
SNP5669	0.1278	0.9954							
SNP5678	0.1406	0.9998							
SNP5681	0.0728	0.9554							
SNP5685	0.3324	1.0000							
SNP5697	0.2944	1.0000							
SNP5700	0.2088	0.9997							
SNP5708	0.1159	0.9950							
SNP5709	0.1393	0.9998							
SNP5720	0.0945	0.9845							
SNP5737	0.3695	1.0000							
SNP5738	0.1739	0.9999							
SNP5748	0.1899	0.9995							
SNP5801	0.1430	0.9975							
SNP5815	0.1333	0.9997							
SNP5822	0.5581	1.0000							
SNP5890	0.1245	0.9967							
SNP5892	0.1313	0.9977							
SNP5904	0.1389	0.9994							
SNP5925	0.1320	0.9972							
SNP5987	0.1484	0.9976							
SNP5991	0.1976	0.9996							
SNP6010	0.1513	0.9988							
SNP6028	0.1330	1.0000							
SNP6034	0.1394	0.9994							
SNP6063	0.1807	0.9992							
SNP6075	0.2588	1.0000							
SNP6096	0.1594	0.9957							
SNP6117	0.1408	0.9995							
SNP6123	0.4740	1.0000							
SNP6130	0.1280	0.9968							
SNP6137	0.2372	1.0000							
SNP6149	0.1926	1.0000							
SNP6188	0.1108	0.9977							
SNP6231	0.1501	0.9980							
SNP6251	0.1060	0.9949							
SNP6278	0.1150	0.9942							
SNP6288	0.1481	0.9997							
SNP6322	0.2113	1.0000							
SNP6353	0.2173	0.9999							
SNP6360	0.1619	0.9999							
SNP6407	0.1339	0.9979							
SNP6408	0.3987	1.0000							
SNP6437	0.1402	0.9982							
SNP6447	0.1345	0.9976							
SNP6477	0.1479	0.9999							
SNP6479	0.1385	0.9979							

Supplemental Table 3 Continue.

SNP6539	0.1007	0.9950							
SNP6558	0.1317	0.9991							
SNP6588	0.2082	1.0000							
SNP6609	0.2730	1.0000							
SNP6663	0.1306	0.9996							
SNP6680	0.2827	1.0000							
SNP6710	0.2269	1.0000							
SNP6730	0.1227	0.9982							
SNP6767	0.1371	0.9968							
SNP6793	0.1276	0.9988							
SNP6812	0.1391	0.9967							
SNP6831	0.1254	0.9950							
SNP6858	0.1131	0.9972							
SNP6859	0.1292	0.9957							
SNP6861	0.2242	1.0000							
SNP6873	0.1896	1.0000							
SNP6891	0.1806	1.0000							
SNP6893	0.2212	1.0000							
SNP6898	0.1421	0.9995							
SNP6921	0.1620	0.9999							
SNP6959	0.1419	0.9995							
SNP6964	0.1678	0.9999							
SNP6971	0.2611	1.0000							
SNP6974	0.1592	0.9998							
SNP6983	0.1618	0.9987							
SNP7030	0.1266	0.9964							
SNP7056	0.1125	0.9966							
SNP7072	0.1240	0.9999							
SNP7089	0.2974	1.0000							
SNP7090	0.1262	0.9970							
SNP7097	0.1440	0.9987							
SNP7137	0.1147	0.9941							
SNP7170	0.1419	0.9986							
SNP7176	0.1646	0.9988							
SNP7213	0.1140	0.9969							
SNP7215	0.1856	1.0000							
SNP7264	0.1468	0.9978							
SNP7272	0.2292	0.9999							
SNP7285	0.2001	0.9996							
SNP7302	0.4191	1.0000							
SNP7303	0.1142	0.9940							
SNP7316	0.1279	0.9972							
SNP7321	0.1470	0.9996							
SNP7358	0.2610	1.0000							
SNP7365	0.1376	0.9969							
SNP7372	0.1741	0.9992							
SNP7397	0.0467	0.8546							
SNP7408	0.1375	0.9978							
SNP7414	0.1292	0.9957							

Supplemental Table 3 Continue.

SNP7425	0.4268	1.0000							
SNP7447	0.2343	0.9999							
SNP7478	0.1612	0.9999							
SNP7480	0.1133	0.9937							
SNP7498	0.1342	0.9995							
SNP7533	0.1429	0.9984							
SNP7542	0.1990	0.9996							
SNP7552	0.1366	0.9997							
SNP7558	0.1371	0.9979							
SNP7563	0.1809	0.9997							
SNP7590	0.1844	0.9997							
SNP7591	0.1246	0.9948							
SNP7594	0.4549	1.0000							
SNP7600	0.1116	0.9963							
SNP7608	0.1682	0.9999							
SNP7635	0.1935	0.9995							
SNP7638	0.1084	0.9962							
SNP7650	0.1453	0.9996							
SNP7655	0.0730	0.9715							
SNP7671	0.1157	0.9972							
SNP7688	0.4303	1.0000							
SNP7726	0.4970	1.0000							
SNP7749	0.0999	0.9987							
SNP7752	0.1125	0.9995							
SNP7764	0.1307	1.0000							
SNP7778	0.1121	0.9990							
SNP7784	0.1390	0.9970							
SNP7832	0.1701	0.9995							
SNP7837	0.1081	0.9972							
SNP7866	0.1242	0.9962							
SNP7870	0.3523	1.0000							
SNP7874	0.1358	0.9999							
SNP7917	0.1026	0.9938							
SNP7920	0.2034	1.0000							
SNP7926	0.1902	1.0000							
SNP7937	0.1644	0.9999							
SNP7939	0.1724	0.9995							
SNP7941	0.1267	0.9966							
SNP7949	0.1472	0.9987							
SNP7952	0.1403	0.9972							
SNP7966	0.1098	0.9974							
SNP7980	0.2961	1.0000							
SNP7982	0.2231	1.0000							
SNP7986	0.1213	0.9941							
SNP8012	0.1872	1.0000							
SNP8043	0.1345	0.9965							
SNP8078	0.1091	0.9975							
SNP8115	1.0000	1.0000							
SNP8130	0.2705	1.0000							

Supplemental Table 3 Continue.

SNP8150	0.1111	0.9962							
SNP8167	0.1034	0.9964							
SNP8172	0.0744	0.9788							
SNP8177	0.1436	1.0000							
SNP8184	0.1068	0.9951							
SNP8186	0.3877	1.0000							
SNP8196	0.2457	1.0000							
SNP8201	0.1398	0.9971							
SNP8208	0.2949	1.0000							
SNP8234	0.1012	0.9952							
SNP8246	0.1230	0.9992							
SNP8259	0.2624	1.0000							
SNP8266	0.1376	0.9997							
SNP8274	0.0474	0.8625							
SNP8277	0.1858	1.0000							
SNP8282	0.1142	0.9983							
SNP8310	0.2278	1.0000							
SNP8327	0.1238	0.9990							
SNP8337	0.2375	1.0000							
SNP8356	0.1134	0.9996							
SNP8365	0.1134	0.9996							
SNP8368	0.1418	0.9995							

Supplemental Table 4 List of individuals (50 *C. galapagensis* and 50 *C. obscurus*) selected as pure parental species based on SNP assignment results from STRUCTURE.

C. galapagensis				*C. obscurus*			
ID	**Location**	**Probability of assignment cluster1 (conf. interval)**	**Probability of assignment cluster2 (conf. interval)**	**ID**	**Location**	**Probability of assignment cluster1 (conf. interval)**	**Probability of assignment cluster2 (conf. interval)**
cgal_KR1	Kermadec	1 (0.998-1.000)	0 (0.000-0.002)	obs_109508	Northern Territory	0 (0.000-0.002)	1 (0.998-1.000)
cgal_KR10	Kermadec	1 (0.999-1.000)	0 (0.000-0.001)	obs_109515	Northern Territory	0 (0.000-0.002)	1 (0.998-1.000)
cgal_K655_236	Kermadec	1 (0.999-1.000)	0 (0.000-0.001)	obs_109523	Northern Territory	0 (0.000-0.001)	1 (0.999-1.000)
cgal_KR2	Kermadec	1 (0.999-1.000)	0 (0.000-0.001)	obs_109507	Northern Territory	0 (0.000-0.002)	1 (0.998-1.000)
cgal_KR12	Kermadec	0.997 (0.985-1.000)	0.003 (0.000-0.015)	obs_109516	Northern Territory	0 (0.000-0.002)	1 (0.998-1.000)
cgal_KR3	Kermadec	1 (0.998-1.000)	0 (0.000-0.002)	obs_109524	Northern Territory	0 (0.000-0.002)	1 (0.998-1.000)
cgal_KR15	Kermadec	1 (0.998-1.000)	0 (0.000-0.002)	obs_109509	Northern Territory	0 (0.000-0.001)	1 (0.999-1.000)
cgal_K655_357	Kermadec	1 (0.999-1.000)	0 (0.000-0.001)	obs_109517	Northern Territory	0 (0.000-0.002)	1 (0.998-1.000)
cgal_KR4	Kermadec	1 (0.998-1.000)	0 (0.000-0.002)	obs_109518	Northern Territory	0 (0.000-0.002)	1 (0.998-1.000)
cgal_KR16	Kermadec	1 (0.999-1.000)	0 (0.000-0.001)	obs_109526	Northern Territory	0 (0.000-0.003)	1 (0.997-1.000)
cgal_K655_389	Kermadec	1 (0.999-1.000)	0 (0.000-0.001)	obs_109519	Northern Territory	0.001 (0.000-0.007)	0.999 (0.993-1.000)
cgal_KR5	Kermadec	1 (0.999-1.000)	0 (0.000-0.001)	obs_109527	Northern Territory	0 (0.000-0.001)	1 (0.999-1.000)
cgal_MG28	Middleton Reef	1 (0.998-1.000)	0 (0.000-0.002)	obs_109510	Northern Territory	0 (0.000-0.004)	1 (0.996-1.000)
cgal_MG15	Middleton Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_132301	Moreton Bay	0 (0.000-0.002)	1 (0.998-1.000)
cgal_MG26	Middleton Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_132300	Moreton Bay	0 (0.000-0.001)	1 (0.999-1.000)
cgal_MG1	Middleton Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_132302	Moreton Bay	0 (0.000-0.002)	1 (0.998-1.000)
cgal_MG16	Middleton Reef	1 (0.998-1.000)	0 (0.000-0.002)	obs_132303	Moreton Bay	0 (0.000-0.001)	1 (0.999-1.000)
cgal_MG29	Middleton Reef	1 (0.996-1.000)	0 (0.000-0.004)	obs_J1180	Western Australia	0.001 (0.000-0.009)	0.999 (0.991-1.000)
cgal_MG4	Middleton Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_J1140	Western Australia	0 (0.000-0.003)	1 (0.997-1.000)
cgal_MG17	Middleton Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_J1187	Western Australia	0.002 (0.000-0.014)	0.998 (0.986-1.000)
cgal_MG30	Middleton Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_J1439	Western Australia	0.001 (0.000-0.008)	0.999 (0.992-1.000)
cgal_MG6	Middleton Reef	1 (0.998-1.000)	0 (0.000-0.002)	obs_J1442	Western Australia	0 (0.000-0.003)	1 (0.997-1.000)
cgal_MG18	Middleton Reef	1 (0.998-1.000)	0 (0.000-0.002)	obs_R1237	Perth	0.001 (0.000-0.008)	0.999 (0.992-1.000)
cgal_ELZEG9	Elizabeth Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_S373	Perth	0 (0.000-0.003)	1 (0.997-1.000)
cgal_ELZEG23	Elizabeth Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_S429	Perth	0 (0.000-0.002)	1 (0.998-1.000)
cgal_ELZEG14	Elizabeth Reef	1 (0.998-1.000)	0 (0.000-0.002)	obs_R1246	Perth	0.001 (0.000-0.005)	0.999 (0.995-1.000)
cgal_ELZEG24	Elizabeth Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_S444	Perth	0 (0.000-0.002)	1 (0.998-1.000)
cgal_ELZEG15	Elizabeth Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_109559	South Australia	0 (0.000-0.002)	1 (0.998-1.000)
cgal_ELZEG25	Elizabeth Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_109560	South Australia	0 (0.000-0.003)	1 (0.997-1.000)
cgal_ELZEG16	Elizabeth Reef	1 (0.998-1.000)	0 (0.000-0.002)	obs_109561	South Australia	0 (0.000-0.002)	1 (0.998-1.000)
cgal_ELZEG28	Elizabeth Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_109562	South Australia	0 (0.000-0.001)	1 (0.999-1.000)
cgal_ELZEG4	Elizabeth Reef	1 (0.999-1.000)	0 (0.000-0.001)	obs_109563	South Australia	0.001 (0.000-0.005)	0.999 (0.995-1.000)
cgal_ELZEG18	Elizabeth Reef	1 (0.996-1.000)	0 (0.000-0.004)	obs_126533	South Africa	0 (0.000-0.001)	1 (0.999-1.000)
cgal_NI312_72	Norfolk	1 (0.998-1.000)	0 (0.000-0.002)	obs_126514	South Africa	0.001 (0.000-0.005)	0.999 (0.995-1.000)
cgal_NI321_81	Norfolk	1 (0.999-1.000)	0 (0.000-0.001)	obs_126532	South Africa	0 (0.000-0.001)	1 (0.999-1.000)
cgal_NI312_73	Norfolk	1 (0.998-1.000)	0 (0.000-0.002)	obs_126502	South Africa	0 (0.000-0.001)	1 (0.999-1.000)
cgal_NI312_78	Norfolk	1 (0.999-1.000)	0 (0.000-0.001)	obs_126530	South Africa	0 (0.000-0.001)	1 (0.999-1.000)
cgal_NI321_82	Norfolk	1 (0.997-1.000)	0 (0.000-0.003)	obs_126505	South Africa	0 (0.000-0.001)	1 (0.999-1.000)

Supplemental Table 4 Continue.

cgal_NI311_97	Norfolk	1 (0.998-1.000)	0 (0.000-0.002)	obs_126531	South Africa	0 (0.000-0.002)	1 (0.998-1.000)
cgal_NI312_85	Norfolk	1 (0.999-1.000)	0 (0.000-0.001)	obs_126520	South Africa	0 (0.000-0.001)	1 (0.999-1.000)
cgal_NI321_83	Norfolk	1 (0.999-1.000)	0 (0.000-0.001)	obs_126538	South Africa	0 (0.000-0.001)	1 (0.999-1.000)
cgal_LH7	Lord Howe	1 (0.999-1.000)	0 (0.000-0.001)	obs_109567	Coffs Harbour	0 (0.000-0.003)	1 (0.997-1.000)
cgal_LH19	Lord Howe	1 (0.999-1.000)	0 (0.000-0.001)	obs_126115	Coffs Harbour	0.003 (0.000-0.016)	0.997 (0.984-1.000)
cgal_LH30	Lord Howe	1 (0.999-1.000)	0 (0.000-0.001)	obs_109568	Coffs Harbour	0.001 (0.000-0.010)	0.999 (0.990-1.000)
cgal_LH8	Lord Howe	1 (0.999-1.000)	0 (0.000-0.001)	obs_126116	Coffs Harbour	0.012 (0.000-0.029)	0.988 (0.971-1.000)
cgal_LH20	Lord Howe	1 (0.998-1.000)	0 (0.000-0.002)	obs_109569	Coffs Harbour	0.001 (0.000-0.004)	0.999 (0.996-1.000)
cgal_LH9	Lord Howe	1 (0.998-1.000)	0 (0.000-0.002)	obs_126117	Coffs Harbour	0 (0.000-0.001)	1 (0.999-1.000)
cgal_LH21	Lord Howe	1 (0.999-1.000)	0 (0.000-0.001)	obs_109570	Coffs Harbour	0.001 (0.000-0.006)	0.999 (0.994-1.000)
cgal_LH10	Lord Howe	1 (0.998-1.000)	0 (0.000-0.002)	obs_126118	Coffs Harbour	0.001 (0.000-0.009)	0.999 (0.991-1.000)
cgal_LH23	Lord Howe	1 (0.999-1.000)	0 (0.000-0.001)	obs_109571	Coffs Harbour	0 (0.000-0.003)	1 (0.997-1.000)

Supplemental Table 5 Identification of *Carcharhinus galapagensis* and *C. obscurus* SNPs with F_{ST}>0.90, calculated in *pegas* R package.

SNP ID	Fit	Fst	Fis	SNP ID	Fit	Fst	Fis
SNP0589	1.00000	1.00000	NA	SNP0491	0.94737	0.94952	-0.04255
SNP0600	1.00000	1.00000	NA	SNP0537	0.94737	0.94952	-0.04255
SNP0605	1.00000	1.00000	NA	SNP0549	0.94737	0.94952	-0.04255
SNP0612	1.00000	1.00000	NA	SNP0607	0.94737	0.94952	-0.04255
SNP0615	1.00000	1.00000	NA	SNP0465	0.94741	0.94870	-0.02510
SNP0616	1.00000	1.00000	NA	SNP0566	0.94741	0.94870	-0.02510
SNP0621	1.00000	1.00000	NA	SNP0574	0.94741	0.94870	-0.02510
SNP0636	1.00000	1.00000	NA	SNP0599	0.94741	0.94870	-0.02510
SNP0639	1.00000	1.00000	NA	SNP0601	0.94741	0.94870	-0.02510
SNP0641	1.00000	1.00000	NA	SNP0608	0.94741	0.94870	-0.02510
SNP0642	1.00000	1.00000	NA	SNP0617	0.94741	0.94870	-0.02510
SNP0656	1.00000	1.00000	NA	SNP0550	0.94743	0.94829	-0.01660
SNP0657	1.00000	1.00000	NA	SNP0552	0.94743	0.94829	-0.01660
SNP0658	1.00000	1.00000	NA	SNP0582	0.94743	0.94829	-0.01660
SNP0661	1.00000	1.00000	NA	SNP0498	0.93617	0.93943	-0.05376
SNP0662	1.00000	1.00000	NA	SNP0505	0.93617	0.93943	-0.05376
SNP0664	1.00000	1.00000	NA	SNP0526	0.93617	0.93943	-0.05376
SNP0668	1.00000	1.00000	NA	SNP0539	0.93617	0.93943	-0.05376
SNP0670	1.00000	1.00000	NA	SNP0680	0.93617	0.93943	-0.05376
SNP0671	1.00000	1.00000	NA	SNP0686	0.93617	0.93943	-0.05376
SNP0676	1.00000	1.00000	NA	SNP0709	0.93617	0.93943	-0.05376
SNP0692	1.00000	1.00000	NA	SNP0522	0.95745	0.93921	0.30000
SNP0699	1.00000	1.00000	NA	SNP0654	0.93628	0.93780	-0.02439
SNP0702	1.00000	1.00000	NA	SNP0494	0.92473	0.92934	-0.06522
SNP0570	0.98990	0.98990	0.00000	SNP0529	0.92473	0.92934	-0.06522
SNP0581	0.98990	0.98990	0.00000	SNP0532	0.92473	0.92934	-0.06522
SNP0619	0.98990	0.98990	0.00000	SNP0545	0.92473	0.92934	-0.06522
SNP0631	0.98990	0.98990	0.00000	SNP0584	0.92473	0.92934	-0.06522
SNP0633	0.98990	0.98990	0.00000	SNP0493	0.94624	0.92912	0.24149
SNP0647	0.98990	0.98990	0.00000	SNP0714	0.94624	0.92912	0.24149
SNP0659	0.98990	0.98990	0.00000	SNP0705	0.92483	0.92812	-0.04573
SNP0665	0.98990	0.98990	0.00000	SNP0508	0.92489	0.92730	-0.03313
SNP0669	0.98990	0.98990	0.00000	SNP0578	0.94635	0.92708	0.26426
SNP0672	0.98990	0.98990	0.00000	SNP0510	0.92492	0.92689	-0.02695
SNP0694	0.98990	0.98990	0.00000	SNP0606	0.92492	0.92689	-0.02695
SNP0701	0.98990	0.98990	0.00000	SNP0715	0.91304	0.91925	-0.07692
SNP0567	0.97959	0.97980	-0.01031	SNP0534	0.91318	0.91783	-0.05660
SNP0576	0.97959	0.97980	-0.01031	SNP0611	0.93488	0.91761	0.20968
SNP0595	0.97959	0.97980	-0.01031	SNP0579	0.91333	0.91620	-0.03430
SNP0614	0.97959	0.97980	-0.01031	SNP0518	0.90110	0.90917	-0.08889
SNP0627	0.97959	0.97980	-0.01031	SNP0527	0.90110	0.90917	-0.08889
SNP0640	0.97959	0.97980	-0.01031	SNP0553	0.90127	0.90754	-0.06780
SNP0643	0.97959	0.97980	-0.01031	SNP0556	0.90140	0.90632	-0.05251
SNP0682	0.97959	0.97980	-0.01031	SNP0655	0.90140	0.90632	-0.05251
SNP0697	0.97959	0.97980	-0.01031	SNP0573	0.90149	0.90551	-0.04255
SNP0577	0.97960	0.97960	0.00000	SNP0618	0.90149	0.90551	-0.04255
SNP0587	0.97960	0.97960	0.00000				
SNP0590	0.97960	0.97960	0.00000				
SNP0626	0.97960	0.97960	0.00000				
SNP0555	0.96907	0.96970	-0.02083				
SNP0568	0.96907	0.96970	-0.02083				
SNP0596	0.96907	0.96970	-0.02083				
SNP0620	0.96907	0.96970	-0.02083				
SNP0637	0.96907	0.96970	-0.02083				
SNP0678	0.96907	0.96970	-0.02083				
SNP0689	0.96907	0.96970	-0.02083				
SNP0690	0.96907	0.96970	-0.02083				
SNP0535	0.96908	0.96930	-0.00685				
SNP0561	0.96908	0.96930	-0.00685				
SNP0591	0.96908	0.96930	-0.00685				
SNP0624	0.98969	0.96908	0.66667				
SNP0540	0.95833	0.95961	-0.03158				
SNP0563	0.95833	0.95961	-0.03158				
SNP0685	0.95833	0.95961	-0.03158				
SNP0706	0.95833	0.95961	-0.03158				
SNP0585	0.95836	0.95900	-0.01554				
SNP0653	0.95836	0.95900	-0.01554				
SNP0648	0.95837	0.95879	-0.01031				
SNP0691	0.95837	0.95879	-0.01031				

Supplemental Table 6 Assignment of putative hybrid individuals according to NEWHYBRIDS using two data subsets: A) 117 SNPs and B) 69 SNPs, based on individual F_{ST} values of >0.90 or 0.95, respectively.

(A)

Hybrid ID	Location	Gal	bCGxCG	bCG	bCOxCG	F_1	F_2	bCGxCO	bCO	bCOxCO	Obs
RSN3	Galápagos	0.0000	0.0000	0.0001	**0.9998**	0.0003	0.0000	0.0000	0.0000	0.0000	0.0000
MX13	Revillagigedos	0.0000	0.0000	0.0000	0.0000	**0.7666**	0.0000	0.0000	0.0692	0.1640	0.0000
MX2	Revillagigedos	0.0000	0.0000	0.0000	0.0000	**1.0000**	0.0000	0.0000	0.0000	0.0000	0.0000
MX1	Revillagigedos	0.0000	0.0000	0.0000	0.0000	0.0008	0.0000	0.0000	0.0024	**0.9967**	0.0000

(B)

Hybrid ID	Location	Gal	bCGxCG	bCG	bCOxCG	F_1	F_2	bCGxCO	bCO	bCOxCO	Obs
RSN3	Galápagos	0.0000	0.0000	0.0001	**0.9998**	0.0000	0.0001	0.0000	0.0000	0.0000	0.0000
MX13	Revillagigedos	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0002	**0.9998**	0.0000
MX2	Revillagigedos	0.0000	0.0000	0.0000	0.0000	0.0000	**1.0000**	0.0000	0.0000	0.0000	0.0000
MX1	Revillagigedos	0.0000	0.0000	0.0000	0.0000	0.0008	0.0000	0.0000	0.0001	**0.9999**	0.0000

Supplemental Table 7 Pairwise Φ_{ST} differentiation indices among *Carcharhinus* spp. and *Sphyrna lewini* (outgroup) based on the mitochondrial control region (549 bp). The asterisks above the diagonal show significance at $p<0.05$.

	S. lewini	*C. brachyurus*	*C. falciformis*	*C. galapagensis*	*C. obscurus*
S. lewini	-	*	*	*	*
C. brachyurus	0.955	-	*	*	*
C. falciformis	0.961	0.737	-	*	*
C. galapagensis	0.993	0.854	0.865	-	*
C. obscurus	0.976	0.793	0.833	0.000	-