Genetic connectivity of the roundjaw bonefish (*Albula glossodonta*) in the Southwest Indian Ocean


Photo credit: Prof. Paul Cowley

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ABSTRACT

The Southwest Indian Ocean (SWIO) islands of Mauritius and Seychelles are both highly dependent on tourism and fisheries for their economies. One of the growing ecotourism sectors is saltwater fly fishing, an industry based on catch-and-release fishing for a host of species, including bonefishes. Bonefishes (*Albula* spp.) have received significant research attention in the Pacific and Atlantic Oceans, with only sporadic research conducted in the Indian Ocean. My project aimed to investigate the genetic connectivity of the roundjaw bonefish (*Albula glossodonta*) in two island states (Seychelles and Mauritius) within the SWIO using a mitochondrial genetic marker (cyt-b) and next generation sequencing (ddRADseq).

Samples collected were grouped based on their spatial distribution. The Seychelles consisted of four island groups (Inner Island Group, Aldabra Group, Amirantes and Alphonse Group, and Farquhar Group) whereas Mauritius was represented by one island group (Saint Brandon). Genetic analyses were undertaken between and within each of these groups. Mitochondrial cytochrome-b identified two species of bonefish: *Albula glossodonta* and *Albula oligolepis*; the latter was only genetically identified from the Inner Island Group. I hypothesise that this is due to habitat partitioning, with *A. oligolepis* being a deeper dwelling bonefish species compared to *A. glossodonta,* which occupies shallow water habitats such as sand flats, atoll lagoons and reef flats.

Neutral SNP loci revealed a panmictic pattern of distribution for *A. glossodonta* throughout the Seychelles Island groups but showed a pattern of weak structure between Seychelles and Mauritius. Genetic diversity indices such as allelic richness, showed low diversity across the sampling sites (AR range: 1.761-1.889). Population structure tests such as pairwise F_{ST} showed low but significant population structure. The highest F_{ST} indices were recorded between the Aldabra and Farquhar Groups, as well as the Aldabra and Saint Brandon Groups (0.044 ± 0.000 and 0.040 ± 0.000, respectively).

Descriptive tests such as PCA and DAPC showed similar trends, whereby Saint Brandon clustered separately from the other samples from the Seychelles Island groups. However, these trends were seen at very low variations (PCA axes 1 and 2 accounted for only 2.0 and 1.9 % of the total variation, respectively). A population assignment test grouped the individuals as one ancestral population. A spatial principal component analysis showed that Saint Brandon was dissimilar to the Seychelles Island groups.

Like other Elopomorph species, bonefishes have leptocephalus larvae capable of long-distance dispersal which may explain the well-mixed genetic population observed within the Seychelles islands. Although currents within the Indian Ocean, especially on a mesoscale, are not well understood, the South Equatorial Current likely facilitates connectivity between the Seychelles islands while also limiting gene flow between Seychelles and Mauritius. Understanding population structure is important for informing the appropriate management and conservation strategies, especially in oceanic nations where data informing important industries like tourism and fisheries are often limited.

The bonefish fly fishing industry is well-known to be a lucrative sector, generating, for example US$ 1.4 million a year in the Bahamas. This study recognised that there are numerous knowledge gaps relevant to the bonefish industry that need to be addressed, including: 1) understanding the socio-economic importance of fly fishing to island states like Seychelles, 2) estimating the abundance and species distribution of bonefishes within Seychelles, 3) understanding effectiveness of MPAs for recreational fishery species like bonefish and, lastly, 4) generating more fishery-relevant biological information on the heavily targeted fly fishing species within Seychelles. These needs must be met to inform management plans and to better manage the fly fishing ventures that target species like bonefish.

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This thesis is dedicated to all the lovely people and furry animals that are part of my life and to the ones that we have lost along the way.

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I am humbled by the incredible community that I am surrounded with and I hope that I can open twice as many doors as has been opened to me.

DECLARATION

The following thesis has not been submitted to any university other than Rhodes University, Grahamstown, South Africa. The work presented here is that of the author.

1 CHAPTER 1: INTRODUCTION

1.1 THE SOUTHWEST INDIAN OCEAN (SWIO)

Ten countries border the Southwest Indian Ocean (SWIO; Figure 1.1) which extends from 0° to 40 ° south of the equator and 30° to 90° east of the African continent (Donohue and Toole 2003; Healey et al. 2018). The geographical spread of these countries encompasses a wide range of unique habitats, with regional endemism around islands and along the African continental coastline (Ridgway and Sampayo 2007). Island nations like the Comoros, Mauritius, Madagascar and Seychelles have been identified as important marine and terrestrial refuges. The Comoros is a haven for dugongs and coelacanths (Olson and Dinerstein 1998; Fricke and Hissman 2000). The Seychelles boasts two UNESCO world heritage sites – the Vallée de Mai and Aldabra – both of which are conservation success stories (Marchese 2015). The Aldabra Atoll is near-pristine, being the home of the Aldabra rail, the only extant flightless bird in the Indian Ocean, over 100,000 giant Aldabra tortoises and a healthy marine habitat conducive to large populations of predators such as blacktip reef sharks and groupers (Stoddart 2015; Stevens 1984; Hamylton et al. 2018).

This rich biodiversity is not limited to the island states but extends to the SWIO countries along the coastline of the African continent (Somalia, Kenya, Mozambique and South Africa). For example, South Africa boasts unique eastern and southern shores with hotspots of biodiversity (Griffiths et al. 2010; Griffiths and Robinson 2016). This high biodiversity is partly due to the contrasting Agulhas and Benguela boundary currents that transport nutrients and produce offshore upwellings, fostering the unique bioregions along the South African coastline (Griffiths et al. 2010; Teske et al. 2011). The

Mozambican coastline straddles a wide latitudinal range, supporting an array of unique habitats and flagship species such as manta rays and humpback whales. The Mozambican coastline is one of the few hotspots for whale shark aggregations worldwide (Warnell et al. 2013; Pereira et al. 2014). The richness of biodiversity makes the SWIO a unique part of the planet.

Although species diversity in the SWIO is high, there is growing concern that increasing human population and infrastructure development, coupled with the lack of research and resource management in the SWIO states, will contribute towards the unsustainable exploitation of marine resources (van der Elst et al. 2009; Worm and Branch 2012).

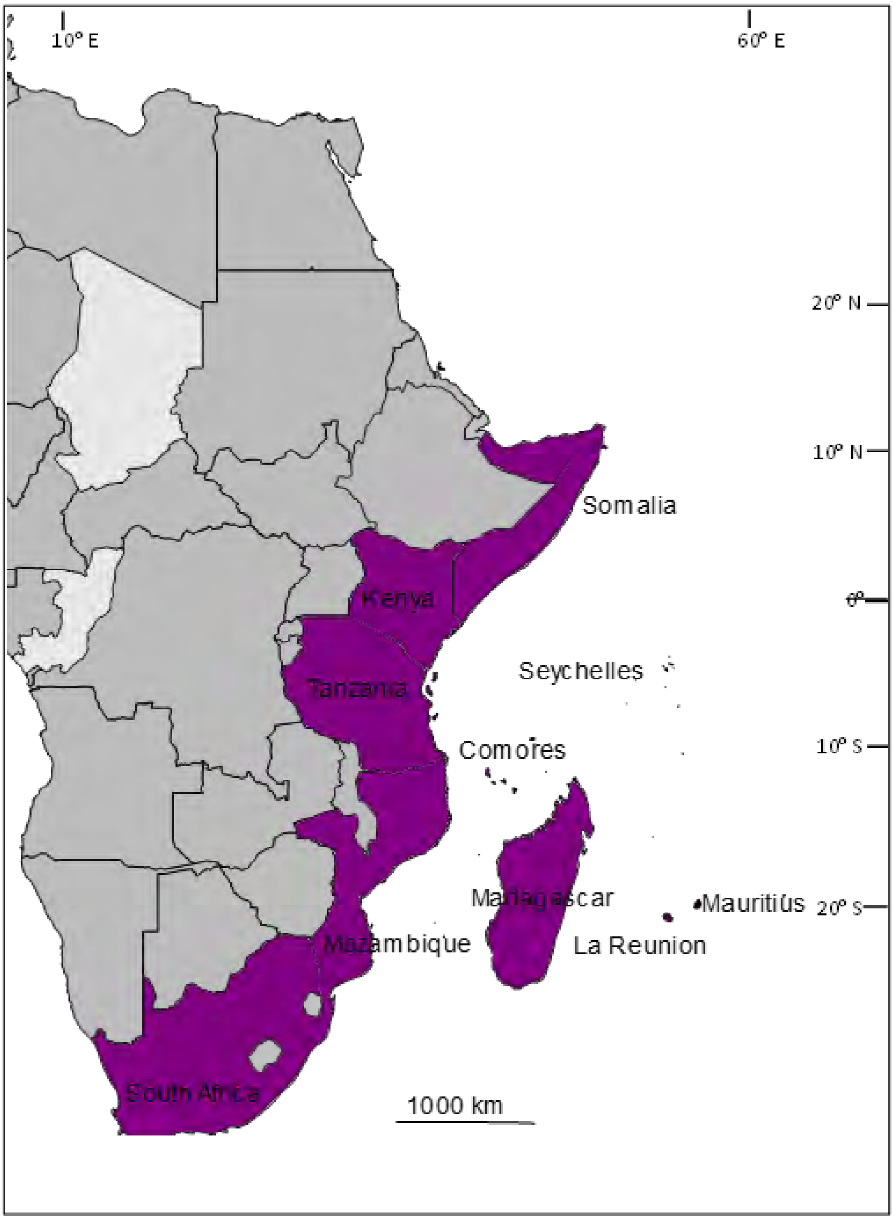

Figure 1.1 Map of countries bordering or located within the SWIO, shown in purple. Five of the ten countries are continental (Somalia, Kenya, Tanzania, Mozambique and South Africa), while the other five countries are island states (Madagascar, Mauritius, Comoros, Reunion and Seychelles).

1.2 THE FISHERIES IN THE SOUTHWEST INDIAN OCEAN

The demand for fish protein has witnessed a substantial increase globally, from 4 kg/capita in 1960 to 22 kg/capita in 2016 (FAO 2018). In the SWIO, fish is a staple consumable and the most accessible source of protein for coastal communities (van der Elst et al. 2009). The Seychelles, for example, was ranked fifth in the world for fish consumption in 2015, with an estimated eight meals containing fish per week (Kimani et al. 2008; Strain 2014; FAO 2018). The fishing industry is paramount to many inhabitants of SWIO countries, providing food security and contributing economically through trade and employment. All SWIO countries have fishery sectors that contribute to their economy, which can be divided into three sub-sectors: commercial, artisanal and recreational fisheries (van der Elst et al. 2005). Each of these fisheries are practised and managed to varying degrees by the governing authorities of the ten nations (van der Elst et al. 2009).

Commercial fishing generally refers to the exploitation of fish for commercial profit purposes and is of tremendous economic importance within SWIO nation states (Groeneveld and Koranteng 2017; FAO 2018). The Seychelles, for example, is one of the highest exporters of yellowfin tuna (*Thunnus albacares*) with more than 45,000 metric tons caught in 2017 (Rattle 2019). Mauritius also supports a large commercial tuna fishery, as well as fisheries for demersal species such as snappers (Lutjanidae; Boistol et al. 2011). South Africa's industrial fisheries are primarily made up of hake (*Merluccius poli)*, horse mackerel (*Trachurus capensis*), and chokka squid (*Loligo reynaudii*). In Tanzania and Kenya, shallow water prawns (*Penaeidae*) are the primary commercial species (Kariuki 2005; Kimani et al. 2008). These commercial fishing industries generate significant revenue – approximately US$ 1.3 billion/year – for countries bordering the SWIO (Andriamahefazafy et al. 2019). The high value of some SWIO commercial fisheries like yellowfin tuna, estimated at US$ 6.5 billion, makes unsustainable fishing practices challenging to control because short-term economic gains outweigh long-term conservation needs (Lecomte et al. 2017).

Artisanal and subsistence fisheries are also well-established in all the SWIO countries. Fishing methods vary depending on targeted species and country; these include the use of handlines, longlines, seine nets, trawls and hand collection (De Young et al. 2003). The marine species targeted in this sector are extremely diverse, ranging from coral reef species such as parrotfish (Scaridae), rabbitfish (Siganidae) and groupers (Serranidae) in the Seychelles and Mauritius, to lobster (Palinuridae), octopus (Octopodidae) and sardines (Clupeidae) in Kenya, and numerous fish species, whelks (Buccinidae), sea squirts (Pyuridae) and white mussels (Mytilidae) in South Africa (Kariuki 2005; FAO 2006; Breuil and Grima 2014). Although artisanal and subsistence fisheries management is quite variable by country (Kimani et al. 2008), these sectors are largely unmanaged in most SWIO countries (FAO 2006).

The fishery sub-sector that is the most data-poor in the SWIO is recreational fishing (FAO 2017). South Africa and Kenya are the only SWIO countries that have recreational fishery management plans (FAO 2006). The best-managed recreational fisheries in the SWIO are in South Africa, which has several monitoring programs and catch restrictions, i.e. bag and size limits (Attwood and Bennett 1995; Cockcroft and Mackenzie 1997; Griffiths et al. 2010). Studies of recreational fisheries in Africa are limited, with the majority reported from South Africa. These studies have covered a range of topics including the evaluation of catch and effort, trends of prominent recreational fisheries and the effects of catch-and-release on fish survival and health (Smale and Buxton 1985; Clarke and Buxton 1989; Cockcroft and Mackenzie 1997; Griffiths et al. 2010; Mann et al. 2011; Butler et al. 2017). There is a dearth of knowledge on recreational fisheries in the other SWIO nations, despite a considerable focus on tourism-based recreational fishing for economic development (FAO 2006). One common type of recreational fishing practised – mainly in South Africa, with increasing popularity in SWIO island nations such as Mauritius and the Seychelles – is fly fishing (FAO 2017).

Marine-based tourism is one of the most significant economic contributors to SWIO island states. In 2016, tourism contributed 22% to the total GDP and created (indirectly), approximately 12,000 jobs within the Seychelles (Turner and Freiermuth 2017). The recreational tourism sector is responsible for the creation of many of these jobs through marine-based activities such as diving, snorkelling and fishing. In Seychelles, the tourism industry was made accessible to a global market with the opening of an international airport in 1972 (SCAA 2017) and subsequent investment in infrastructure at the nation's isolated coralline atolls, collectively referred to as the outer islands. These factors have enabled the establishment of a thriving, elite tourism industry (Campling et al. 2011). Tourism is also an important economic pillar in other SWIO states like Mauritius and Madagascar (Sobhee 2006). In 2018, Mauritius received more than 1.3 million tourists, accounting for 8.6% of the GDP and providing employment for more than 9.6% of its 1.265 million population (MMT 2019). Mauritius - similar to Seychelles - has had an increase in visitors due to the improvement of flight networks, and the majority of tourists partake in marine-based activities (Sobhee 2006). Although, Seychelles and Mauritius' tourism industries are well-established, the tourism potential of SWIO states like Madagascar and Comoros remains largely untapped (Christie et al. 2003; World Bank 2013a, 2013b, 2018; Sambaouma et al. 2016).

Recreational fly fishing is a growing attraction, drawing tourists to SWIO island nations such as Seychelles and Mauritius. Fly fishing requires specialised equipment and a technique different from other forms of hook and line fishing as it is not a fishing technique used by artisanal and subsistence fishers (De Lestang 2005). This contrasts with countries such as the U.S.A and U.K., where fly fishing has been an established recreational fishing method for decades (Hoogendoorn 2014). In South Africa, freshwater fly fishing was introduced during the 1890’s with the introduction of several freshwater fish species (Du Preez and Hosking 2011; Davies 2002). Introduced species like rainbow trout (*Oncorhynchus mykiss*), large and smallmouth bass (*Micropterus* spp.), and native species like yellowfish (*Labeobarbus* spp.) are important for generating revenue for recreational fisheries in many rural areas of South Africa (Du Preez and Lee 2010; Hoogendoorn et al. 2017) and numerous small towns surrounding dams in the Northern Cape Province (Du Preez and Hosking 2011; Hoogendoorn 2017; Butler and Rogerson 2016). Whilst South Africa is known for its freshwater fly fishing opportunities, salt-water fly fishing is less popular, largely due to the unpredictable and unfavourable conditions of the coastline (Hoogendoorn 2014). Although coastlines are perceived as unfavourable for fly fishing, anglers still fly fish in estuaries, many of which are overfished or polluted (Hoogendoorn 2014). The shallow waters and sand flats of destinations such as Seychelles and Mauritius are ideal for fly fishing.

1.3 FLY FISHING IN THE SEYCHELLES

In the Seychelles, the increasing popularity of fly fishing has led to infrastructure investment and development in the outer coralline islands, where fly fishing tours are offered. Currently, the development of the fly fishing industry in the Seychelles outer islands is at an advanced stage, where popular big game fishes (e.g., trevallies, bonefishes and sailfish) and unchartered waters attract high-end tourists that pay for the experience of fishing in isolated, pristine locations that are less developed than the more-populated inner islands (Cooke et al. 2008).

The saltwater fly fishing industry in the Seychelles comprises two sectors: lodge- and yacht-based operators and independent operators. Similar to operations in other parts of the world, such as the Bahamas and Belize, the lodge- and yacht-based fly fishing operators are well-established businesses that employ many people, including skilled guides that offer fishing clinics and take guests fishing (Fedler and Hayes 2008; Fedler 2010). The independent operators, on the other hand, are less structured and more opportunistic, with fly fishing being one of several activities offered to their clientele. The occurrence of these two types of fly fishing operations observed in the Seychelles, both lodge- and yacht-based and independent operators, is standard for the global fly fishing industry, and similar business strategies have been described in Belize, Venezuela and the Bahamas (Debrot and Posada 2005; Fedler and Hayes 2008; Fedler 2010).

There is a distinct division between geographic areas in the Seychelles used by the two groups of fly fishing businesses. The independent fly fishing operators fish mostly within the Inner Island Group and part of the Alphonse and Amirantes Island Groups (Figure 1.2). The lodge- and yacht-based fly fishing companies operate primarily in the outer islands, including the Alphonse and Amirantes Island Group, Farquhar Island Group, and Aldabra Island Group (Figure 1.2). Only one company operates both in Seychelles and the Saint Brandon Island Group, a Mauritian territory (Figure 1.2).

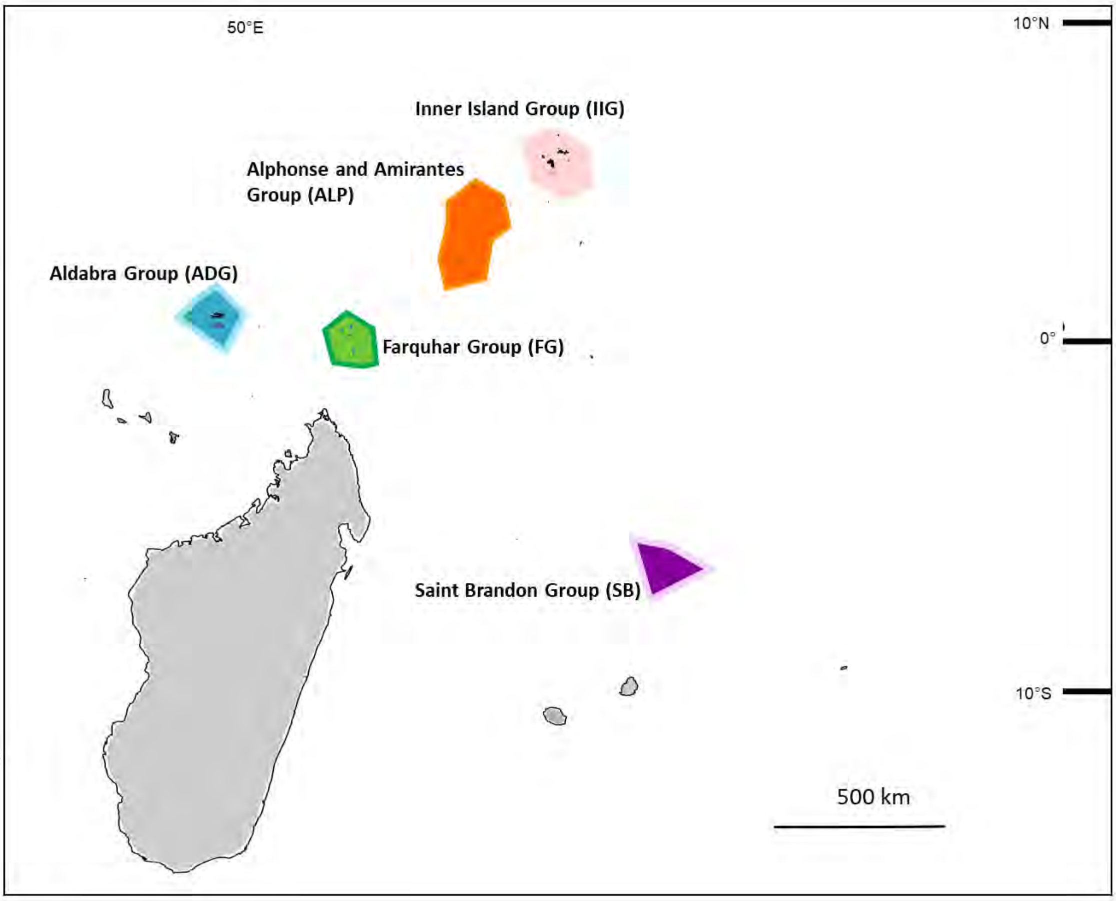

Figure 1.2. The Seychelles and Mauritius Islands divided into the following island groups (illustrated using coloured polygons) based on fly fishing operations in this study: Inner Island Group (IIG; pink), Alphonse Island Group (ALP; orange), Farquhar Island Group (FG; green), Aldabra Group (ADG; blue) and Saint Brandon Island Group (SB; purple).

Some of the most popular saltwater fly fishing targeted species in Seychelles (and globally) include trevallies (Carangidae) and bonefishes (Albulidae; Danylchuk et al. 2007; Cooke et al. 2008). Bonefishes dominate catches at the Alphonse and Amirantes Island Group and Saint Brandon, while trevallies are the most sought-after in the Farquhar Group and the Aldabra Islands Group (Cosmoledo and Astove Islands). An assessment of catch from internet fishing blogs and surveys of the two largest lodge- and yacht-based fly fishing operators in the Seychelles and Mauritius (FlyCastaway and Alphonse Fly Fishing Company) between January 2016 – December 2017 revealed that bonefishes made up the majority of the catch per season (~1000 fish/season). The popularity of bonefishes in the global saltwater fly fishery is well recognised and contributes significantly to nations' economies. For example, in the Bahamas, the bonefish fishery is estimated to contribute US$ 1.4 million to the local economy (Danylchuck et al. 2008). To-date, there has been no economic evaluation of the fly fishing industry in the Seychelles, but several components of this industry contribute to the economy of the Seychelles, including consumables purchased for operating remote lodges (e.g., food, hotel supplies), petrol costs for operating yachts, contributions from fly fishing tourists towards the airline and hotel industries, as well as the employment of guides and hospitality staff.

In other parts of the world, including Florida, Bahamas, Venezuela and Belize, the importance of the bonefish fly fishery has generated considerable scientific and socioeconomic research interest (Debrot et al. 2005; Fedler et al. 2008; Fedler 2010; Larkin et al. 2010; Larkin 2011; Frezza and Clem 2015; Santos et al. 2017). Scientists have worked with fly fishers, fly fishing guides and fly fishing establishments to carry out surveys to understand how the bonefish fly fishery functions differently at various locations. For example, in Kiribati it was estimated that having only 20 local guides involved in the industry could generate more than US$ 2.5 million for the local economy (Campbell and Hainich 2014; Filous et al. 2019a).

Apart from fishery socioeconomic studies, bonefishes have received additional research attention worldwide, including studies focused on the effect of catch-and-release on mortality, as well as movement patterns (Humston et al. 2005; Danylchuk et al. 2007; Fedler 2010; Moxham 2019). The post-release mortality of bonefishes ranges between 15 – 100% (Humston et al. 2005). Moxham et al. (2017) found that 90% of bonefish caught and tagged at the Saint Joseph Atoll in Seychelles were preyed upon in an area of high predator abundance; a trend also noted at Eleuthera (Bahamas; Danylchuk et al. 2007). Additionally, many studies have focused on the distribution, genetics and species delimitation of bonefishes worldwide (Colborn et al. 2001;

Obermiller and Pfeiler 2003; Friedlander et al. 2007; Pfeiler et al. 2008; Wallace 2014; Pickett 2020).

1.4 BONEFISHES (ALBULIFORMES)

Bonefishes belong to the order Albuliformes, nested within the clade Elopomorpha, which also contains Anguilliformes (true eels), Elopiformes (ladyfishes and tarpons), and Notacanthiformes (spiny eels) (Inoue 2004; Adams 2014). The uniting feature of Elopomorpha is the presence of leptocephalus larvae, a character only present in these fishes (Pfeiler 1986; Filleul and Lavoué 2001). Although, certain life history and larval biology characteristics differ between species; several characteristics are shared across all elopomorph taxa (Pfeiler 1999). One of the distinct features of the larvae is the presence of a transparent, laterally compressed body and a highly hydrated transparent extracellular gelatinous matrix (Ault 2007). The larval bodies are all very thin, enabling a high surface area to volume ratio. Unlike other larval types, leptocephalus larvae may not feed exogenously after their yolk supply is depleted. Therefore, it is assumed that the primary method of nutrient acquisition is through the uptake of dissolved organic matter (Pfeiler 1986, 1999).

The combination of these described characteristics enables leptocephalus larvae to survive for several months at sea (Chen et al. 2014). European eels, for example, can spend as much as six to nine months in the pelagic marine environment (Wang and Tzeng 2000; Bonhommeau et al. 2010). Larval tarpons (Megalopidae) were reported to spend a maximum of two and a half months at sea, whereas bonefishes can have wildly varying pelagic larval durations (PLDs). For example, species like *A. vulpes* and *A. oligolepis* are estimated to have a pelagic larval duration of one to two and a half months, whereas tropical eastern Pacific species can have a PLD of six to seven months (Ault 2007; Friedlander et al. 2007; Adams 2014).

These larval characteristics may be responsible for elopomorph fishes' ability to migrate between oceanic basins and maintain genetic mixing over vast distances (Gagnaire et al. 2011; Miller et al. 2006; Robinet et al. 2007; Bonhommeau et al. 2010; Wallace 2015). Consequently, many studies on Elopomorpha have focused on the effects of long-distance migration on the genetic variability and connectivity between populations (Humston et al. 2005; Bonhommeau et al. 2010; Gagnaire et al. 2011; Wallace 2015).

The Albulidae family is still under taxonomic revision (Pickett et al. 2020). Currently, the family is divided into two genera: *Albula* and *Pterothrissus* (Nelson 2006; Wallace 2016). Species of *Pterothrissus* live in deep waters in subtropical and temperate regions (Adams et al. 2014). In contrast, *Albula* species inhabit shallow waters and were once considered monotypic (*Albula vulpes*) because of the few morphological features available to delimit species (Whitehead 1986). Previously, it was assumed that *A. vulpes* had a global distribution and its connectivity was attributed to the species' ability to disperse via its leptocephalus larvae using oceanic currents. Only after the 1980s, with improved morphological methods and genetic resources, was it suggested that more than two species of bonefish existed (Shaklee and Tamaru 1981; Hidaka et al. 2008). The improved morphological and genetic methodologies enabled a revision of the globally identified *A. vulpes* complex. During the re-evaluation process, more than 20 previously assigned species names were re-evaluated and changed (Hidaka et al. 2008). In some cases, up to three names have now been synonymised to one species of *Albula* (Whitehead 1986; Randall and Bauchot 1999). Presently, genetic, and morphological studies have contributed to the recognition of 12 species of *Albula* worldwide that are characterised into three species complexes: *Albula argentea*, *Albula vulpes* and *Albula nemoptera* (Table 1.1 and Figure 1.3; Colborn et al. 2001, Wallace and Tringali 2010, Pickett et al. 2020).

Globally, bonefish research has focused primarily on the Atlantic and Pacific Oceans, with few studies occurring in the Indian Ocean (Danylchuk et al. 2011; Wallace and Tringali 2010, 2016; Wallace 2015). A recent review on albulid research revealed that 56% of studies were conducted within the Atlantic Ocean, 41% in the Pacific Ocean and only 3% in the Indian Ocean (Moxham 2017). One important emphasis of recent reviews is that genetic studies have proven vital for delimiting bonefish species worldwide (Pickett et al. 2020; Moxham 2019).

Table 1.1. The three *Albula* species complexes (from Pickett et al. 2020).

Species complex	Daughter species
Albula vulpes complex (Shaklee and Tamaru 1981; Pfeiler 1996; Colborn et al. 2001)	*A. vulpes, A. glossodonta, A. esuncula A. sp. Cf. vulpes, A. koreana, A. gilberti,* and *A. goreensis*
Albula argentea complex (Hidaka et al. 2008)	*A. argentea, A. oligolepis,* and *A. virgata*
Albula nemoptera complex (Pickett et al. 2020)	*A. nemoptera* and *A. pacifica*

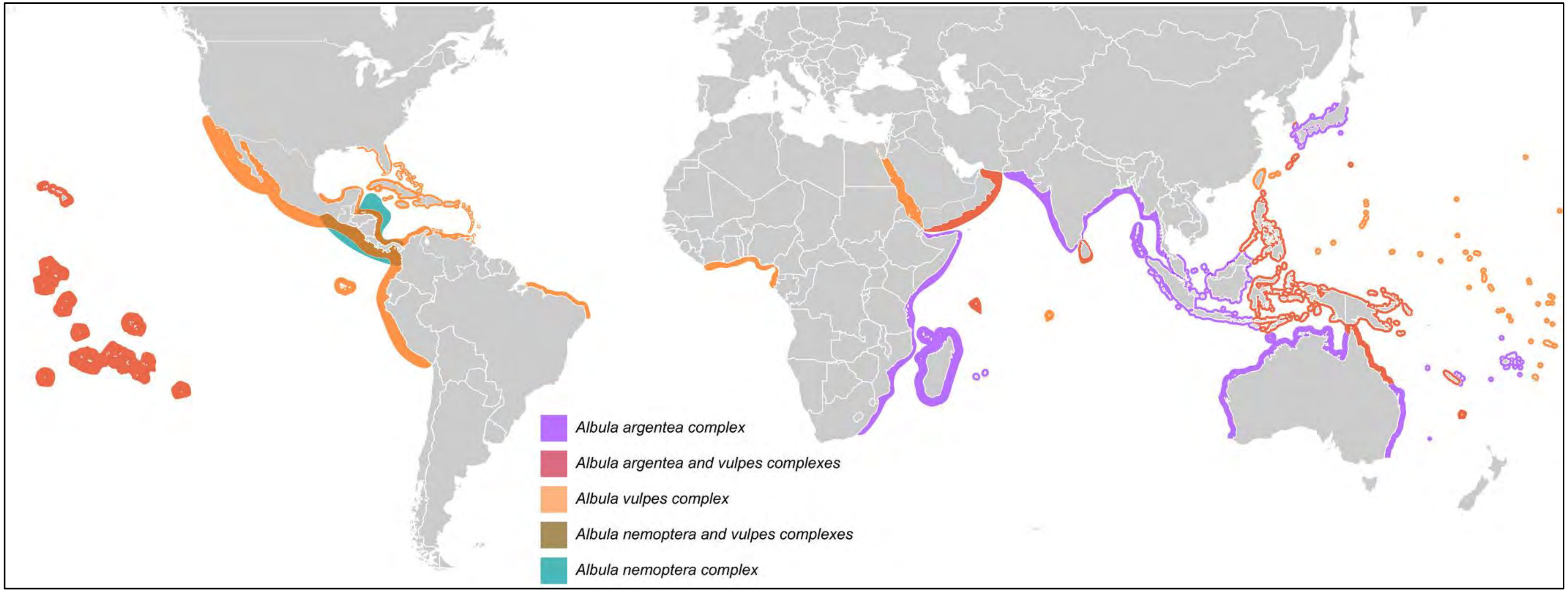

Figure 1.3 Global distribution of *Albula* species complexes each represented by a colour (Pickett et al. 2020). Overlapping distributions are also highlighted in a distinct colour.

1.5 GENETIC MARKERS

To quantify genetic diversity and divergence, most studies target a combination of genetic markers, including mitochondrial and nuclear genes, microsatellites, and single nucleotide polymorphisms (SNPs). Sequencing regions of the mitochondrial genome has historically been useful for identifying population bottlenecks, migration and for tracing evolutionary history at timescales shallower than nuclear markers (Moritz et al. 1987; Harrison 1989; Song et al. 1998; Wallace 2015; Villacorta-Rath et al. 2016). MtDNA has also commonly been used for the assignment of unknown individuals to species, known as genetic species identification (GSI; Hebert et al. 2004; Stoeckle 2003). In bonefish studies, the mitochondrial gene cytochrome b (cyt-b) has been used to describe species occurrences, for GSI, and for estimates of haplotype diversity and population differentiation (Colborn et al. 2001; Obermiller and Pfeiler 2003; Pfeiler et al. 2006; Pfeiler et al. 2008; Díaz-Viloria et al. 2017). Many scientists, however, recognise the limitation of mtDNA, faulting this type of marker for rendering ambiguous outcomes and arguing that the use of mtDNA alone returns low-resolution results, making the delineation of stocks difficult (Morin et al. 2004; Hurst and Jiggins 2005). For such reasons, mtDNA studies are often coupled with additional genetic markers, such as microsatellites.

Microsatellites are short tandem repeats, usually composed of 1–6 nucleotide repeats, distributed evenly throughout a gene region (Miah et al. 2013). These markers are generally abundant, highly polymorphic and inherited as codominant markers. Although frequently used for population-level studies, microsatellite primers are time-consuming to develop and difficult to amplify across species (Wallace 2015). A 2015 study on bonefish, for example, generated 19 microsatellite loci for *Albula vulpes*, but only 12 of these microsatellites amplified successfully for bonefish species in the SWIO (Wallace 2015). Due to the limitations mentioned above with mtDNA and microsatellite markers, the development of next-generation sequencing techniques has transformed the field of population genetics (Davey and Blaxter 2010; Peterson et al. 2012; Grewe et al. 2017).

Next-generation sequencing (NGS) is increasingly accessible and cost-effective for population genetics research (Davey and Blaxter 2012; Peterson et al. 2012; Valenzuela-Quiñonez 2016). NGS technologies allow for millions of reads from multiple individuals to be sequenced in parallel (Hudson 2008; McCormack et al. 2012). One NGS method, double digest restriction site-associated DNA sequencing (ddRADseq), uses restriction enzymes to digest genomic DNA (Peterson et al. 2016) and is advantageous in that this method requires only a small quantity of high-quality DNA to generate millions of sequence reads at a relatively low cost (Hudson 2008; McCormack et al. 2012). Compared to microsatellites, ddRADseq protocols are less prone to human error and have a shorter processing pathway (Thrasher et al. 2018).

NGS methods have transformed conventional population genetics into population genomics, enabling population delineation to be conducted on a genomic scale and resolving intraspecific relationships where other markers such as mtDNA and microsatellites had previously provided little resolution (Wagner et al. 2013; Stinchcombe and Hoekstra 2008). Although NGS methods generally come at a higher upfront financial cost than traditional Sanger sequencing methods (e.g., mtDNA, microsatellites), they are more cost-effective because they generate substantially more amounts of data per individual (Miah et al. 2013). Consequently, the use of NGS methods is becoming more popular for fisheries’ management purposes, such as stock structure identification (Knutsent et al. 2011; Mullins et al. 2018). NGS platforms can also be used to trace illegal, unreported and unregulated-caught species and investigate fishes’ abilities to adapt to climate change (Valenzuela-Quiñonez 2016). Although there has been an increase in the number of studies using NGS platforms for marine fishes, few studies to-date have employed the use of NGS platforms to elopomorphs, and none to bonefishes (Valenzuela-Quiñonez 2016; Benestan et al. 2016; Babin et al. 2017; Moxham 2017). Given the ability to detect fine-scale population structure through methods such as ddRADseq and the recent identification of new bonefish species through genetic techniques, the application of ddRADseq to bonefishes in the SWIO would shed light on the population structure of these important fly fishery species in an understudied region.

1.6 OBJECTIVES

My study aimed to investigate the species identification, genetic diversity and population structure of *Albula* species (bonefishes) in the SWIO.

These aims were achieved by addressing the following objectives:

i) Identify the occurrence of *Albula* species in SWIO island states and use genetic species identification to assign samples to species.

ii) Reveal patterns of genetic diversity of the dominant bonefish species, *A. glossodonta*, using both mtDNA and SNPs.

iii) Outline the fisheries management implications for bonefishes based on the results of the genetic analyses.

1.6.1 THESIS OUTLINE

This thesis is divided into four chapters. Chapter 1 (Introduction) provides an overview of the fly fishing industry and bonefish fishery within the SWIO. It further outlines the importance of using DNA platforms for cryptic species such as bonefishes. Chapter 2 (Materials and Methods) provides an overview of the sampling sites and the methods employed to generate data for genetic species identification and the population structure of bonefishes within the SWIO. The chapter is separated into two sections: the Sanger sequencing method (cyt-b), and the next-generation sequencing method (ddRADseq). Chapter 3 (Results) follows a similar structure as Chapter 2 with two main sections: results obtained using cyt-b, and results from ddRADseq. Chapter 4 (Discussion) focuses on the broader context of the study's results, specifically:

i) The bonefish species found within the Seychelles.
ii) The genetic diversity found within the SWIO.
iii) The possible reasoning behind the genetic structure between Seychelles and Mauritius.
iv) The possible habitat partitioning between the two species of bonefish (*A. glossodonta* and *A. oligolepis*).
v) Implications for conservation and management.

2 CHAPTER 2: MATERIALS AND METHODS

2.1 COLLECTION OF GENETIC MATERIAL

Bonefish tissue samples were gathered from a variety of sources over a period of five years (2013–2017;Figure 2.1). Fly fishing guides collected samples from the coralline atoll of Saint Brandon, Mauritius (SB) and the outer island groups of Seychelles, specifically Farquhar Group (FG) and Alphonse and Amirantes Group (ALP) (Figure 2.1). Samples from the Aldabra Group (ADG) were collected during a scientific cruise in 2013, whereas samples from Mahé Island within the Inner Island Group (IIG) were obtained from seine net fishers and fishing markets (Table 2.1). During collection, there was no record of bonefishes size classes because collection events were opportunistic and largely dependent on the bonefish fishery stakeholders (fly fishing companies and small-scale fishers). A visual infographic was provided to each fly fishing guide to ensure that a standard protocol was performed during sampling to avoid contamination. All fly fishing guides were asked to collect a 1 cm clip of any fin tissue, which was stored in 99% ethanol in a conventional freezer (-4° C). All tissues were deposited and catalogued at the South African Institute for Aquatic Biodiversity in Makhanda, South Africa (www.saiab.ac.za). Sampling numbers (N) varied between locations based on availability of samples during DNA extraction for the two sampling methods as well as prioritisation of the DNA samples for ddRADSeq analyses over Cyt-b sequencing. Prior to all genetic analysis (ddRADSeq and Cyt-b), each sample was genetically identified as described in Section 2.4.1.

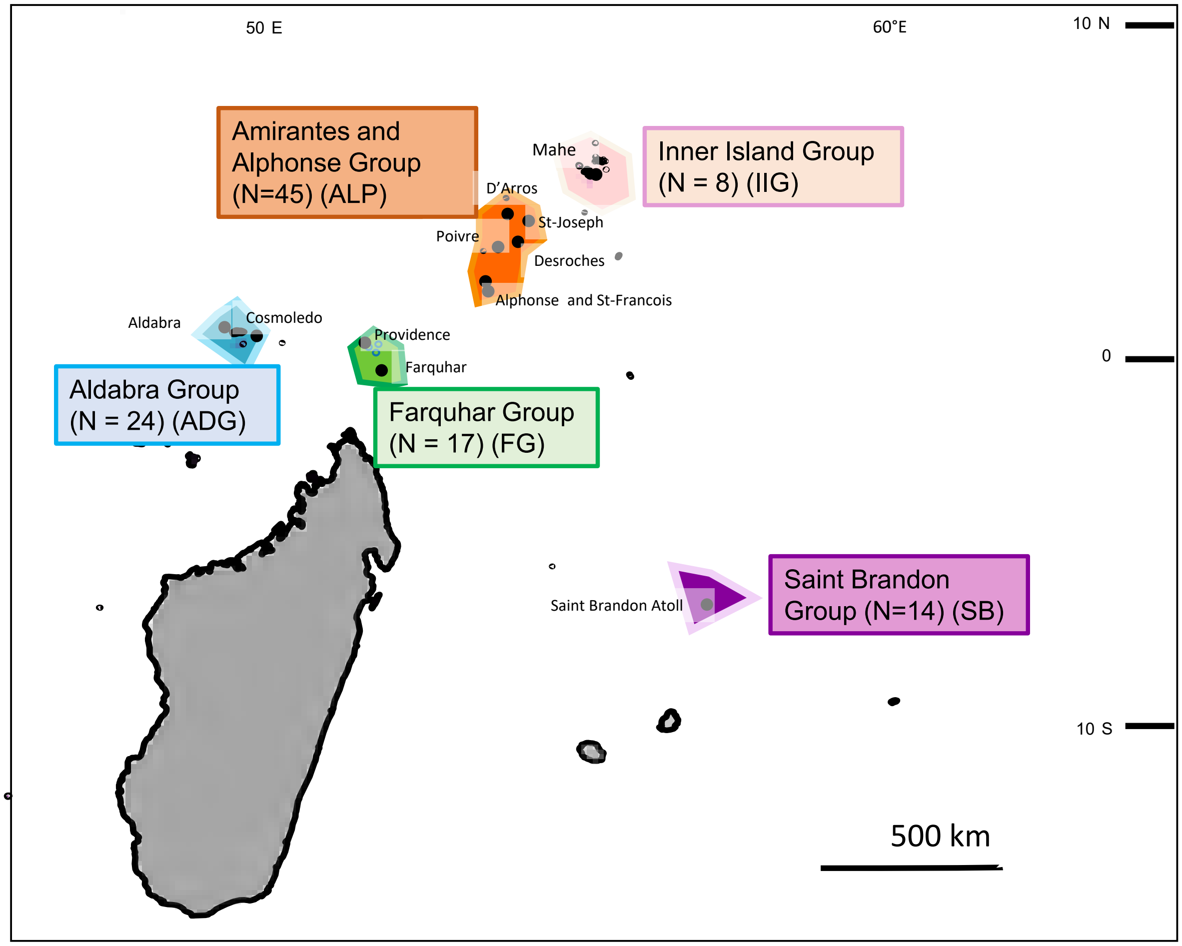

Figure 2.1. Eleven sampling sites (islands) in the Seychelles and Mauritius, each of the islands are demarcated with a vector shape with a specific colour. Samples were aggregated into five larger island groups: Saint Brandon (SB), Aldabra (ADG), Alphonse and Amirantes (ALP), Farquhar (FG), and Inner Islands (IIG). Each box indicates N number of individuals collected from each island group.

Table 2.1. Bonefish tissue collection details, including the name of the island group, locality, number of samples for each sequencing method, and sample source. Each of the samples were genetically identified to species level.

Island groups	**Locality**	**Number of samples (N)**		**Source**
		cyt-b	ddRADseq	
Inner Island Group (IIG)	Mahe Island	8	0	Seine net fishery
Amirantes and Alphonse Group (ALP)	Saint Joseph Atoll	30	17	Scientific research team
	Poivre Atoll	18	3	Scientific research team
	Desroches Island	4	0	Fly fishing guides
	Alphonse Atoll	20	1	Fly fishing guides
	Saint François Atoll	25	1	Fly fishing guides
Farquhar Group (FG)	Farquhar Island	8	9	Fly fishing guides
	Providence Island	6	8	Fly fishing guides
Aldabra Group (ADG)	Aldabra Atoll	12	11	Fly fishing guides
	Cosmoledo Atoll	12	7	Fly fishing guides
Mauritius (SB)	Saint Brandon Atoll	14	18	Fly fishing guides
Total		157	75	

2.2 DNA EXTRACTION

DNA was extracted using the GeneJET genomic DNA purification kit (ThermoFisher Scientific, USA) using the manufacturer’s instructions. Quality tests were performed on the extracted DNA, using gel electrophoresis by running 5 µl of the DNA on a 2% agarose gel with ethidium bromide and 1XTBE buffer at 100V for 30 minutes to visualise bands. The gel was then photographed under ultraviolet light using the UV transilluminator (UVITEC, Cambridge, UK). A Qubit® 3.0 Invitrogen fluorometer (ThermoFisher Scientific, USA) was used to measure the extracted DNA concentrations. All extracted DNA was stored at -20° C.

2.3 MITOCHONDRIAL DNA METHODS

2.3.1 MITOCHONDRIAL GENE SEQUENCING

Mitochondrial DNA (mtDNA) was always believed to be only maternally transmitted in animals, however, recent studies have shown that recombination may occur in fish species (Guo et al. 2005). Whilst recombination is now a confirmed artefact, mtDNA is still powerful as rarely does recombination result in new haplotypes due to homoplasy (Tsaousis et al. 2005; Wang et al. 2021). Therefore, mtDNA is advantageous for several aspects of genetics research such as tracing historical events, identifying species, and conducting connectivity studies (Billington 2003). In this study, the mitochondrial gene cytochrome-b (cyt-b) was used for genetic species identification and preliminary tests of genetic connectivity.

2.3.2 CYTOCHROME-B PRIMERS

The Alba-1 (5'-GTCTCCAAGAAGGTTAGGCGA-3') and Alba-6 (5'-GACAAACCCTAACAAGTC- 3') forward primers, and Alba-3R (5'-TGCTAGGGTTGTGTTTAATTA-3) reverse primer were used for all cyt-b sequencing (Colborn et al. 2001). These primers are *Albula*-specific and were used for previous studies on bonefishes (Colborn et al. 2001, Seyoum et al. 2008, Wallace 2015, Wallace and Tringali 2016).

2.3.3 PCR AMPLIFICATION AND SEQUENCING

Using the above-mentioned primers, a 600 bp fragment was amplified using Polymerase Chain Reaction (PCR). PCR was performed on a 96 welled plate in 20 µl reaction solutions. Each well contained 10 µl of BigDye^{TM} Terminator v3.1 standard kit master mix (ThermoFisher Scientific, USA), 0.5 µl of forward and 0.5 µl of reverse primers, 7 µl of deionized H_2O and 2 µl of DNA. The amplification profile used was adjusted from previous bonefish studies (Wallace 2014; Wallace 2015). Thermocycler parameters consisted of denaturing at 94°C for one cycle for 2 minutes, one cycle at 94°C for 1 minute, 50° for 30 seconds, followed by 72°C for 1 minute. This was followed by 36 cycles at 94°C for 30 seconds, followed by a further 55°C for 30 seconds, 72°C for 90 seconds, and a final extension at 72°C for 8 minutes. All PCR products were tested on a 2% agarose gel and carried forward to the clean-up process, whereby any excess primers and nucleotides were removed using a Thermo Scientific ExoSAP protocol and BigDye^{TM} Terminator v3.1 Cycle Sequencing kit (ThermoFisher Scientific, USA). The purified product was then stored at 4°C for immediate sequencing or -20°C for downstream processing. All cleaned products were sequenced at the Molecular Laboratory of the South African Institute for Aquatic Biodiversity (SAIAB), Makhanda, South Africa.

2.4 CYTOCHROME-B (CYT-B) ANALYSES

2.4.1 GENETIC SPECIES IDENTIFICATION

All cyt-b sequences were trimmed and visualized using *Geneious v11.1.* (Drummond et al. 2010). To genetically identify species, each sample was treated as unknown and run through the *Basic Local Alignment Search Tool (BLAST)* available at https://blast.ncbi.nlm.nih.gov/Blast.cgi. The cleaned and verified sequences were then analysed using *DnaSP* v5 (Librado and Rozas 2009) and *Arlequin* v3.4.2.2 (Excoffier and Lischer 2010) for genetic structure and diversity.

2.4.2 GENETIC DIVERSITY AND HAPLOTYPE MAP

The number of haplotypes within each individual and each island group was used for the calculation of haplotype diversity (Nei 1987). Haplotype diversity (*h*) refers to the probability that if any two random individuals were drawn out of a population, they would have different haplotypes (Billington 2003). Private haplotypes (*P*) were also calculated; these are haplotypes that are unique to one population and are therefore informative about local adaptation (Sjöstrand et al. 2014; Slatkin 1985). Generally, these haplotypes arise due to differences in external factors that may be unique to the geographical region that the population inhabits (Slatkin 1985; Langille et al. 2014). Lastly, nucleotide diversity (π; Nei 1987) was calculated to estimate the number of nucleotide differences between any two randomly chosen haplotypes (Tajima 1983). These genetic diversity indices were calculated in *DnaSP* v*5* (Librado and Rozas 2009). To visualize haplotypes based on their similarity and geographic distribution, haplotype maps were drawn using *Networks* (Network © Copyright Fluxus Technology Ltd., 1999-2019) and *GeoViz* (Hardisty and Robinson 2011).

2.5 NEUTRALITY TESTS AND DEMOGRAPHIC HISTORY

2.5.1 NEUTRALITY TESTS

Hardy Weinberg Equilibrium (HWE) tests are essential to perform when dealing with variants of allele frequencies. The HWE principle states that organisms in HWE are diploid, have no overlapping generations, no natural selection, no mutation, and that large populations are characterised by random mating and no migration (Waples 2015). Screening for HWE and other neutrality tests is considered an integral part of quality control checks in genetic studies (Gomes et al. 1999).

Five tests were used to identify potential evolutionary patterns within the population (Fu 1997; Table 2.2). These tests are widely used in population genetic studies and have been categorised as class I (based on the differences between estimators of the population mutation rate and frequency of mutations), class II (most-affected by recombination-based on haplotype distribution) and class III (based on the distribution of pairwise differences; Table 2.3; Ramírez-Soriano et al. 2008).

1. Tajima's *D* was used to test neutrality (Tajima 1989). This test calculates the difference between the observed nucleotide heterozygosity (θπ) and the observed number of segregating sites (θk) (Tajima 1989). A negative *D* value suggests a recent population bottleneck, e.g., due to drift or selective sweep, whereas a positive *D* value indicates population expansion, e.g., purifying selection. The Tajima's *D* test was performed in *Arlequin* v3.4.2.2 (Excoffier and Lischer 2010).
2. Fu and Li's *D** (Fu and Li 1993) was calculated using the total number of segregating sites (Simonsen *et al*. 1995; Rozas *et al*. 2017). Similar to Tajima's *D* statistic, Fu and Li's *D** can either be greater or less than zero. This test is useful for identifying background selection. A

negative value is associated with an excess of rare alleles, which could indicate a selective sweep, whereas a positive value suggests balancing selection (Panhuis and Sanson 2006). When performing this test, no outgroups are considered.

3. Fu and Li's F^* (Fu and Li 1993) follow the same principle as Fu and Li's D^*; the main difference with this test is that the use of an outgroup is not employed (Simonsen et al. 1995; Rozas et al. 2017; Panhuis and Sanson 2006).
4. Fu's F_S is a statistical class II test based on Ewens' sampling distribution that uses the infinite sites model of mutation with the assumption of no recombination and is best at identifying genetic hitchhiking (Ewens 1972). The test is based on the probability that haplotypes sampled at random will have the same or greater number of alleles than observed in the test population. A positive value of F_S is indicative of a recent bottleneck event (deficiency of alleles) or the selection of specific alleles. A negative F_S value is indicative of genetic hitchhiking or recent population expansion. Unlike other described neutrality tests, statistical significance is tested under the coalescent algorithm (Excoffier and Lischer 2010). The tests were calculated in *Arlequin* v3.4.2.2 (Excoffier and Lischer 2010) with 10,000 simulated samples.
5. The R_2 test investigates the difference between the number of SNPs and the number of nucleotide differences (Ramos-Onsins and Rozas 2002). The test is effective for low sample sizes (Ramírez-Soriano et al. 2008). The significance of the R_2 test is simulated under the standard coalescent model. The test was performed in *DnaSP v5* (Librado and Rozas 2009).

6. Lastly, a mismatch distribution test was performed. This is a statistical class III test, which assesses the null hypothesis that populations have undergone a recent demographic expansion (Ramos-Onsins and Rozas 2002). The pairwise haplotype differences – as expected under the demographic expansion model – are plotted against the observed distribution of haplotype differences for each putative population (Rogers and Harpending 1992). The resulting plot illustrates the demographic history of that population. An L-shaped plot suggests a reduction in population size, a unimodal plot is indicative of a population expansion event, whereas a bi- or multi-modal distribution implies a population that is at demographic equilibrium (Rogers and Harpending 1992). To examine the significance of the model, a sum of squared differences (SSD) test was calculated for each putative population in *Arlequin* v3.4.2.2 (Excoffier and Lischer 2010). In addition, the Harpending's raggedness index (*Hri*; Harpending 1994) was calculated to measure the appropriateness of fit of the model of population expansion. The significance of the SSD and *Hri* were calculated using 10,000 iterations.

Table 2.2. Summary of neutral statistical tests performed with the cytochrome b dataset. The table is adapted from Ramírez-Soriano et al. (2008).

Test	Statistical class	Description	Reference
Tajima's *D*	I	Compares estimates of the number of segregating sites and the mean pairwise difference between sequences.	Tajima 1989
Fu and Li's *D**	I	Compares the number of derived singleton mutations and the total number of derived nucleotide variants without the use of an outgroup.	Fu and Li 1993
Fu and Li's *F**	I	Compares the number of derived singleton mutations and the mean pairwise difference between sequences without the use of an outgroup.	Fu and Li 1993
Fu's F_s	II	Based on Ewens' sampling distribution, taking into account the number of different haplotypes in the sample.	Fu 1997
R_2	I	Compares the difference between the number of singleton mutations and the average number of nucleotide differences.	Ramos-Onsins and Rozas 2002
Mismatch Distribution	III	Uses information from the pairwise sequence differences.	Slatkin and Hudson 1991; Rogers and Harpending 1992

2.5.2 DEMOGRAPHIC INFERENCE

Haplotype (*h*) and nucleotide diversity (π) indices (Table 2.3) can be used simultaneously with the mismatch distribution test to infer population demographic histories because population size changes influence these genetic indices. Table 2.3 provides a summary of different scenarios for population demographic histories (Grant and Bowen 1998; Mullins 2017). The *h* and π indices can be compared to the indices of Tajima's *D* and Fu's F_S, as well as the mismatch distribution shape, to better understand the demographic history of the population in question.

Table 2.3. Population demographic history for marine fishes with varying levels of haplotype (*h)* and nucleotide (π) diversity and their influence on effective population size (N_e). Table from Mullins (2017).

	Low π (<0.005)	High π (>0.005)
Low *h* (<0.5)	Suggests recent bottleneck/ founder event	Suggests recent bottleneck event acting on population with a previously large N_e, or secondary contact
High *h* (>0.5)	Suggests historical population expansion event from a small N_e	Suggests population at demographic equilibrium with large, stable N_e, or secondary contact

2.6 POPULATION STRUCTURE

2.6.1 ANALYSIS OF MOLECULAR VARIANCE (AMOVA)

Analysis of molecular variance (AMOVA) uses hierarchical levels to estimate genetic indices of structure (Excoffier et al. 1992). The test is divided into two components. First, a matrix of Euclidean distances is calculated using the mtDNA haplotype divergence between all haplotype pairs (Excoffier et al. 1992). Second, an assessment of covariance is performed at each hierarchical level of genetic structure. For this study, the hierarchical levels were the five island groups, based on population pairwise fixation index (F_{ST}) comparisons (Duncan 2013; Section 2.6.3). The AMOVA tests were conducted in *Arlequin* v3.4.2.2, with 10,000 permutations to test the significance of the covariance components and fixation indices (Excoffier and Lischer 2010).

2.6.2 POPULATION DIFFERENTIATION

To investigate the null hypothesis of no population differentiation between island groups, an exact test of population differentiation was performed (Raymond and Rousset 1995). The test explores the estimated probability of observing a contingency table (island groups multiplied by different haplotypes) that is less or equally likely than the observed sample configuration. The test was conducted in *Arlequin* v3.4.2.2 with a significance level of 0.05 using 100,000 steps between different states of a Markov chain and 10,000 de-memorisation steps (Excoffier and Lischer 2010).

2.6.3 PAIRWISE F_{ST}

The fixation index (F_{ST}) is a measure of population structure and tests the genetic divergence among subpopulations within the total population by measuring the ratio of differences between inter-population allele frequencies and the expected heterozygosity of the entire population (Nei 1987).

The test generates an index ranging from 0 to 1, where F_{ST} = 0 is synonymous with no genetic differentiation, whereas 1 suggests no sharing of genetic material and allele frequencies are fixed in populations (Davey et al. 2011). Pairwise F_{ST} tests were conducted between island groups in *Arlequin* v3.4.2.2 with 10,000 permutations (Excoffier and Lischer 2010).

2.7 DOUBLE DIGEST RESTRICTION SITE ASSOCIATED SEQUENCING (DDRADSEQ) METHODS

2.7.1 LIBRARY PREPARATION

Double digest RADseq (ddRADseq) is used for SNP genotyping and discovery and employs two restriction enzymes to digest genomic DNA (Andrews et al. 2016). The laboratory protocol I used was a modified version of Peterson et al. (2012), developed by the Near Laboratory in the Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut, USA (https://nearlab.yale.edu/).

A Qubit® 3.0 Invitrogen fluorometer (ThermoFisher Scientific, USA) was used to normalise DNA samples to target molecular weights between 150–200ng. DNA was digested using the PstI (0.4 µl) and MspI (0.4 µl) restriction enzymes. These enzymes, as well as 2 µl of 10xNEB cutsmart buffer, were added to each well on a 96 well plate. Samples were then incubated in a thermocycler at 37˚C for 8 hours and held at 4˚C until ligation. The ligation step involved attaching adapters to digested DNA fragments. The MspI adapters were uniform, whereas the PstI adapters had unique Illumina barcodes to enable the identification of individuals (Peterson et al. 2012). A master mix was made with three reagents: T4 DNA Ligase buffer (4µl), T4 DNA Ligase (1.28 µl), and DNase free water (9.72 µl). A 15 µl volume of the master mix, together with 4 µl of a 1 µM PstI adapter solution and 1 µl of 10 µM MspI solution, was added to each well. The plate containing a ligation solution was then placed in a thermocycler and run for 3 hours at 22˚C. The DNA fragments that were not ligated or digested were extracted from the solution using the QIAquick® PCR purification kit (QIAGEN, Hilden, Germany) and samples were then pooled into groups of eight. Quality checks on the purified DNA were performed using a Qubit fluorometer to ensure as a minimum of 100 ng of template DNA.

For PCR, 10 µl 5 Phusion Buffer, 1 µl 10 mM dNTPs, 1 µl 10 µM PstI Primer, 1 µl 10 µM MspI Primer, 1.5 µl DMSO, 1 µl Phusion Polymerase and 29 µl DNase free water were added to each 6 µl sample of DNA and placed in a thermocycler for 40 minutes. The PCR protocol included a digestion temperature of 98˚C for 30 seconds, run for one cycle. This was followed by 12 cycles at 98˚C, 62˚C, and 72˚C (each run for 30 seconds), then a final step at 72˚C for 10 minutes.

A 2100 Bioanalyzer (Agilent, Santa Clara, USA) was used to ensure the success of PCR amplification. Samples were then normalised, pooled into a single well, and size-selected to a range of 300–500 bp using a BluePippin (SageScience; Massachusetts, USA) DNA size selection system. This was followed by a final Bioanalyzer run to ensure the success of size selection and to calculate molarity. Sequencing (150 bp single-end) was performed at the University of Oregon Genomics and Cell Characterization Core Facility (https://gc3f.uoregon.edu) on an Illumina HiSeq 4000 (San Diego, USA).

2.8 DDRADSEQ DATA ANALYSIS AND FILTERING

2.8.1 FILTERING AND DATA ALIGNMENT

The program *ipyrad* v 0.7.11 (https://github.com/dereneaton/ipyrad*)* was used for sequence demultiplexing and filtering (Eaton et al. 2012; Eaton 2014). The recommended workflow, adapted from (Eaton 2014), was followed to attain aligned sequences for downstream analyses (Figure 2.2).

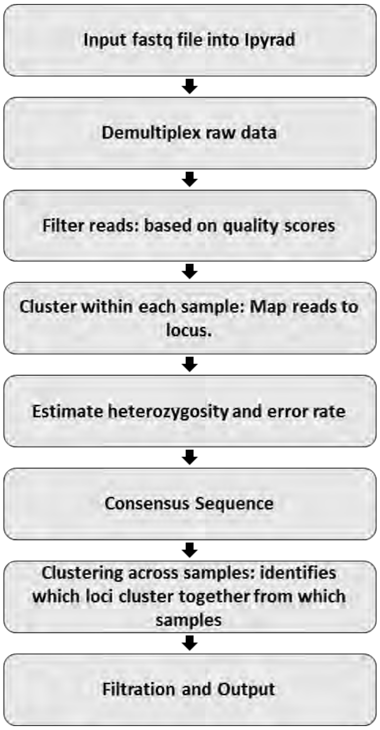

Figure 2.2 Overview of the *ipyrad* workflow used in this study, adapted from (Eaton 2014) and (Grewe et al. 2017).

The sequenced library was first demultiplexed using the Illumina barcodes. This was followed by filtering reads based on their quality scores (Phred Qscore offset parameter of 33) and removing Illumina adapters, barcodes, and cut sites (Eaton 2014). Once cleaned, the samples went through the first clustering process using the *de novo* clustering method. The sequences were matched based on sequence similarity and aligned using MUSCLE (Edgar 2004). Sequencing error rates and heterozygosity were calculated by setting the parameter “max_alleles_consens” to the default of 2. Consensus sequences were clustered again and aligned for a second time using MUSCLE. To reduce the amount of missing data, the “min_samples_locus” parameter was set to 30, meaning each locus must have a minimum of 30 samples to be retained for downstream steps. Other filtering parameters were kept to *ipyrad*’s default settings (https://ipyrad.readthedocs.io/en/latest/index.html). The filtered and aligned samples were then output for downstream analysis.

2.8.2 MISSING LOCI

Missing data is a prominent and inevitable bi-product of ddRADseq and all reduced representation sequencing approaches (Baird et al. 2008). The significant causes of unevenness in restriction sites between samples, leading to missing data, are often attributed to mutations in restriction recognition sites as well as low sequence coverage and the inability of bioinformatics pipelines to identify sites of homology fully (Eaton 2014).

How missing data should be handled is still under debate. Gautier et al. (2013) and Arnold et al. (2013) state that including missing data increases biases and inflates false positives, which are especially prevalent in long fragment sequence data (350 – 450 bp) as opposed to shorter fragments (150 – 250 bp). Opposing arguments, however, have shown that including missing loci can be beneficial, as these loci are often linked with highly mutated sites with the highest rates of polymorphism and allelic dropout (Huang and Knowles 2014; Chattopadhyay et al. 2014, and Eaton et al. 2017). It is also argued that genetic distance values such as F_{ST} are more reliable if missing data are retained within the dataset (Arnold et al. 2013). Based on the various opposing arguments, we decided to retain missing loci in the dataset.

When undertaking multivariate analyses, the dataset was treated by using the “mean” option to replace missing loci with the mean for that locus, as recommended by Jombart et al. (2009), using the “missingno” function in the R package *poppr* (Kamvar et al. 2014). Additional filtering processes were employed to remove uninformative and non-neutral loci (Figure 2.3). Unless otherwise noted, all data filtration and subsequent steps were performed in R v3.4.

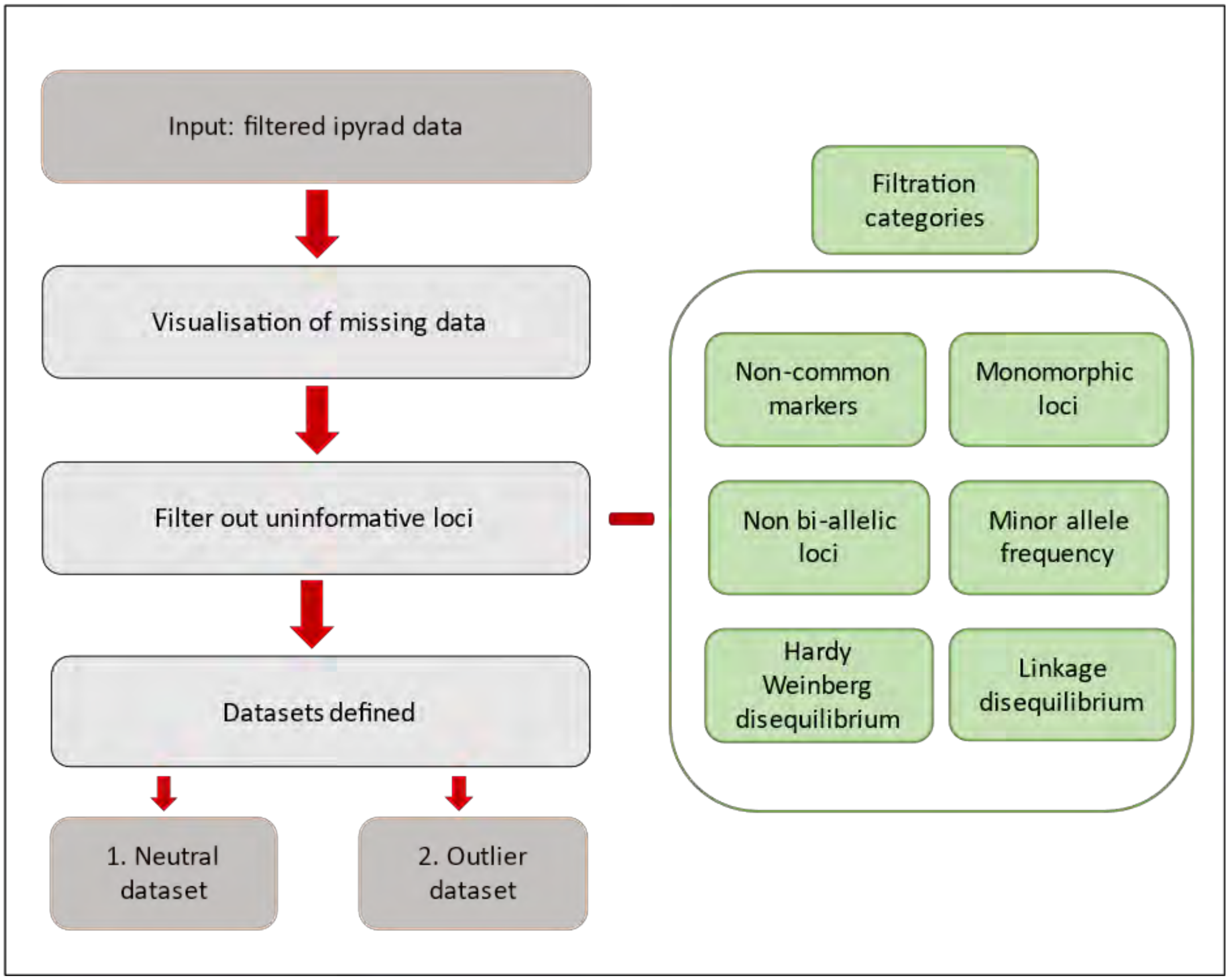

Figure 2.3. The workflow of downstream filtering and analyses of the dataset. The red minus sign and green boxes represent loci that were removed before statistical analyses. Black boxes indicate the steps that were taken for filtration, and the orange boxes indicate the input (filtered *ipyrad* data) and output datasets (neutral and outlier datasets).

2.8.3 MINOR ALLELE FREQUENCY

Minor allele frequency (MAF) refers to the rate at which the second-most common allele occurs within a population (Linck and Battey 2019). A minimum MAF of polymorphic loci was tested using the package *poppr* (Kamvar et al. 2014) using a minimum MAF of 5%. Loci that had a minimum MAF greater than 5% were removed from the dataset. This allowed SNPs to be filtered from rare allelic variants (Mullins 2017; DiBattista et al. 2017).

2.8.4 COMMON MARKERS

A filter was run using the "common.markers" function in the package *radiator* (Gosselin 2017) to retain only markers that were genotyped across more than one of the sampling sites.

2.8.5 NON-BI-ALLELIC LOCI

Monomorphic individuals only have one allele at a specific locus. Monomorphic loci are said to be uninformative and therefore create noise within the dataset (Kamvar et al. 2014). Monomorphic sites were removed using *poppr*, *dartR* (Gruber et al. 2018) and *radiator* (Gosselin 2017). Each program was used to identify the monomorphic sites, and all identified sites were removed. As most software packages have been designed only to handle bi-allelic loci, all multi-allelic loci were also removed using the R package a*degenet* (Jombart 2017a).

2.8.6 LOCI IN DISEQUILIBRIUM

2.8.6.1 *HARDY WEINBERG EQUILIBRIUM TESTS*

During this study, Hardy Weinberg equilibrium (HWE) tests were conducted in *PopGenReport* (Adamack and Gruber 2014) and each locus was tested for its departure from HWE. Samples with a significant departure ($\alpha > 0.05$) were removed from the dataset. After filtering, the dataset was further analysed using the *dartR* package (Gruber et al. 2018), which tests for loci in HWE while applying a Bonferroni correction to the dataset.

2.8.6.2 *LINKAGE DISEQUILIBRIUM*

Linkage disequilibrium (LD) refers to the non-independence of alleles; when alleles do not occur randomly in association with each other (Gaut and Long 2003). Tests of LD are performed to identify loci that are thought to be under selection (Hamblin et al. 2004).

To test for LD, the “gl.report.ld” function in the package *dartR* (Gruber et al. 2018) was used to generate an LD pairwise report. The output is a data frame giving test statistics of pairwise LD across all loci in the dataset. These statistics include:

i. *D*: a raw estimate of the difference between the observed and expected number of loci;
ii. *D*’: Lewontin’s *D*’ is the standardised value of *D*. When two SNPs are in complete linkage disequilibrium, the value of *D*’ is 1;
iii. r^2: a correlation coefficient between the markers spanning 0 – 1;
iv. *p*-values: estimate the significance of *D*’ and r^2 (Marroni et al. 2011)

Loci that had (i) significant *p*-values, (ii) were reported as duplicates, (iii) had a value of *D*’= 1, and (iv) had an r^2> 0.5 were considered in LD. These loci in disequilibrium were removed from the dataset.

2.8.7 OUTLIER TESTS

Outlier tests were performed to identify loci that were putatively under selection, which are generally identified by a significant difference in allele frequencies between populations (Luu et al. 2017). Two tests were implemented in R to identify outlier loci: *PCAdapt* (Luu et al. 2017) and *BayeScan* (Foll and Gaggiotti 2008). Outlier loci identified from both methods, regardless of overlap between methods, were separated from the neutral dataset.

2.8.7.1 *PCADAPT METHOD FOR OUTLIER DETECTION*

The assumption made by *PCAdapt* is that loci superfluously associated with population structure are under selection. The *PCAdapt* method has the advantage of being fast and able to handle large numbers of loci (Luu et al. 2017). The number of principal components (K-value) were chosen based on a scree plot of the eigenvalues of a covariance matrix. Choosing the K-value from the scree plot was based on Cattell’s rule that principal components corresponding to values up to the point where the line plateaus horizontally should be maintained (Cattell 1966; Ledesma et al. 2015). Catell’s rule assumes that the scree plot should plateau when eigenvalues are explaining random variation, as opposed to a deviation from the plateau, when population structure is observed (Cattell 1966; Ledesma et al. 2015). Once the K-value was chosen, the Mahlanobis distance (*D* test statistic) was calculated using multiple linear regression of the number of SNPs by the number of selected principal components (Martins et al. 2016; Luu et al. 2017). The Mahlanobis test statistic outputs a range of *p*-values that can be investigated visually using a Q-Q plot and a histogram. If the graph displays an excess of small *p*-values inconsistent with the general trend of the plot, this is indicative of the presence of outliers (Luu et al. 2017).

The disadvantage of using the Mahlanobis test static is that there is a tendency to experience genomic inflation in the *p*-values generated (Luu et al. 2017). Therefore, to account for this inflation and control false discovery rates, the *p*-values generated using the Mahalanobis distance *D* were transformed to q-values using the *q-value* package (Storey et al. 2020) and the cut-off point (α) was set to 10% (0.1) as recommended in the *PCAdapt* manual (Duforet-Frebourg and Blum 2014).

2.8.7.2 *BAYESCAN METHOD FOR OUTLIER DETECTION*

The *Bayescan* method measures allele frequencies between different populations and identifies loci that are perceived to be undergoing natural selection based on their F_{ST} values (Vitalis et al. 2001; Beaumont and Balding 2004). Historically, models assumed that if the observed F_{ST} value for a given locus is significantly different from the F_{ST} value expected from neutral theory, it is likely that locus is an outlier (Foll and Gaggiotti 2008). *Bayescan* uses two models: one includes the effects of selection, whereas the other excludes the effects of selection (Foll and Gaggiotti 2008). The settings used in *Bayescan* for the model parameters were chosen based on the recommended settings in the manual (Foll 2012; Table 2.3). For the Reversible-jump Markov chain Monte Carlo (rjMCMC) settings, the posterior probability at each locus for each of the models was calculated by setting 20 pilot runs of 5,000 iterations. The rjMCMC iterations were run 50,000 times (thinning intervals of 10 and 5,000 and an output iteration of 100,000) with a burn-in value of 50,000.

Table 2.3. Parameters used in *BayeScan* as recommended by Foll and Gaggiotti (2008).

Parameter	**Value**
Iterations (-n)	5,000
Thinning interval (-thin)	10
Number of pilot runs (- nbp)	20
Length of pilot runs (-pilot)	5,000
Burn in length (-burn)	50,000
Prior odds (-pr_odds)	100

2.8.8 DEFINING DATASETS

Two final datasets, outlier loci and neutral loci, were used in downstream analyses. The neutral dataset was expected only to be influenced by neutral processes, whereas the outlier loci were expected to be influenced by non-neutral processes.

2.9 DDRADSEQ STATISTICAL ANALYSES

The aims of the ddRADseq analyses were to address (i) genetic diversity, (ii) population structure, (iii) population assignment, and (iv) spatial influences of genetic patterns between and within geographically separated sites.

2.9.1 GENETIC DIVERSITY

Genetic diversity indices were calculated for each locus using an array of packages in R. Indices including allelic richness (*Ar*), inbreeding coefficient (F_{IS}), gene diversity (*H*), total number of alleles, and number of private alleles were calculated using packages *PopGenReport* (Adamack and Gruber 2014), *dartR* (Gruber et al. 2018), and *poppr* (Kamvar et al. 2014). The observed and expected heterozygosities and the Bartlett test and paired t-test of significance were performed using *hierfstat* (Goudet 2005).

2.9.2 POPULATION STRUCTURE

2.9.2.1 *PAIRWISE* F_{ST}

Pairwise F_{ST} calculates the unbiased F_{ST} between two putative populations to compare similarity in allele frequencies between them (Weir and Hill 2002; Section 2.6.3). The pairwise F_{ST} function in *hierfstat* (Goudet 2005) was used to calculate Nei's pairwise F_{ST} between the island groups. The statistic was calculated with 9,999 permutations to test for significance.

2.9.2.2 *Analysis of Molecular Variance (AMOVA)*

An analysis of molecular variance (AMOVA) was conducted on the SNP dataset, following the same approach as the cyt-b dataset (Section 2.6.1) The AMOVA tested the allele frequencies at different stipulated hierarchical levels (between and within island groups) to test three hypotheses based on the sampling sites and pairwise F_{ST} results (Table 2.4).

Table 2.4. Hypotheses for population structure between island groups based on geographic distribution and F_{ST} tests. Each hypothesis was tested using a three-level AMOVA.

	Putative population grouping I	Putative population grouping II
Hypothesis 1: between sampling sites	ADG, ALP, SB, FG	ADG, ALP, SB, FG
Hypothesis 2: based on pairwise F_{ST} values	ADG	ALP, SB, FG
Hypothesis 3: based on pairwise F_{ST} values	FG, SB	ADG, ALP

2.9.2.3 *Principal component analysis*

A principal component analysis (PCA) refers to the Eigen analysis of the dispersion matrix by transforming possibly related variables into a new set of linearly uncorrelated variables (Rao 1964; Janžekovič and Novak 2012). The most beneficial aspect of PCA is that it takes a large dataset (such as SNPs) and condenses it into a few dimensions (principal components), which account for variability within the dataset. The first principal component (PC) explains the most significant amount of variance, the second PC explains the second-largest amount, and the successive PCs encompass the remaining variance (Lever et al. 2017). A PCA biplot is a scatterplot of selected PCs. It reflects variation within the dataset and clusters data points based on similarity. In this study, a PCA biplot was used as a clustering method based on variation in allele distributions without pre-assigning populations (Reich et al. 2008). PCA was performed using the R packages *adegenet* (Jombart 2017a) and *ade4* (Dray et al. 2007).

2.9.2.4 *DISCRIMINANT ANALYSIS OF PRINCIPAL COMPONENTS (DAPC)*

Discriminant analysis of principal components (DAPC) is a combination of discriminant analysis and PCA (Jombart et al. 2010). This multivariate method can be used to infer population structure using cluster analysis by optimising the variance between groups while minimising variance within groups. The "find.clusters" function in the package *adegenet* was used to choose the number of optimal clusters (Jombart 2008; Jombart 2012a). In this study, *K* clusters 1–4 were tested, and only the models with the optimal clusters are presented. The optimal cluster was verified using the lowest value of the Bayesian Information Criterion.

The validation step of DAPC divides the data into a training set (containing >90% of the data) and a validation set (containing the remaining data). The objective is that random samples are picked out of the validation set so there is a good representation of the sampling groups or populations in each of the two datasets. The number of PCs retained is based on the degree to which the DAPC accurately predicted group membership of the individuals in the validation set. The "xvaldapc" function in a*degenet* was used to validate the number of PCs to be retained for the DAPC analysis. This was first performed on a large number of PCs (300) with a training set of 90. Based on that output, a second "xvaldapc" analysis was run with a reduced number of PCs, with 999 permutations. This cross-validation technique was carried out for each of the chosen *K* values before running the DAPC analysis.

2.9.3 POPULATION ASSIGNMENT

Sparse non-negative matrix factorisation (sNMF) in the R package *LEA* (Frichot and Francois 2015) explores population structure on large datasets (Frichot et al. 2014; Frichot and François 2015). Using the least-squares optimisation algorithm (Engelhadt and Stephens 2010), the function estimates the number of ancestral populations and the probability of the number of gene pools from which each individual originated by calculating an ancestry coefficient and investigating the model's fit through cross-entropy criterion (Shryock et al. 2017). To estimate the number of ancestral populations, a range of *K* values between 2–5 were run with 30 repeats and 99,999 iteration steps per replicate, generating an assignment bar chart of lowest entropy scores for each individual (Weigand et al. 2017; Weiss et al. 2018). The sNMF analyses were conducted for both outlier and neutral datasets.

2.9.4 SPATIAL ANALYSES

2.9.4.1 *ISOLATION BY DISTANCE*

Isolation by distance (IBD) refers to the restriction of an individual's capacity to disperse over space, a well-known pattern described by Wright (1942). A Mantel test is the comparative statistical test performed between pairwise genetic distances and spatial distances (Mantel 1967; Excoffier and Lischer 2010). Tests for IBD were performed using the *adegenet* and *dartR* packages in R. The tests were performed to compare IBD trends between island groups and between individuals using the Slatkin's pairwise F_{ST} and Euclidian geographic distance. To test for significance between the genetic and geographic distances between island groups and individuals, a Mantel test was performed using 99,999 permutations. Despite the Mantel test being a reliable indicator for the presence and absence of IBD and spatial patterns, it did not show individuals within the dataset responsible for the patterns. To investigate this further, a spatial principal component analysis was computed for both datasets.

2.9.4.2 *SPATIAL PRINCIPAL COMPONENT ANALYSIS*

Spatial principal components analysis (sPCA) complements classic PCA whilst being more sensitive to otherwise overlooked spatial genetic patterns, but differs in two main aspects (Jombart et al. 2008; Jombart 2012b). Firstly, sPCA uses the product of the variance and Moran's *I* (a measure of spatial autocorrelation) to divide positive, negative and null components (Jombart et al. 2008; Jombart 2012b). On the other hand, PCA decomposes the variance into decreasing components. Secondly, sPCA keeps all the eigenvalues, dividing them into global or local scores whereas PCA makes use of the highest eigenvalue scores (Jombart et al. 2008 Jombart 2012b; Whitney et al. 2012; Basto et al. 2016; Jombart 2017). sPCA, unlike PCA, investigates genetic variability without the ability to show cryptic spatial patterns which Jombart et al. (2008b) describes as "spatial patterns that are not associated to the highest genetic variation."

sPCA was run in R v.3.5.0 (R Development Core Team, 2013) and made use of the *adegenet* (Jombart et al. 2008, Jombart 2012b), *ade4* (Dray and Dufour 2007) and *spdep* (Bivand 2007) packages. To run the sPCA, all the spatial co-ordinates of the individual samples were randomly shifted (jittered) by a factor of 10, as recommended by Jombart et al. (2008b). I used the Delaunay triangulation network because it can deal with irregular spread of points (for details see: Jombart et al. 2008; Whitney et al. 2012; Basto et al. 2016; Jombart 2017). Running sPCA requires genetic data in the form of allele frequencies and Moran's *I* as a measure of spatial structure. To choose whether there were any spatial trends to be inferred, the genetic variance of allele frequencies was plotted against Moran's I (Supplementary Figure 6.4). This produced a screeplot which is visually used to decipher whether there is spatial structure. In addition, a Monte-Carlo test with 100,000 iterations was performed using the spatial weightings and genetic data to statistically test the presence of local and global scores. Once I observed the presence of global and local artefacts using the generated scree plot and eigenvalue graph (Supplementary Figure 6.4). I used these to determine which eigenvalues would be most appropriate (Jombart 2012b). The eigenvectors for each of the eigenvalue scores were plotted on a map corresponding to the appropriate individual sample (co-ordinate).

Within a biological context, the sPCA scores that are generated and mapped are used to interpret whether they get categorised as local or global scores. Local scores refer to genetic differentiation between two individuals and occur when individuals either attract or repulse each other (e.g., via mating systems or spatially influenced factors). The global scores differentiate between two spatially distributed groups or find a cline (Huxley 1938).

3 CHAPTER 3: RESULTS

3.1 MITOCHONDRIAL DNA RESULTS

3.1.1 GENETIC SPECIES IDENTIFICATION

Following trimming and editing in the software, Geneious a 517 base pair (bp) region of cyt-b was recovered for 153 individuals from 11 islands within the Seychelles Archipelago and Saint Brandon's Atoll (Figure 3.1). Samples were assigned to one of five island groups based on their collection location (Figure 3.1). Two genetically distinct species were identified: *Albula glossodonta* and *Albula oligolepis* (Figure 3.1). *Albula glossodonta* occurred throughout the entire sampled region; 143 individuals were identified with 99.6 – 100% pairwise identity match using the NCBI GenBank reference library. *Albula oligolepis*, however, was only identified from Mahé within the Inner Island Group (IIG), with a 99.2 – 100% pairwise identity match to eight *A. oligolepis* samples previously collected from the Indo-Pacific. Due to low sample size and the occurrence only on Mahé, samples identified as *A. oligolepis* (N = 7) were excluded from downstream analyses. The single *A. glossodonta* sample collected from the Inner Island Group (IIG) was also omitted from all analyses due to low sample size from this site. Therefore, all analyses were conducted using samples from four of the island groups: Saint Brandon Atoll (SB), Farquhar Island Group (FG), Alphonse and Amirantes Island Group (ALP) and Aldabra Island Group (ADG).

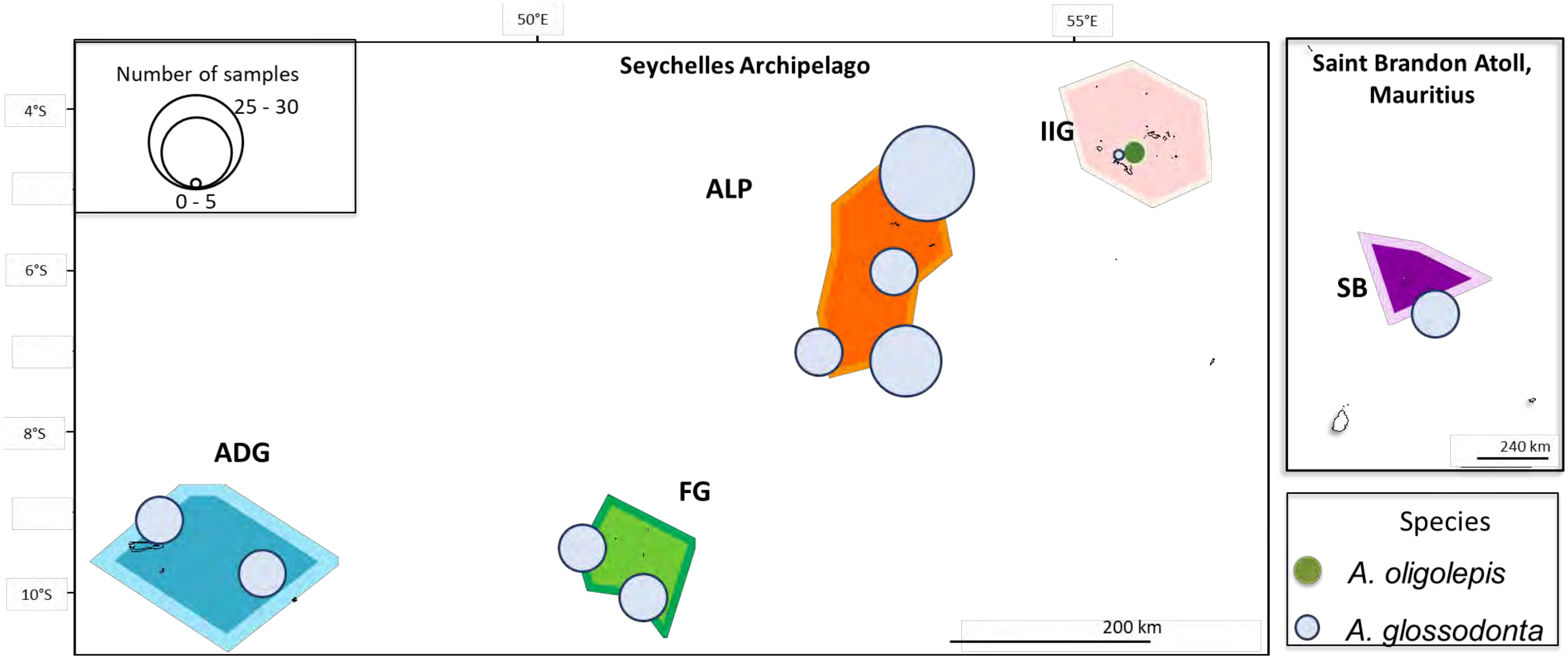

Figure 3.1. Collection sites of genetically identified *Albula* species throughout the Seychelles Archipelago (IIG = Inner Island Group, ALP = Alphonse and Amirantes Group, FG = Farquhar Group, ADG = Aldabra Group) and Mauritian atoll (SB = Saint Brandon Atoll). *Albula glossodonta* is represented as red circles, and *Albula oligolepis* is represented as green circles. The circle sizes are indicative of the number of samples per bonefish species.

3.1.2 GENETIC DIVERSITY AND HAPLOTYPE MAP

The haplotype map indicated no evidence of geographic structuring. One haplotype (Hap1) was shared among all sampling sites, while private haplotypes were observed within the FG and ALP groups (Figure 3.2). The highest nucleotide diversity (π) was observed in the FG group (Table 3.1). In general, however, overall nucleotide diversity was low (Table 3.1). Saint Brandon (SB) shared only one haplotype with the other island groups; therefore no genetic diversity indices could be generated for this site. Overall, genetic diversity indices (excluding SB) suggested low haplotype numbers (H = 7), low diversity (*h* = 0.1375) and few polymorphic sites (S = 8). The average number of nucleotide pairwise differences (k) was 5.97. The highest number of polymorphic sites was seen within the ALP group, which also had the highest number of haplotypes (H = 6), private haplotypes (P = 3) and pairwise nucleotide differences (*k* = 0.24). The FG group had the second-highest number of haplotypes (H = 3), but the highest haplotype diversity (*h* = 0.23), nucleotide diversity (π = 0.0005) and pairwise nucleotide differences (*k* = 0.24). The Aldabra Group (ADG) had two haplotypes and no private haplotypes. It also had low haplotype and nucleotide diversity (*h* = 0.083, π = 0.0002, respectively) compared to the other island groups.

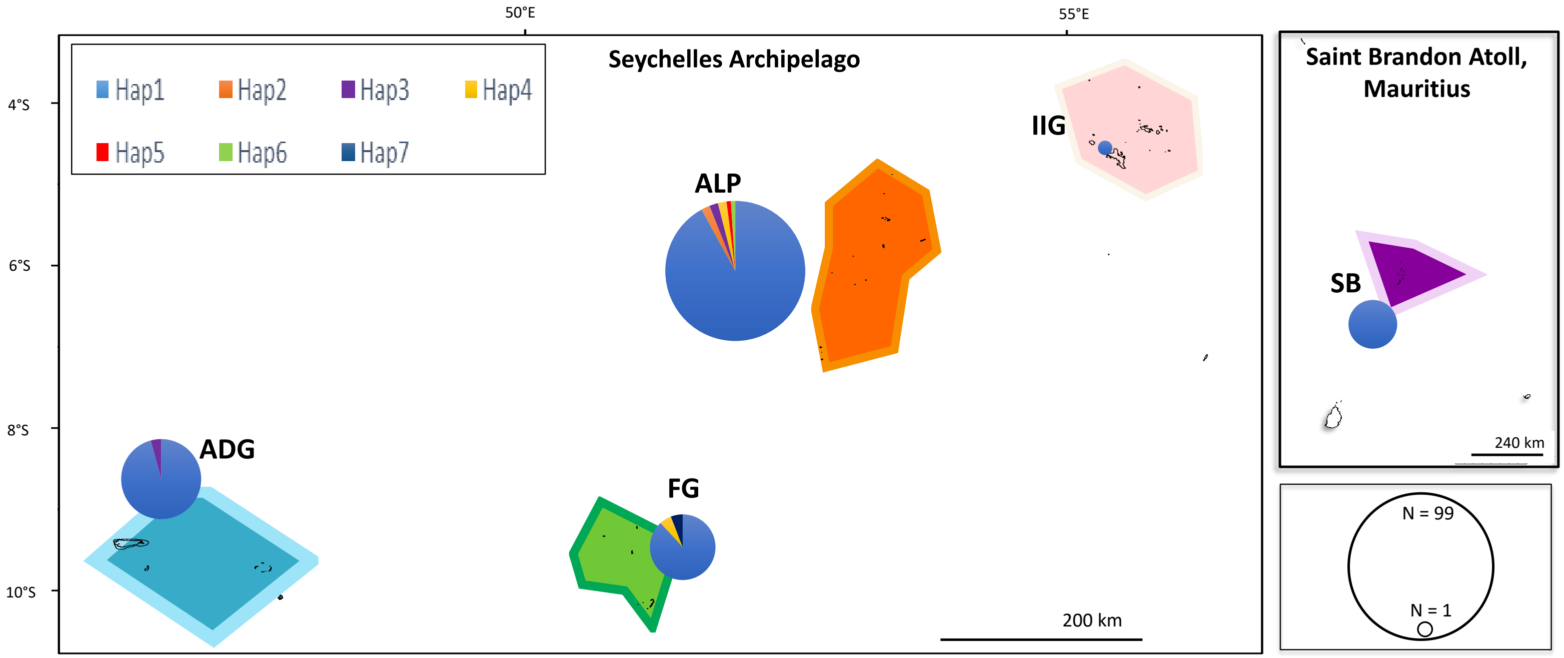

Figure 3.2. Cytochrome-b haplotype map for *Albula glossodonta*. The pie charts are sized according to sample size, and each haplotype is characterised as a unique colour. Island groups are abbreviated as in Figure 2.1 and Table 2.1

Table 3.1. Genetic diversity indices for 153 individuals sequenced for cyt-b. Displayed are the number of samples from each location (N), number of polymorphic sites (S), number of haplotypes (H), number of private haplotypes (P), haplotype diversity (*h*), nucleotide diversity (π) and the average number of pairwise nucleotide differences (*k*).

Island group	N	S	H	P	*h*	π	*k*
FG	17	2	3	1	0.2279	0.0005	0.2353
ADG	24	1	2	0	0.0833	0.0002	0.0833
ALP	99	7	6	3	0.1552	0.0005	0.2404
SB	14	0	1	0	0.0000	0.0000	0.0000
Total	153	8	7	4	0.1376	0.0005	0.1933

3.1.3 NEUTRALITY TESTS AND DEMOGRAPHIC HISTORY

3.1.3.1 *NEUTRALITY TESTS AND DEMOGRAPHIC INFERENCE*

Selective neutrality tests were performed on each island group, as well as the entire pool of samples representing one population (Table 3.2). The SB group had no polymorphic or segregating sites and tests on this group could not be conducted. However, SB samples were still included with the pooled group. The ALP group showed significant departure from neutrality for all tests except Fu and Li's *D* and *F** (Table 3.2). The FG group showed significant departure from neutrality with the Tajima's *D* and Fu's F_S tests (Table 3.2). For the pooled data, only the R_2 test indicated a departure from neutrality ($R_2 = 0.020$, $p = 0.001$).

Table 3.2. Selective neutrality tests for spatially grouped samples conducted on the cyt-b dataset. Significance at α = 0.05 is indicated with bold formatting (ns = non-significant).

	Tajima's *D*		Fu's Fs		Fu and Li's D		Fu and Li's F*		R_2	
Island group	D	*p*	Fs	*p*	D	*p*	F*	*p*	R_2	*p*
FG	-1.503	0.047	-1.680	0.015	-1.953	ns	-2.097	ns	0.161	ns
ADG	-1.931	0.002	-5.294	0.002	-0.551	ns	-1.202	ns	0.025	0.001
ALP	-1.159	ns	-1.028	ns	-1.606	ns	-1.704	ns	0.200	ns
Sites pooled	-1.972	ns	-7.715	ns	-1.340	ns	-1.858	ns	0.020	0.001

The mismatch distribution indicated a stable population (Figure 3.3). Harpending's raggedness index was relatively high (Hri = 0.580) but non-significant ($p = 0.425$), indicating a good fit of the observed data to the expected model of a stable population. The pooled samples showed a haplotype diversity < 0.5 ($h = 0.138$) and a nucleotide diversity < 0.005 ($\pi = 0.00037$), which is indicative of a recent bottleneck event.

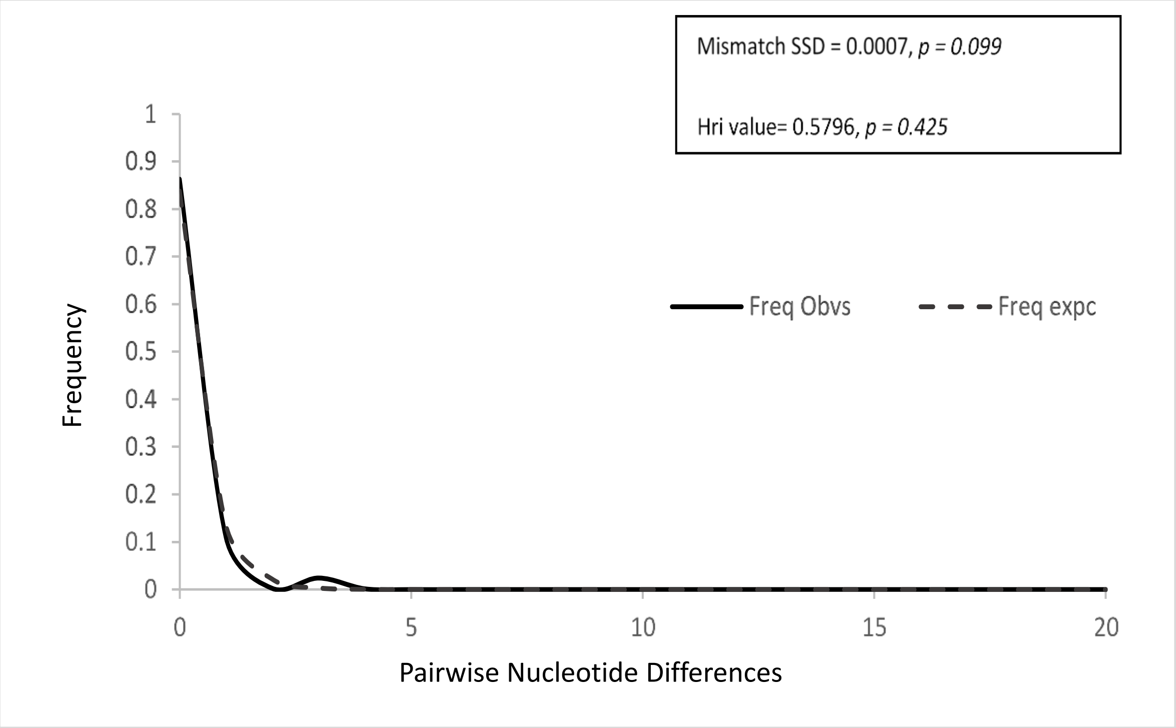

Figure 3.3. Mismatch distribution of observed and expected pairwise nucleotide differences between haplotypes.

3.1.4 POPULATION STRUCTURE

3.1.4.1 *ANALYSIS OF MOLECULAR VARIANCE (AMOVA)*

The AMOVA test showed that none of the fixation indices differed significantly from the null distribution, indicating no structure between or within islands and island groups. The highest variation was seen within samples (101.64%), whereas variation between (-0.99%) and within (-0.65%) island groups was low (Table 3.3).

Table 3.3. Results of AMOVA analysis indicating genetic variance between the four island groups (FG, SB, ALP, ADG), islands within the four groups, and all individuals. The inner Island group was excluded due to low sample size.

Source of Variation	Degrees of freedom	Sum of squares	Variance components	Percentage of variation	Fixation indices	*p*-value
Between groups	3	0.137	-0.0007	-0.99	F_{CT}: -0.0065	0.5836
Within groups (islands)	7	0.447	-0.0005	-0.65	F_{SC}: -0.0164	0.5357
Within samples	142	9.939	0.0700	101.64	F_{ST}: -0.0099	0.7996

3.1.4.2 *POPULATION DIFFERENTIATION*

Pairwise tests of exact differentiation among haplotypes conducted between island groups showed that differentiation among haplotypes was not significant (Table 3.4).

Table 3.4. Significance values (*p*-values) for pairwise exact tests of genetic differentiation.

	FG	ALP	ADG
ALP	0.3582		
ADG	0.3706	0.8783	
SB	1.0000	1.0000	1.0000

3.1.4.3 *PAIRWISE F_{ST}*

Pairwise F_{ST} values between island groups ranged from -0.0245 – 0.0003, but none were significantly estimated at $\alpha = 0.05$. The highest pairwise F_{ST} values were observed between the FG and ADG groups (Table 3.5).

Table 3.5. Pairwise F_{ST} values for the island groups (ADG, FG, ALP and SB). The lower triangle represents the pairwise F_{ST} values, whereas the upper triangle indicates the *p*-values.

	ADG	FG	ALP	SB
ADG		0.4920	0.8950	0.9900
FG	0.0003		0.5650	0.5630
ALP	-0.0153	-0.0129		0.5880
SB	-0.0245	-0.0171	-0.0116	

3.2 DOUBLE DIGEST RESTRICTION SITE ASSOCIATED SEQUENCING (DDRADSEQ)

3.2.1 DDRADSEQ DATA ANALYSIS AND FILTERING

3.2.1.1 *FILTERING*

An average of 280,631 loci per individual were generated, which, after filtering in *ipyrad*, were reduced to an average of 167,500 loci per individual. We used the *ipyrad* output dataset of unlinked SNPs, for which 109,200 SNPs were recovered. Following filtering and alignment, a total of 75 individuals were retained for analysis. For this study, loci that had more than 40% missing data were removed (Huang and Knowles 2014; Grewe et al. 2017; Lewis et al. 2017). The mean number of missing loci in the dataset was 24.89%. After filtering, the final datasets consisted of 9,180 neutral and 49 outlier loci (Figure 3.4).

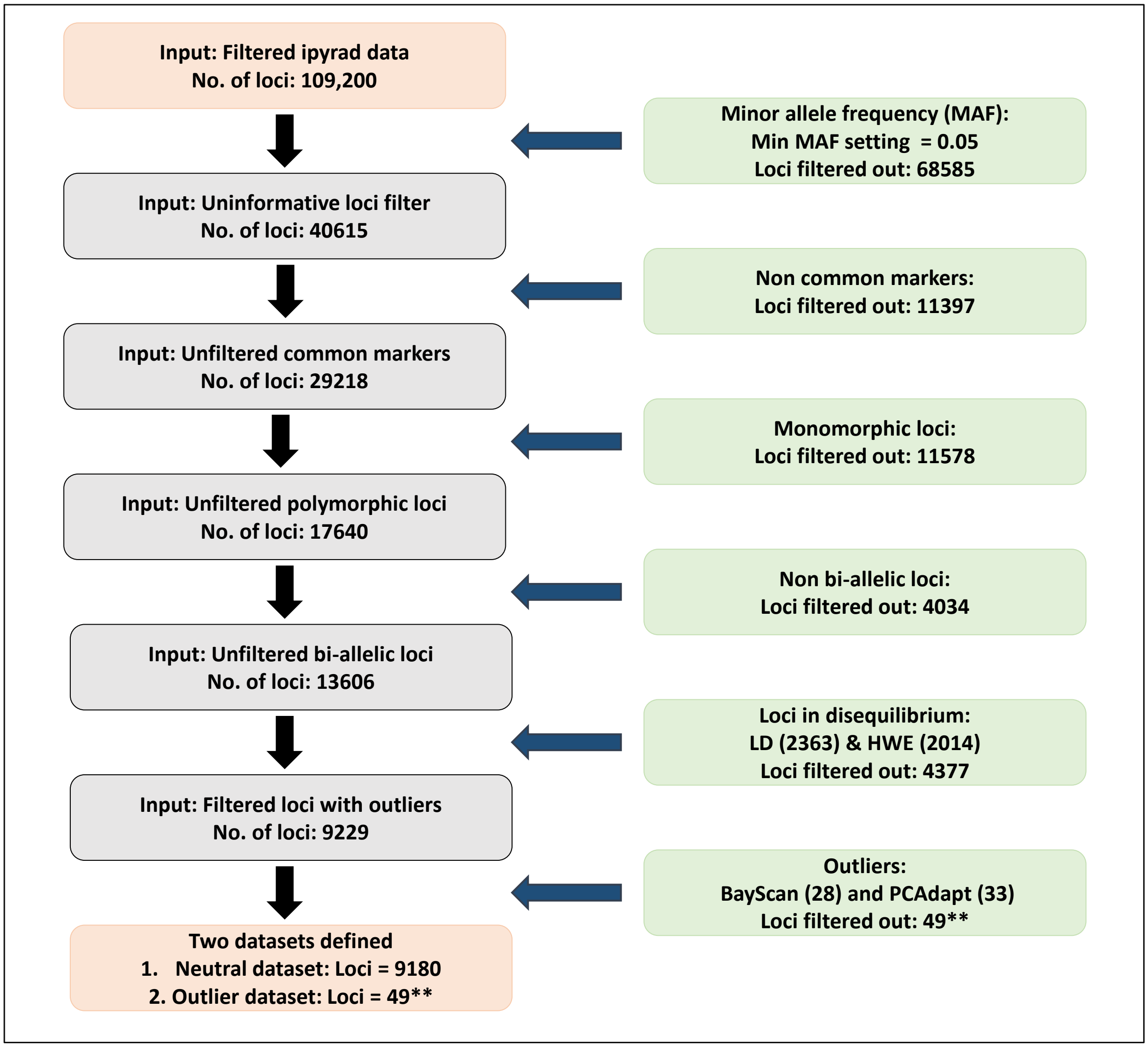

Figure 3.4. Summary of locus filtering steps. Orange boxes show the input and output data; grey boxes indicate loci remaining after each filtering step and green boxes show the types of filters and number of loci removed. ** 61 loci were identified as outliers; however, an additional 12 loci in linkage disequilibrium were removed

3.2.2 OUTLIER TESTS

The *PCAdapt* outliers were identified using a value of $K = 2$, chosen based on visualisation of the scree plot (Supplementary Figure 6.1) and an excess of *p*-values with a peak distribution around 0. BayeScan outlier loci were all under positive selection ($\alpha < 0.0$; outlier loci *p-values*) whereas *PCAdapt* indicated a mixture of diversifying (12 loci) and balancing (14 loci) selection. None of the identified loci overlapped between the two outlier detection methods.

3.2.3 GENETIC DIVERSITY

3.2.3.1 *NEUTRAL DATASET*

The overall observed heterozygosity (H_O) was 0.273 and the overall expected heterozygosity (H_E) was 0.2925. The paired t-test indicated H_E was significantly different from H_O (t = 25.45, df = 9179, $p < 0.0001$). The Bartlett's k-squared test of homogeneity of variances also showed significant differences between H_O and H_E (k-squared = 24.184, df=1, $p < 0.0001$). The calculated H_E of individual populations ranged from 0.267 – 0.280 and was higher than the observed H_O in ADG and ALP, whereas H_O was greater than H_E in FG and SB (Table 3.6).

The ADG group exhibited the lowest genetic diversity across all indices (Table 3.6). The FG and SB groups displayed the highest genetic diversity, relative to the other groups. Private alleles and allelic richness were highest in the FG group and lowest in the ADG group (Table 3.6). Generally, an observed H_O lower than H_E is associated with inbreeding whereas an H_E value lower than H_O suggests mixing of two previously isolated populations. To test this, Wright's F_{IS} was calculated for each island group. All F_{IS} estimates rendered low values (-0.022 – 0.021) with only the ADG group yielding significant results (F_{IS} = 0.021, CI =-0.05 – 0.002).

3.2.3.2 *OUTLIER DATASET*

The overall observed heterozygosity (H_O) was 0.170 and the overall expected heterozygosity (H_E) was 0.204. A pairwise t-test showed significant differences between H_O and H_E (t(48) = 4.564, p = 1.750e^{-05}). The Bartlett's k-squared test of homogeneity of variances was not significant (k-squared (1) = 0.951, p = 0.329). The H_E and H_O values were similar across all the island groups except for ADG, where H_O was lower than H_E, suggestive of inbreeding (Table 3.6) The F_{IS} values ranged from -0.012 – 0.197 but were only significant for the FG group (Table 3.6) The dataset showed a higher number of private alleles (22) and allelic richness within the SB group (Table 3.6)

Table 3.6. Genetic diversity summary indices for neutral and outlier datasets, averaged for each island group, including the number of samples (N), number of private alleles, allelic richness (AR), observed heterozygosity (H_O), expected heterozygosity (H_E) and Wright's inbreeding coefficient (F_{IS}) with 95% confidence intervals.

		Neutral Dataset					Outlier Dataset				
Island group	N	Private Alleles	AR	H_O	H_E	F_{IS}	Private Alleles	AR	H_O	H_E	F_{IS}
ADG	18	32	1.761	0.254	0.267	0.021 (CI = -0.057 - 0.002)	0	1.610	0.141	0.1770	0.116 (CI = -0.085 – 0.190)
FG	17	102	1.885	0.286	0.283	-0.022 (CI = -0.074 - -0.035)	2 (2.5%)	1.565	0.173	0.173	0.171 (CI = -0.128 – 0.008
ALP	22	58	1.847	0.273	0.277	0.002 (CI = -0.055 - -0.011)	4 (4.65%)	1.659	0.179	0.179	-0.012 (CI = -0.063 – 0.233)
SB	18	77	1.889	0.279	0.279	-0.003 (CI = -0.052--0.014)	22 (24.17%)	1.723	0.174	0.174	0.197 (CI = 0.012 – 0.208)

3.2.4 POPULATION STRUCTURE

3.2.4.1 *PAIRWISE* F_{ST}

Pairwise F_{ST} values indicated weak structuring between the four island groups for the neutral dataset (Table 3.7). The highest pairwise F_{ST} value was observed between FG and ADG (F_{ST} = 0.0442; P = 0.001), while the lowest was observed between FG and SB (F_{ST} = 0.023; P = 0.038).

Table 3.7. Pairwise F_{ST} estimates between sampling sites for the neutral SNP dataset. Pairwise F_{ST} values are below the diagonal, and corresponding *p*-values are above the diagonal. Bold numbers represent significant tests ($p < 0.05$).

	ADG	ALP	FG	SB
ADG		0.009	0.001	0.001
ALP	**0.024**		0.009	0.024
FG	**0.044**	**0.025**		0.038
SB	**0.040**	**0.023**	**0.023**	

Outlier Pairwise F_{ST} values indicated an overall trend of weak structuring between the four island groups for the neutral dataset (Table 3.8). However, moderate differentiation was seen between the FG and SB groups (F_{ST} = 0.073; *p* = 0.017), SB and ADG groups (F_{ST} = 0.059; *p* = 0.001) and ADG and SB groups (F_{ST} = 0.058; *p* = 0.001). In addition, there was low genetic differentiation between all island group comparisons made with ALP.

Table 3.8. Pairwise F_{ST} estimates between sampling sites for the outlier SNP dataset. Pairwise F_{ST} values are displayed below the diagonal, and pairwise F_{ST} *p*-values are above the diagonal. Bold numbers represent significant estimates of F_{ST} ($p < 0.05$).

	ADG	ALP	FG	SB
ADG		0.090	0.001	0.001
ALP	0.030		0.040	0.001
FG	**0.058**	0.005		0.017
SB	**0.059**	**0.037**	**0.073**	

3.2.4.2 *Analysis of Molecular Variance (AMOVA)*

The AMOVA tests on the neutral dataset indicated no genetic differentiation between island groups or between islands (Table 2.4). Significant variation, however, was observed between samples within island groups (103.64%; Table 3.9). AMOVA results from the outlier dataset indicated little differentiation between islands within groups, whereas comparisons between island groups (12.50%) and between individuals (92.20%) were both high and significant.

Table 3.9. AMOVA tests of genetic differentiation displaying the degrees of freedom (d.f.) the mean sum of squares (μSS), the percentage of variance (% var), the Phi statistic (φ), as well as the *p*-value associated with each test of genetic differentiation between island groups, within island groups, and between individuals within an island. Bold indicates significant *p-values* (<0.05).

		Neutral dataset				Outlier dataset			
Test	DF	μ_{SS}	% var	φ	*p-value*	μ_{SS}	% var	φ	*p*-value
Between island groups	3	3384	4.100	0.041	1.000	32.120	12.500	0.126	**0.007**
Between islands within island groups	6	3921	-7.160	-0.075	1.000	14.940	-4.794	-0.055	0.944
Between samples within islands	65	70443	103.060	-0.031	**0.044**	230.180	92.200	0.123	**0.014**
Total	75	77749	100	-	-	277.24	100	-	-

3.2.4.3 PRINCIPAL COMPONENT ANALYSIS

In the neutral dataset, a visualisation of the first two principal components indicated many individuals from the SB group clustering separately from the Seychelles island groups (ADG, ALP and FG) along PC 1 and PC2 (Figure 3.5A), although PC1 and PC2 accounted for just 2.0% and 1.9% of the variance, respectively. In the outlier dataset, 25.5% of the variance was accounted for by the first three principal components. The FG and SB groups both formed distinct clusters from ALP and ADG along PC1 and PC2 (Figure 3.5B), accounting for 12.0% and 7.2% of the variance, respectively.

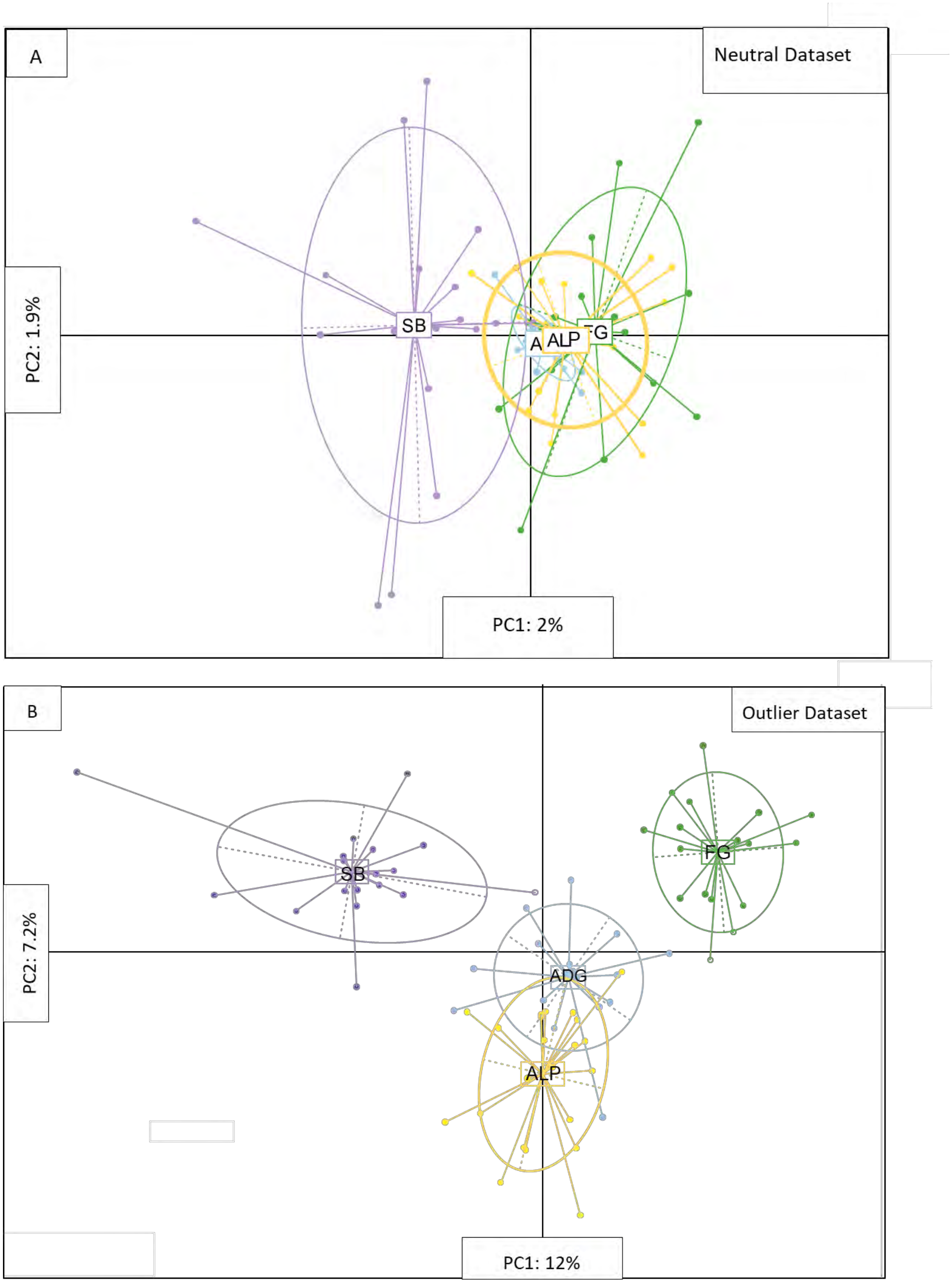

Figure 3.5 . PCA biplots of the A) neutral and B) outlier datasets showing variance along PC1 and PC2. Each colour corresponds to a different island group (green = ALP, blue = ADG, purple = SB, red = FG), and each point represents a different individual.

3.2.4.4 *Discriminant analyses of principal components (DAPC)*

Discriminant analyses of principal components with and without pre-defined populations were run. For the neutral dataset, a cluster size of *K* = 2 was chosen based on Cattell's rule and Bayesian Information Criteria (Cattelle 1966; BIC; Supplementary Figure 6.3). Ten PCs were retained, which gave a good fit of the DAPC model with a mean successful assignment (MSA) of 0.926 and an error rate (MSE) of 0.181 (Supplementary Table 6.1), meaning that with the use of 10 PCs the data could be assigned to two clusters with a successful model assignment of 92.6% and an error rate of 18.1%. The SB group clustered separately from the other sites on the first discriminant function, similar to the trend seen in the PCA analysis. Both *K* = 2 and *K* = 3 clusters were highly supported by BIC for the outlier dataset. The analysis in support of *K* = 2 clusters had an MSA of 0.98 and an MSE of 0.09, whereas the analysis *of K* = 3 clusters had an MSA of 0.97 and MSE of 0.08 (Supplementary Table 6.2; Supplementary Table 6.3). Although only two clusters were supported, the outlier dataset showed both FG and SB island groups clustering separately from ADG and ALP (Figure 3.6).

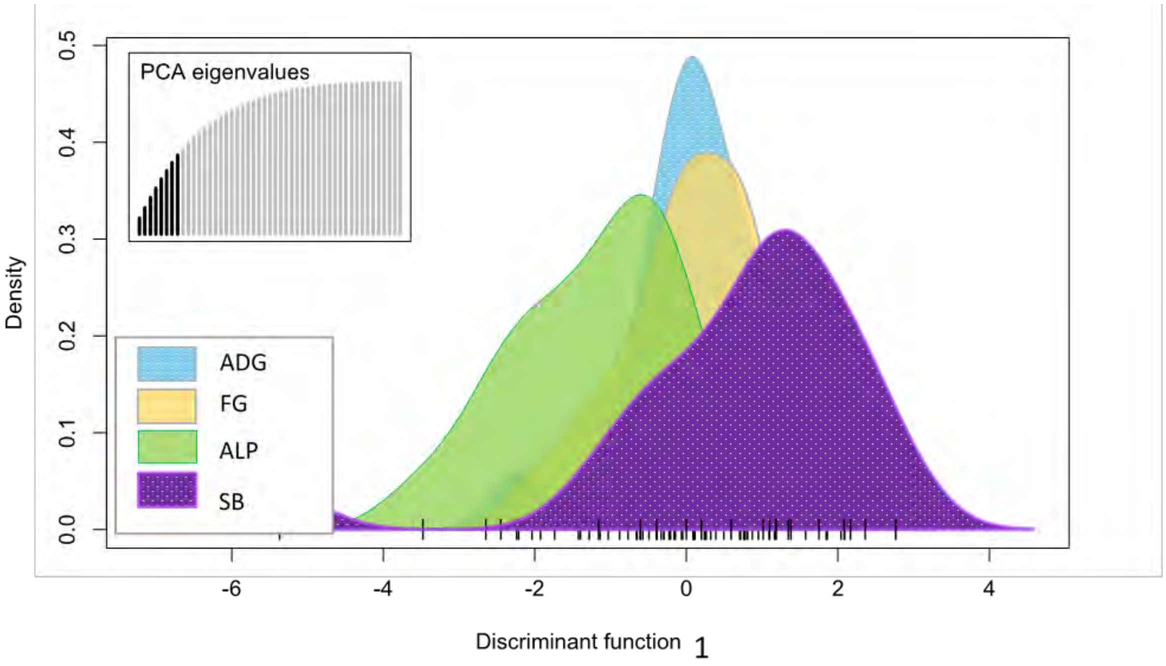

Figure 3.6 DAPC exploring the best fitting number of population clusters (*K* = 2) in the neutral dataset. The graph shows the plot densities of individuals from different island groups along the first retained discriminant function 1. The shaded areas show individual densities for each of the island groups.

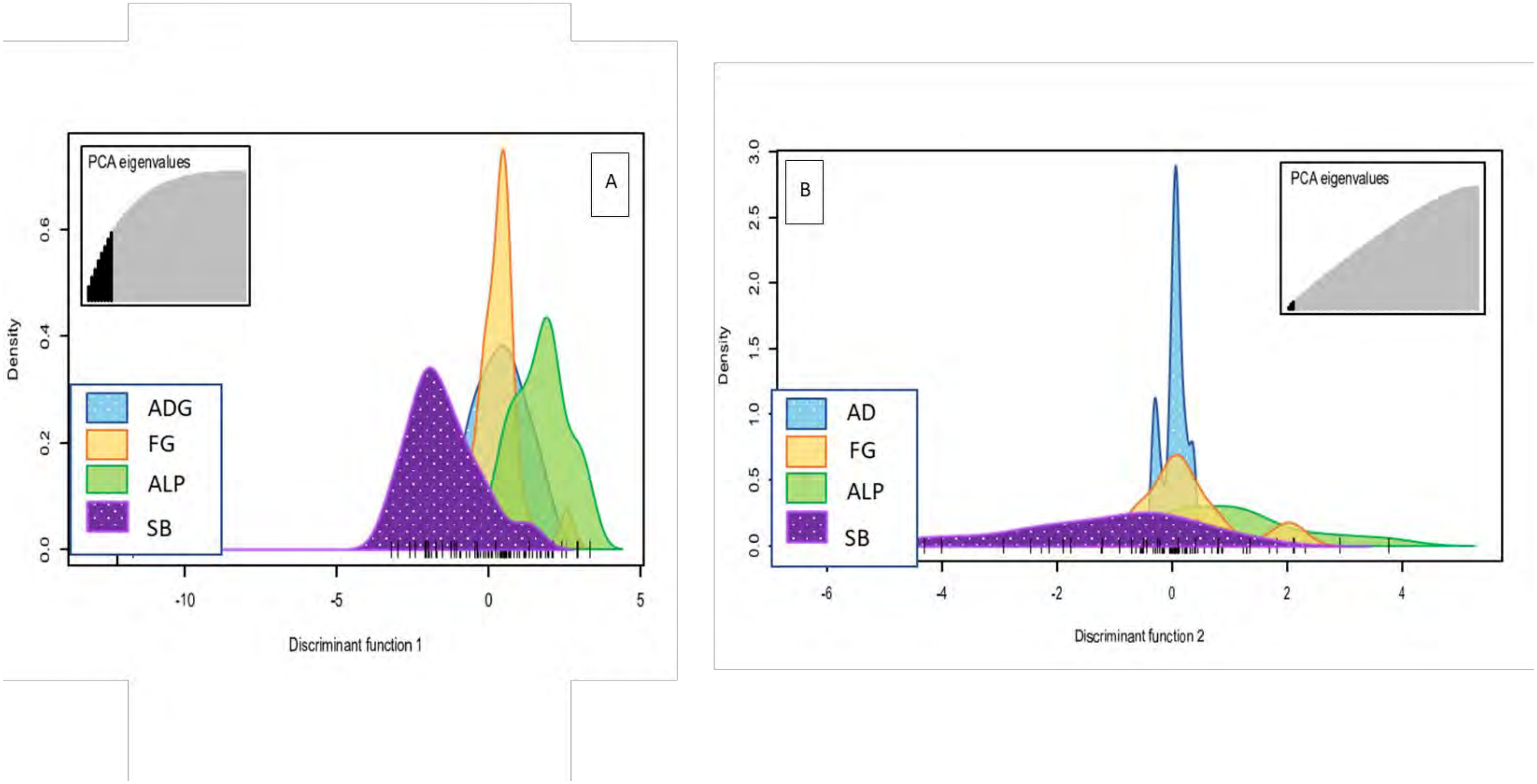

Figure 3.7 DAPC of the outlier dataset for clusters A) *K* = 2 and B) *K* = 3 along the 1st Discriminant Function. Each colour represents a different island group and the shaded areas show individual densities for each of the island groups.

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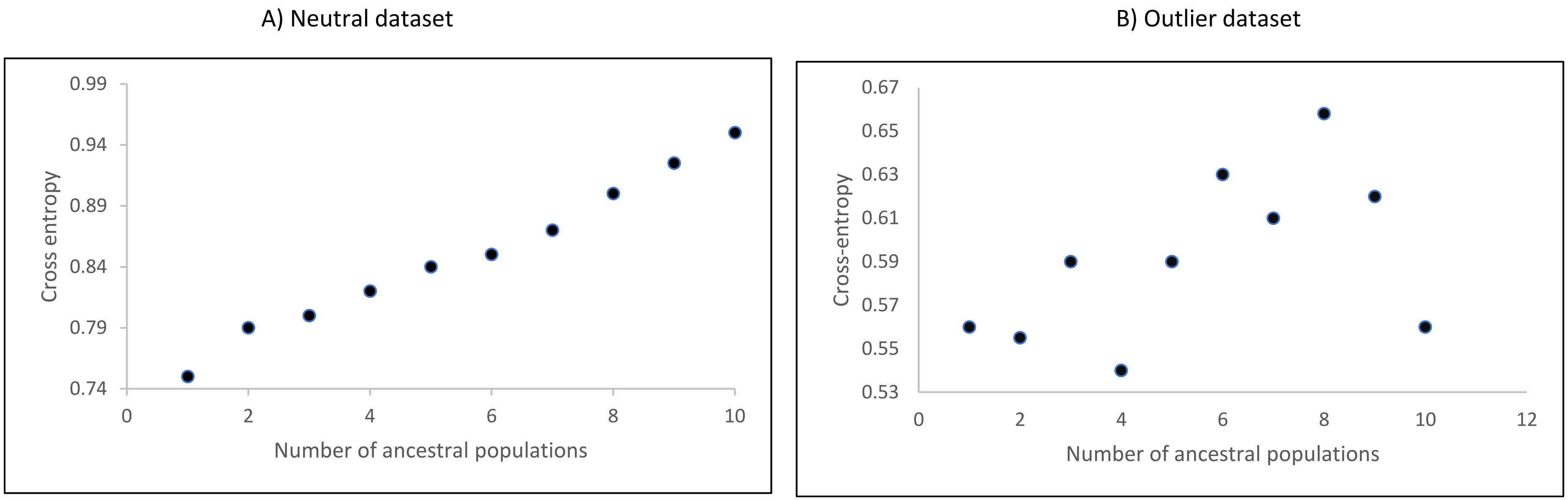
3.2.4.5 *POPULATION ASSIGNMENT*

3.2.4.5.1 SPARSE NON-NEGATIVE MATRIX FACTORISATION (SNMF)

The population assignment analysis, sNMF, indicated the most support for *K* = 1 cluster for the neutral dataset (Figure 3.8A) and *K* = 4 clusters for the outlier dataset (Figure 3.8B). For both datasets, individual ancestry plots showed no geographic structure by island group.



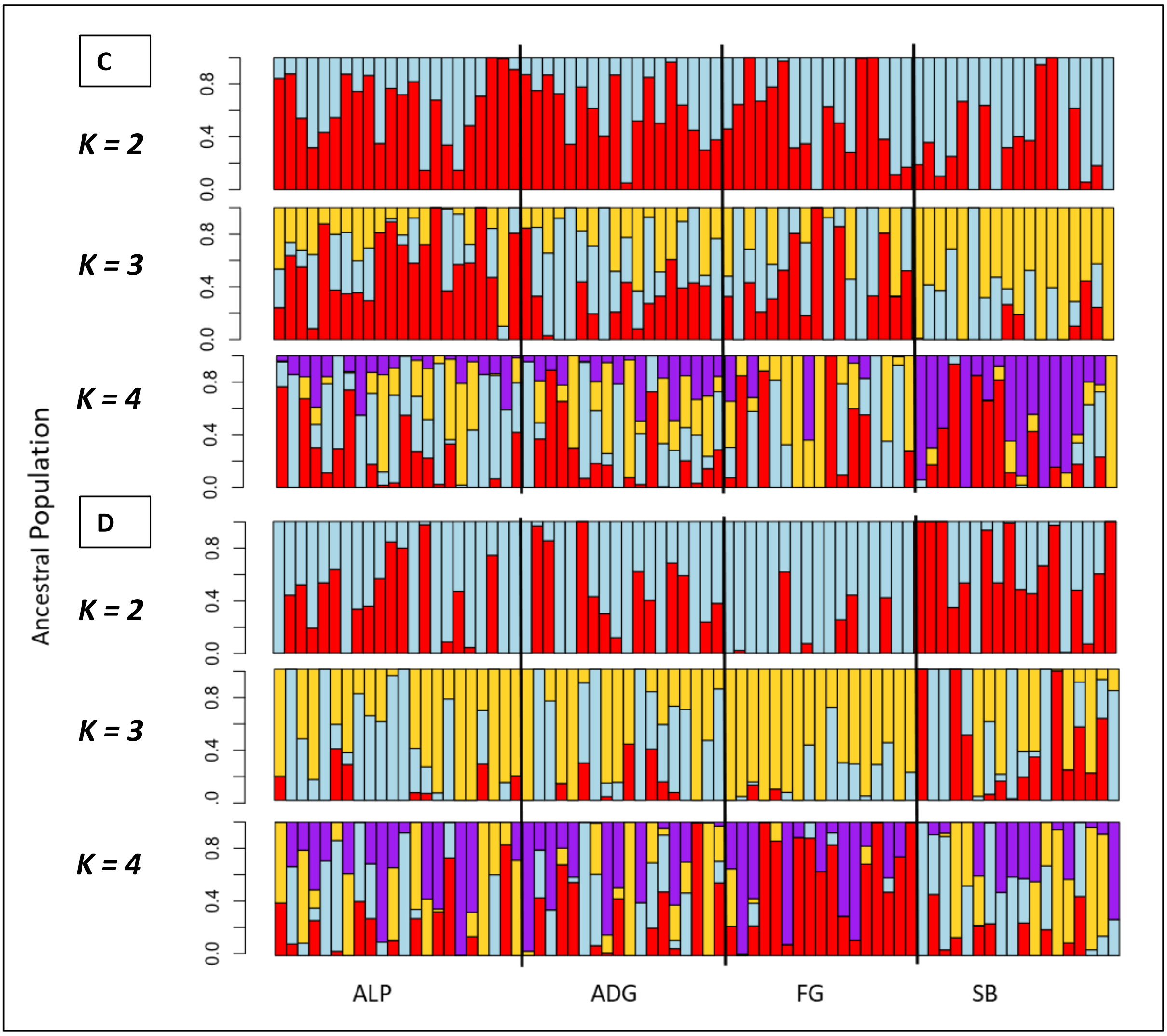


Figure 3.8. sNMF plots from LEA showing entropy scores for best *K* in the (A) neutral data set and the (B) outlier data set. The bar chart of ancestral population assignment of each individual in the (C) neutral data set and the (D) outlier dataset for *K* = 2 to 4. Different colours represent a different ancestral population.

3.2.5 SPATIAL ANALYSES

3.2.5.1 *ISOLATION BY DISTANCE (IBD)*

The IBD tests for pairwise island groups showed two genetic patches for both outlier and neutral datasets (Figure 3.9). The Mantel Monte Carlo randtest for significance showed that genetic and spatial distances were not correlated in either the neutral (Mantel's $r = 0.02487$; $p = 0.4583$) or outlier (Mantel's $r = -0.259$; $p = 0.708$) datasets. In addition, the Mantel test only accounted for 0.06% of the overall genetic divergence explained by geographical distance in the neutral dataset, and 6.25% in the outlier dataset

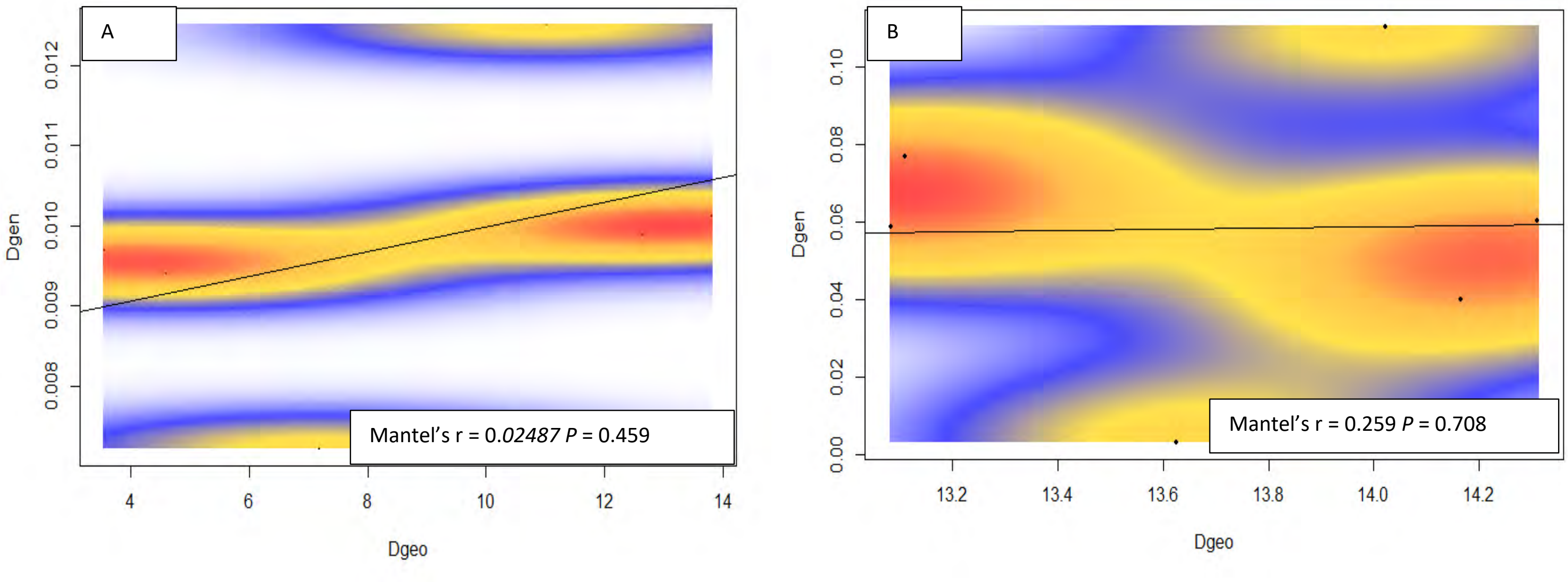


Figure 3.9. Isolation by distance of 75 *A. glossodonta* individuals sampled from four island groups across the SWIO. The colours show a two dimensional kernel density estimation between geographic and genetic distance for A) neutral and B) outlier datasets. Red is demonstrative of high density correlation between geographic and genetic distance whereas blue demonstrates low density between genetic and geographic distance.

3.2.5.2 *Spatial principal component analysis (sPCA)*

The sPCA analysis using a Delaunay network revealed the presence of one global and one local pattern. However, only the first global score (denoted as λ1) was supported, based on the sPCA eigenvalue bar chart and the scree plot showing decomposed eigenvalues into variance and spatial autocorrelation components (Supplementary Figure 6.4). This means that only the eigenvalue of the first global score showed a distinct genetic variance and geographical distinction. Monte Carlo randtests were significant for both the global (randtest = 0.035, *p* = 0.001) and local (randtest = 0.0159, *p* = 0.027) structures in the neutral dataset (Supplementary Figure 6.4D). The global structures were positively autocorrelated, indicating a spatial pattern of differentiation between the northwest Seychelles island groups and the Saint Brandon group (Supplementary Figure 6.4D). In this case, the first global sPCA for the neutral dataset showed two groups: one contained only individuals from SB and the other consisted of all the Seychelles island groups (FG, ADG, ALP; Figure 3.10A). The outlier dataset showed similar results as the neutral dataset. One global pattern was identified (Supplementary Figure 6.5). The Monte-Carlo randtest performed on global structures was significant (randtest = 0.034, *p* = 0.023) whereas the local score was not (randtest = 0.027, *p* = 0.123) (Supplementary Figure 6.5). The trend seen with the outlier data set emphasised that the SB samples grouped into one cluster with only negative scores, whereas the samples from of ADG and FG grouped into a different cluster with positive entity scores and the ALP group had entity scores from both groups, hence not adhering to any of the clusters (Figure 3.10B).

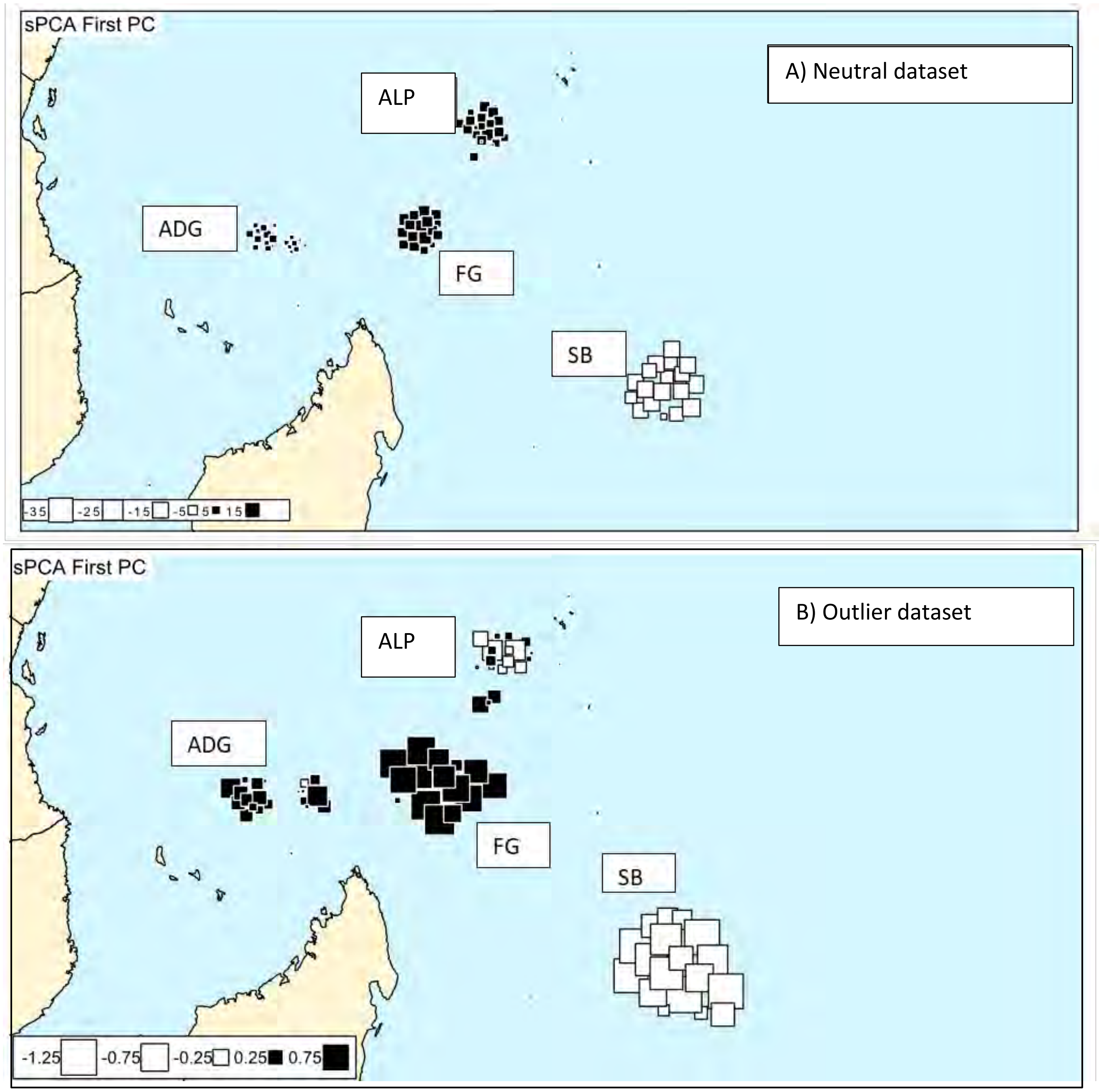


Figure 3.10. Lagged scores of neutral and outlier datasets for the first global sPCA along the first PC. Each square is representative of an individual, positioned using the geographical coordinates of its sampling location. The black boxes are representative of positive lagged scores, whereas the white boxes are negative lagged scores. The different coloured boxes shows the allocation of individuals to two different clusters. The size of the boxes shows the likelihood that each individual fits into the designated cluster.

4 CHAPTER 4: GENERAL DISCUSSION

4.1 THE INDIAN OCEAN

4.1.1 Southwest Indian Ocean Oceanography

The Indian Ocean (IO) is the third largest water mass on the planet, covering an estimated 18% of the Earth's surface (Eakins and Sharman 2010). The coastal waters of the IO host more than 30% of the world's coral reefs, over 40,000 km^2 of mangroves, as well as some of the world's largest estuaries (Wafar et al. 2011). The IO is subjected to two monsoons: the Northeast monsoon during the Austral summer months (November to February) and the Southwest monsoon during the Austral winter months (March to October). Both monsoons are caused by strong winds that flow in a westerly direction during the winter months and an easterly direction during the summer months, directly influencing surface currents. The change in the wind direction during the year strengthens the East Africa Coastal Current during the Southwest monsoon and weakens it during the Northeast monsoon which, in combination, reverses the Somali current (Crochelet et al. 2016; Figure 4.1). During the summer months, the Counter Equatorial Current and the North Equatorial Currents are absent (Benny 2002).

The Southwest Indian Ocean (SWIO) is a region of the IO that is understudied by oceanographers (Paula 2015). A combination of surface winds and bathymetric structures such as sea mounts, continental shelves and ocean trenches govern the currents in the SWIO (Paula 2015; Groeneveld and Koranteng 2016). The dominant currents of the SWIO are the Agulhas Current, Somali Current, South Equatorial Current (SEC), South Equatorial Counter Current (SECC), East Madagascar Current and the East Africa Coastal Current (Figure 4.1). The SEC flows in a westerly direction and bifurcates as it approaches the Mascarene plateau, flowing towards northern Madagascar, down the slope of the Mascarene plateau towards Mauritius, and further flowing into the East Africa Coastal Current, as well as through the Mozambique channel and into the Agulhas current (Badal et al. 2009). These currents are important for the SWIO by creating nutrient-rich upwelling zones such as the Somali currents and eddies in the Mozambique channel (Crochelet et al. 2016). Whilst research on the SWIO currents and their specific biological roles in the dispersal, larval distribution and gene flow of fishes continues to grow, the effects that ocean currents have on migration patterns, larval swimming ability and larval mortality and recruitment, especially in elopomorphs still require extensive research (Badal et al. 2009; Bonhommeau et al. 2009; Miller et al. 2019; Zeng 2019) .

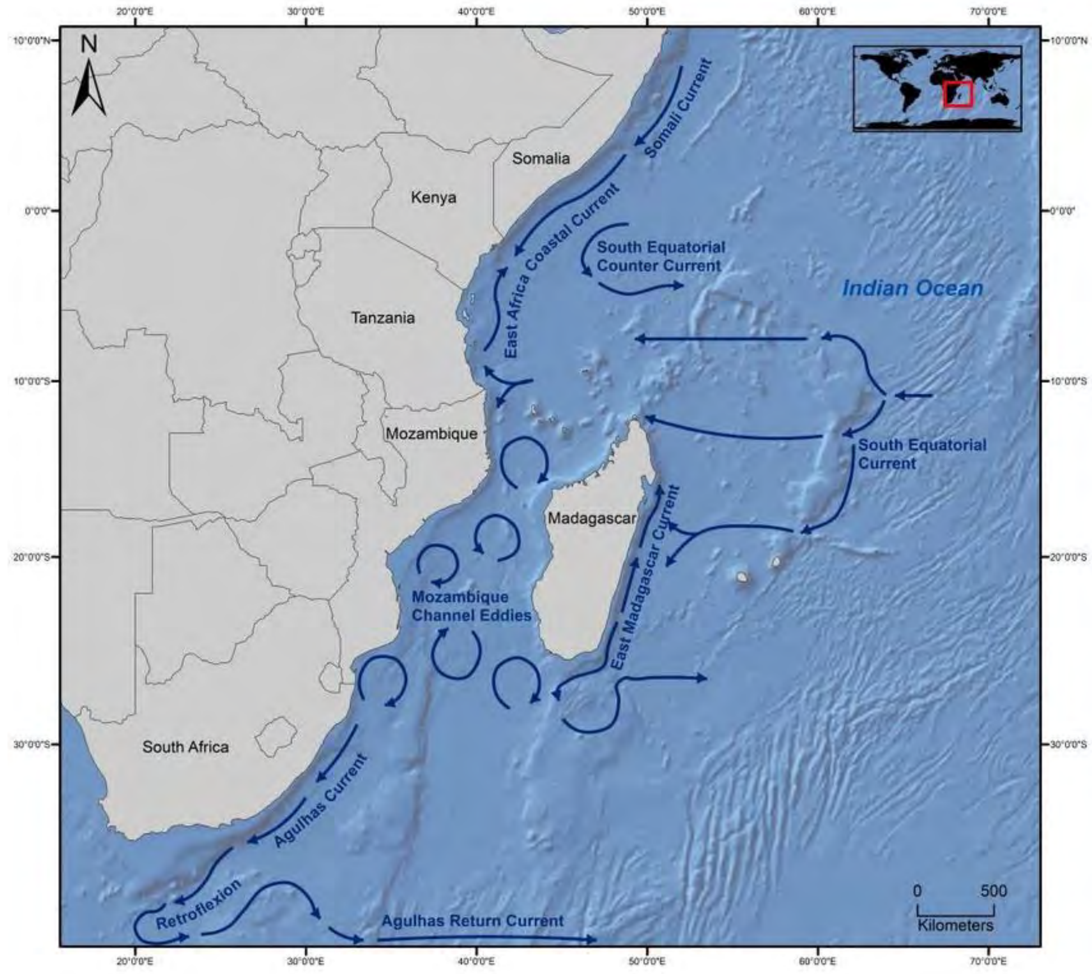


Figure 4.1 Dominant currents of the Southwest Indian Ocean (Crochelet *et al.* 2016).

4.1.2 MIGRATION OF ELOPOMORPHS

Hundreds of fish species partake in highly migratory behaviour, ranging from circumnavigation of the globe to shorter distances within or between countries (Lascelles et al. 2014). In the Indian Ocean, some of the economically valuable fishery species that undergo long-distance migrations for spawning and feeding are tunas (Hallier and Fonteneau 2015). Tuna species, such as yellowfin (*Thunnus albacares*) and bigeye (*Thunnus obesus*) have strong schooling behaviour and are capable of migrating long distances between the equator and the southern waters of South Africa (Sabarros et al. 2017; Sinan and Bailey 2020). Many species of

Elopomorpha, such as some members of the family Anguillidae, also undertake long migrations for spawning (Pous et al. 2010; Minegishi et al. 2012; Chang 2018; Miller et al. 2019) . Given the historical economical value of eels more information is available for this group of elopomorphs compared to other elopomorphs like bonefishes which have become of interest with the expanding fly fishing industry (Pous et al. 2010; Wallace 2015; Moxham et al. 2017; Pickett 2020). Adult bonefishes are known for high site fidelity with relatively short migrations (10–100 km) occurring only during the spawning season (Larkin et al. 2011; Perez et al. 2019; Moxham et al. 2019). Whilst there have been anomalous recordings of bonefishes migrating more than 300 km from Florida (Larkin 2011), to sightings in Virginia and New York (Larkin 2011; Boucek et al. 2019), as well as across Miami to the Bahamas (Boucek et al. 2019), these records are often linked to single individuals. It is thus speculated that these recordings are due to rogue bonefish displacements resulting from strong storms (Larkin 2011; Boucek et al. 2019).

Due to the lack of studies on the migratory habits of bonefishes in the SWIO, it is assumed that adult *A. glossodonta* behave similarly to bonefish species in the Atlantic and Pacific Oceans (e.g., *A. vulpes*). Consequently, my observed trend of panmixia in *A. glossodonta* between the SWIO islands of Seychelles and Mauritius, spanning distances of 400 to >1000km (Figure 4.2) is probably not a result of adult long-distance migrations, but of pelagic larval dispersal, the primary dispersal mechanism for most bonefish species (Danylchuk et al. 2011; Wallace and Tringali 2016; Perez et al. 2019; Zeng et al. 2019). Larval bonefishes in the *A. vulpes* complex, with a prolonged pelagic larval duration of 41 to 72 days, can disperse long distances (Mojica 1995; Friedlander et al. 2007; Filous et al. 2019). Whilst knowledge on the migration patterns of large marine species has been facilitated through satellite and acoustic tagging, quantifying the pelagic drift of ichthyoplankton, such as leptocephalus larvae, to measure dispersal is difficult. Population genetics thus serves as a tool to investigate these patterns of connectivity using DNA.

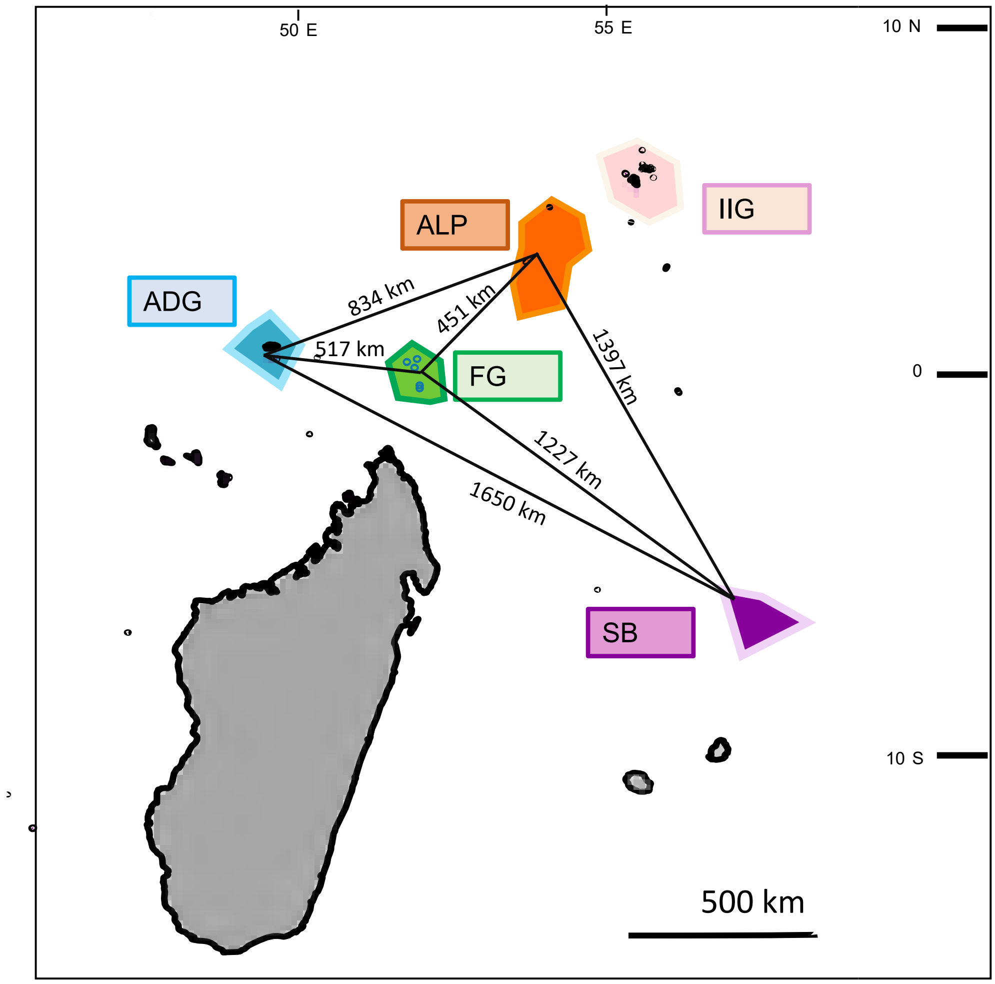


Figure 4.2. Distance (km) between island groups (ALP = Alphonse Group, SB = Saint Brandon, ADG = Aldabra Group, FG = Farquhar Group) sampled in this study. Coloured polygons show the locations of each of the island groups.

4.2 GENETICS AND POPULATIONS

4.2.1 BONEFISHES AS CRYPTIC SPECIES

Genetics is a rapidly advancing field used for the description of new species, understanding population structure and connectivity, and identifying cryptic species, all of which assist national and transnational species management (Jombart et al. 2008; McKeown *et al*. 2016, 2017). Genetic techniques have, for example, led to the discovery of new cryptic fish species worldwide, including bonefishes (Pickett et al. 2020). The cryptic morphological features of bonefishes have confused scientists for decades, and bonefishes were initially identified as a single species (Colborn et al. 2001). Bonefishes remain understudied, with gaps in the knowledge of species distributions, movement behaviour and habitat partitioning (Colborn et al. 2001; Wallace 2015; Kamikawa et al. 2015; Santos et al. 2017). In fact, new bonefish species are still being described. For example, *Albula koreana,* a species native to Korea and Taiwan, was only identified and described in 2011 (Kwun and Kim 2011). In the Pacific Ocean, *A. virgata* (resurrected name; Jordan and Jordan 1922; Hidaka et al. 2008), was one of the few endemic bonefishes identified during the last decade, whereas in the Indian Ocean, *A. oligolepis* was identified in 2008 using genetic and morphological methods (Hidaka et al. 2008). These studies validate the need to use genetics to investigate cryptic species, particularly in understudied regions such as the Indian Ocean. Only two species of *Albula* are known from the Indian Ocean: *A. glossodonta* and *A. oligolepis* (Hidaka et al. 2008; Wallace 2014; Pickett et al. 2020; Williams 2020). Given the difficulty of differentiating between species of bonefishes, most Seychelles artisanal fishers are not aware of the presence of more than one species of bonefish. This is based on the observation that all bonefishes within the Seychelles are referred to as one common Creole name, “banane,” similar to how bonefishes (*A. virgata* and *A. glossodonta*) in Hawaii are referred to as “O’io” regardless of the species (Kamikawa et al. 2015).

Genetic techniques like next generation sequencing, by generating large, genome-wide datasets, have facilitated delineating population structure where conventional markers have been insufficient (Willette et al. 2014; Gagnaire et al. 2015). Although this is the first study to employ ddRADseq to investigate the population genetics of any bonefish species, next-generation sequencing methodologies have become more accessible and are being employed to other non-model clades within Elopomorpha, namely the economically valuable anguillids.

In this study, I coupled ddRADseq and mtDNA to identify cryptic bonefish species and analyse population genetic structure of *A. glossodonta*. A total of 9,180 loci were analysed, expanding the genomic coverage considerably from a previous study on *A. glossodonta* using 19 microsatellites (Wallace 2015). Similar transitions to genomic sequencing methods for intraspecific population analyses have occurred for other fish species such as yellowfin tuna (*Thunnus albacares;* Mullins et al. 2018) and the American eel (*Anguilla rostrata*; Babin et al. 2017). These studies add to the growing list of literature using large genomic datasets to investigate connectivity in open-ocean environments.

4.3 GENE FLOW OF ALBULA GLOSSODONTA IN THE SOUTHWEST INDIAN OCEAN

4.3.1 4.4.1 OVERVIEW OF RESULTS

Many marine fishes migrate long distances, either as adults or during pelagic larval phases, which can result in genetically connected metapopulations, sometimes referred to as panmixia (Palumbi 1994; Gagnaire et al. 2012). I found that *A. glossodonta* exhibits high levels of gene exchange throughout four locations sampled in the SWIO when only interpreting mtDNA data. I then showed that ddRADseq revealed patterns of population structure not observed using mtDNA, specifically between the Saint Brandon Island group (Mauritius) and the Seychelles islands.

The variety of genetic diversity tests performed using mtDNA indicated panmixia via low nucleotide diversity and low haplotype diversity. Low genetic diversity has also been observed in other *Albula* species and elopomorphs, such as anguillids, in the Indo-Pacific (Han et al. 2010; Wallace 2015), Pacific (Pfeiler et al. 2008; Gubili et al. 2019) and Atlantic Oceans (Palm et al. 2009; Als et al. 2011; Wallace et al. 2014). All the afore-mentioned studies showed no genetic structure of bonefish and anguillids at their respective study sites and indicated panmixia. This could be due to the presence of a leptocephalus larval stage, common to elopomorphs, which can drift at sea for extended periods of several months prior to post-larval metamorphosis (Filous et al. 2019b). However, panmixia is not always an outcome of long pelagic larval duration (PLD), as demonstrated by *Anguilla mormorata*, where 2–5 stocks have been identified in the Indo-Pacific Ocean (Gagnaire et al. 2011; Donovan et al. 2012), and *A. glossodonta,* where putative stocks between the Indian Ocean and the Pacific Ocean have been suggested (Williams et al. 2020). Prior genetic studies on *Albula* used mtDNA or microsatellites, and the authors proposed using next generation sequencing to decipher intraspecific trends (Pfeiler et al. 2008; Wallace et al. 2014; Wallace 2015).

Whilst I generated a larger genomic dataset using ddRADSeq, my overall results also show low genetic diversity (Table 4.1). These results are partially attributed to the fact that the SNPs analysed were biallelic and biased towards giving low genetic indices compared to polymorphic multiallelic markers such as microsatellites (Ryynänen et al. 2007; Helyar et al. 2011; DeFaveri et al. 2013; Liu et al. 2020). Lack of polymorphism has been described as a potential bias of using reduced representation sequencing, and therefore may reflect that ddRADseq does not ensure random distribution across the genome and that the composition of bases and polymorphisms are linked (Andrews and Luikart 2014; Andrews et al. 2016). Lack of polymorphism may result in genetic diversity measures being underestimated, especially for putatively neutral loci (Cariou et al. 2016). However, a comparison of genetic diversity indices from my study with indices generated from microsatellite data for bonefish species in the Atlantic revealed little dissimilarity in diversity between *A. glossodonta* and other bonefishes, which could be a result of species-specific variability (Table 4.1). Although statistical estimates using RADseq approaches may underestimate polymorphisms, this underestimation is not of huge significance if the number of polymorphisms are below 2%, which is the case for most animal species (Cariou et al. 2016).

Table 4.1. Observed heterozygosity (Ho) and expected heterozygosity (He) estimated for different Albula species using different data types (ddRADseq and microsatellites).

Species	**Type of marker**	**Citation**	H_O	H_E
A. glossodonta	ddRADseq	This study	0.2541	0.2674
A. vulpes	Microsatellite	Wallace & Tringali 2016	0.378	0.524
A. goreensis	Microsatellite	Wallace & Tringali 2016	0.247	0.298
A. spc.cf.vulpes	Microsatellite	Wallace & Tringali 2016	0.239	0.312

4.3.2 BREAK BETWEEN SAINT BRANDON AND SEYCHELLES

Noteworthy from this study is that specimens from the Saint Brandon Atoll (SB) were distinct from the Seychelles island groups in all PCA-based analyses (sPCA, PCA and DAPC) of population structure and pairwise F_{ST} comparisons based on both the neutral and outlier datasets. The results from the AMOVA analysis supported differentiation between island populations in the outlier dataset. This multi-method approach provides support for restricted gene flow between the Seychelles Islands and Saint Brandon Atoll.

Saint Brandon Atoll is part of the Mauritian Archipelago and is nestled along the Saya de Malha bank, 1000 – 2000 km away from the Seychelles Islands. Consequently, this genetic structuring was unexpected, given the long PLD (57 days) for *A. glossodonta* in the Pacific (Friedlander et al. 2007; Filous et al. 2019b). However, evidence of limited gene flow between the Seychelles and Mauritian islands has been demonstrated in other marine species, including lethrinid fishes (Healey et al. 2018), lutjanid fishes (Muths et al. 2012) as well as corals (Crochelet et al. 2016; Mzingirwa et al. 2019).

I attribute the genetic structure observed between Seychelles and Saint Brandon's Atoll to the complex current system of the SWIO. Saint Brandon's Atoll is in the direct path of one of the bifurcated arms of the South Equatorial Current (SEC) as it passes through the Mascarene Plateau (Figure 4.1). The SEC pushes water westwards, which may create a barrier to gene flow between continental Africa and the northern Mascarene Islands (Mzingiwa et al. 2019; Gamoyo et al. 2019). Due to the dearth of research in this region, there may be other localised oceanographic features (e.g., eddies) that have not been studied and form additional oceanographic barriers between Seychelles and Mauritius. Whilst there are currently no bonefish or elopomorph larval dispersal models for the Indian Ocean, other models exist that may explain the observed reduction in gene flow between the Mauritian Archipelago and the Mascarene plateau. Reef cluster simulations (Crochelet et al. 2016) and reef dispersal simulations (Gamoyo et al. 2019) displayed in Figure 4.2.A and Figure 4.2.B, respectively, show that with an increase in PLD there will also be an increase in connectivity or cluster overlap. Even with a PLD of 50 to 60 days, connectivity to the SB group would be limited. However, both models considered passive coral larvae, which may be completely reliant on the currents for their dispersal (Otwoma et al. 2018; Gamoyo et al. 2019; Zeng et al. 2019). Little is known about the dispersal behaviour of bonefish larvae relative to other elopomorph species with long pelagic larval durations (e.g., anguillids), but bonefishes – being closely related to and sharing some early life history traits with anguillids – presumably disperse greater distances than corals by having the ability to swim (e.g., *Anguila japonica*; Chang et al. 2018) or may even take part in vertical migrations (e.g., *Anguila japonica*; Kudo 2001; Shinoda et al. 2011).

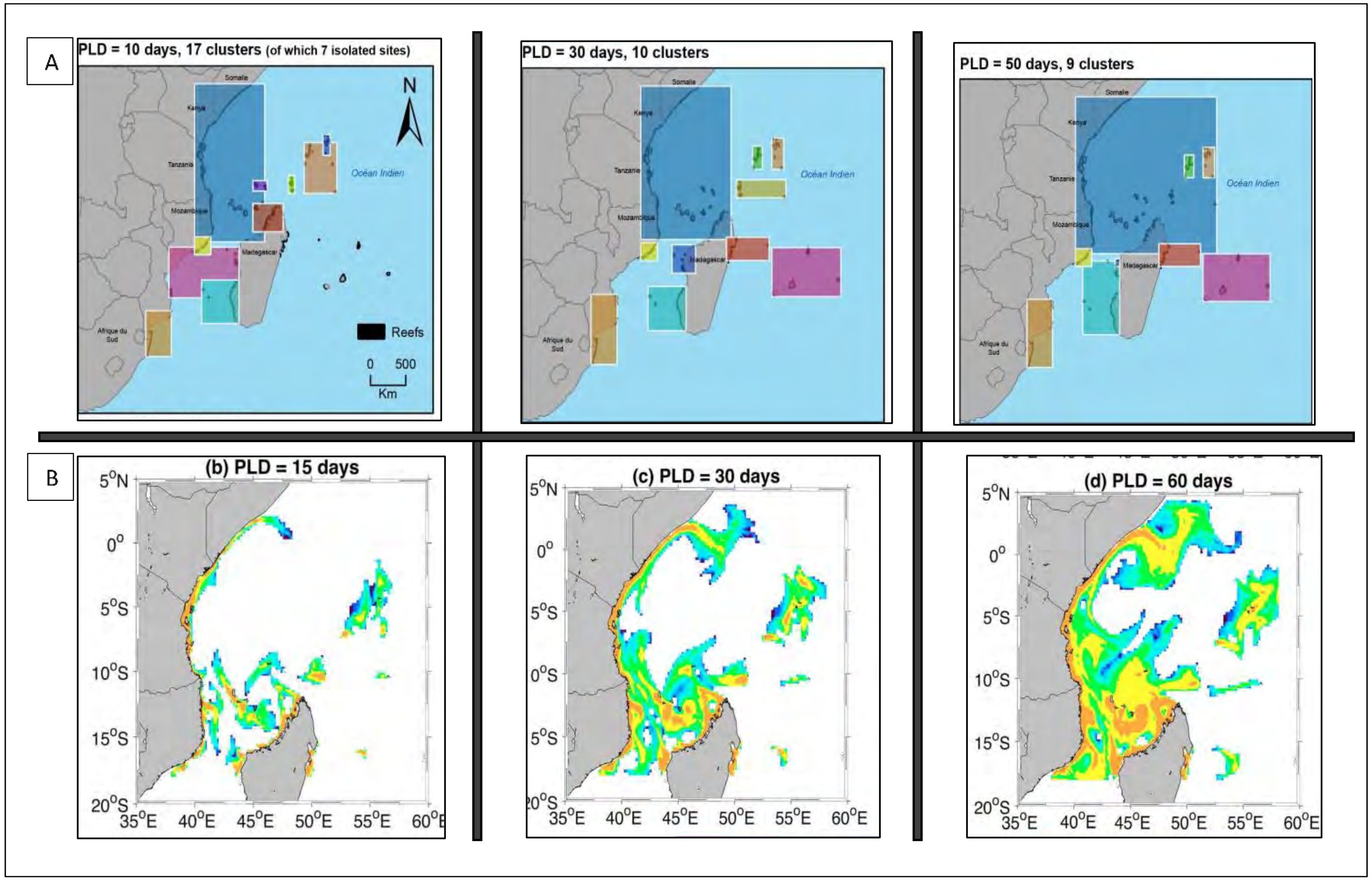


Figure 4.2. Simulated larval dispersal of reef systems throughout the SWIO showing A) reef clusters extending with an increase in PLD from 10–50 days (Crochelet et al. 2016), and B) simulated larval densities over three different simulated PLD groups (15–60 days; Gamoyo et al. 2019).

4.4 HABITAT PARTITIONING

4.4.1 BONEFISH HABITAT

Bonefishes have been collected in low to high saline environments throughout their lifecycle (Ault 2007; Santos 2019). In my study, while the sampling locations were classified as having a granitic (IIG) or coralline origin (ALP, ADG, FG and SB), most had an array of habitat features such as lagoons, bays, seagrass and algal beds, mangroves and sand flats, all of which are suitable habitats for bonefish (Wallace 2015; Brownscombe et al. 2019). The highly populated islands of the IIG such as Mahé, Praslin and La Digue have limited suitable habitat for bonefish (some grass beds, sandflats, and sheltered bays), and these are constantly experiencing anthropogenic pressure such as land reclamation, pollution and development (Adams et al. 2013). It is therefore more challenging to catch bonefish within the populated IIG islands compared to the less-populated coralline islands, where bonefishes have more-suitable and less-impacted habitats. Sightings of bonefishes within the IIG are rare, but they are occasionally caught as bycatch in the seine net mackerel fishery and sold in the markets very cheaply (approximately US$ 1.75 per fish). This contrasts with the outer islands (ADG, ALP and FG), where bonefishes are abundant and sought-after in an active, and high-value catch-and-release recreational fishery.

In the coralline islands, fly fishing methods were employed to collect samples. Samples were collected from a variety of habitat types, including sand flats, lagoons and seagrass beds, as well as a range of sampling depths (0–10m). The IIG islands, on the other hand, support a small fly-fishing industry which does not specifically target bonefish, and only one individual was collected via fly fishing. All samples collected in this study using fly fishing were identified as *A. glossodonta* (N =153), whereas all samples identified as *A. oligolepis* (N = 7) were collected from a single fishing event as bycatch from the mackerel fishery, which generally occurs in shallow bays extending 5–10 metres deep, between 50–100 metres offshore. This suggests the two bonefish species may have specific habitat requirements or different foraging behaviours. However, little evidence exists on how fishing methods influence the catches of different bonefish species, since most records do not explicitly state the fishing methods used to catch different species of bonefish. Anecdotal records suggest that both *A. oligolepis and A. glossodonta* may be caught using a variety of methods. For example, *A. oligolepis* has been caught in South Africa and Mozambique using traditional baited hook and line methods (PD Cowley, South African Institute for Aquatic Biodiversity, pers. comm.), as well as via seine net in Mauritius (Hidaka et al. 2017). On the Pacific island of Kiribati and the Anaa Atoll, French Polynesia, there are artisanal and subsistence bonefish fisheries, and residents have used stone weirs for generations to capture *A. glossodonta* (Johannes et al. 2008, Filous et al. 2019a). *A. vulpes* has also been caught using conventional baited hook and line fishing (Humston et al. 2005). The different sampling methods employed are often due to the popularity of fishing at certain destinations, such as the predominance of fly fishing by tourism establishments in Seychelles and Mauritius. However, different fishing methods may target specific habitat types, which may bias the species of bonefish caught.

4.4.2 BONEFISH HABITAT PARTITIONING

Habitat partitioning is not unique to *Albula oligolepis* and *A. glossodonta*; it has also been suggested for *A. virgata* and *A. glossodonta* in Hawaii, USA (Donovan et al. 2015) and *A. vulpes* and *A. goreensis* in the Bahamas (Haak et al. 2019). These bonefishes are either rarely caught within the same habitat or empirically demonstrate spatial partitioning due to feeding regimes and habitat preferences (Adams 2007; Donovan et al. 2015; Kamikawa 2015; Filous et al. 2019; Haak et al. 2019). For example, *A. vulpes* and *A. goreensis* have a West Atlantic sympatric distribution but appear from fishery data to have different habitat preferences (Haak et al. 2019; Wallace and

Tringali 2016). *A. goreensis* have been caught away from sandy flats and in deeper waters, but juveniles are generally collected and sighted on exposed beaches (Wallace and Tringali 2016). *A. goreensis* has also been caught in high wave energy areas, generally connected to coral reefs (Haak et al*.* 2019). *A. vulpes*, on the other hand, are more commonly caught along flats in sheltered bays (Wallace and Tringali 2016; Haak et al. 2019).

A. goreensis and *A. virgata* are hypothesised to be deeper-dwelling species and are more often caught in deeper coastal bays and along continental shelves instead of on shallow sandy flats (Rennert et al*.* 2019). *A. goreensis* has also been reported to co-occur with *A. vulpes* along the Cuban coast and occurs in depths >2 m, whereas its shallower dwelling counterpart is generally caught in waters <1 m (Wallace and Tringali 2016; Rennert et al*.* 2019). *A. virgata*, on the other hand, is uniquely found along the coastal waters of the Hawaiian islands (Kamikawa et al. 2015; Pickett et al*.* 2020) and is caught in greater numbers on the island of O'ahu, especially on the steep western coastline and sheltered bays, as well as the steep volcanic island of Kauai (Kamikawa et al. 2015). Although widespread, *A. glossodonta* is more prevalently caught in Hawaii in areas with extensive shallow habitat, such as reef flats on O'ahu's south-eastern coastline (Donovan et al. 2015; Kamikawa et al. 2015).

A. glossodonta appears to have similar habitat preferences as *A. vulpes*, whereby it is generally caught on shallow coastal flats – a commonly a feature of coral atolls - or shallow bays and seagrass beds at granitic and volcanic islands. This is possibly why *A. glossodonta* and *A. vulpes* are the species most commonly caught by fly fishing. Most bonefish fly fishing destinations have long stretches of reef flats and seagrass beds (e.g., Saint Brandon Atoll in Mauritius, O'ahu Island of Hawaii, the Bahamas and the Florida Keys).

The fishery-independent, species occurrence studies available for bonefishes, especially *A. glossodonta* and *A. oligolepis,* are generally conducted on a large geographic scale, covering oceanic regions, but lacking detail on exact location, depth and habitat (Colborn et al. 2001; Obermiller and Pfeiler 2003; Pfeiler et al. 2008; Hidaka et al. 2008, 2017;Wallace 2014, 2015; Pickett et al. 2020; Williams 2020). However, even coarse occurrence maps and available data from scientific studies allude to a trend of habitat partitioning between *A. oligolepis* and *A. glossodonta* (Figure 4.3). It is possible that *A. oligolepis* in the SWIO occupies similar niches as *A. virgata* in Hawaii, as well as *A. goreensis* in the Atlantic. All of the aforementioned bonefish species have shown similarities in depth ranges, having been caught in waters >2 m deep. However, fine-scale spatial habitat data for most of these species, especially those in the Indian Ocean, are not available (Wallace and Tringali 2016; Brownscombe et al. 2019; Pickett et al. 2020). Williams *et al.* (2020) investigated the occurrence of bonefishes in the Gulf of Aden and the Red Sea with the aim to genetically identify bonefish species from this region. Interestingly, the bonefish samples collected from different locations in the Red Sea were exclusively identified as *A. glossodonta* (Figure 4.3). The areas sampled, namely Thuwal, Yanbu, Farassan islands and the coasts of Sudan and Egypt, exhibited similar characteristics to the coralline sites in this study. A single *A. oligolepis* individual was collected off the coast of Yemen on the Island of Socotra (Williams *et al.* 2020), which has a rocky habitat that resembles the IIG of Seychelles. Wallace (2015) also suggested *A. oligolepis* and *A. glossodonta* demonstrate habitat partitioning in a similar way to *A. vulpes*, predominantly caught by anglers on inshore flats, and *A. goreensis*, usually caught in deep waters in the Caribbean and on exposed beaches in the Florida Keys (Colborn 2001 ; Wallace and Tringali 2016; Wallace 2014).

Habitat partitioning amongst species with overlapping ranges is common due to the theory that it may drive speciation and also reduce interspecific competition (Donovan et al*.* 2015; Galaiduk et al*.* 2017; Haak et al*.* 2019). For example, the Japanese eel (*Anguilla japonica*) and Giant-mottled eel (*Anguilla marmorata*) co-inhabit rivers in East Asia, exhibiting interspecific competition. In the Fengshan River in Japan, the Japanese eel is more common in the lower and middle sections of the river whereas the giant mottled eel is caught in the upper and middle sections of the river (Hsu et al*.* 2019). Similarly, two Tripterygiidae species (*Bellapiscis lesleyae* and *Bellapiscis medius*) along the coast of New Zealand have largely sympatric distributions but occur in distinct littoral habitats (Hilton et al*.* 2008). These two tripterygiid species demonstrate a classic example of habitat partitioning as both species occupy distinct rock pools in the intertidal zone (Hilton et al. 2008). Other notable examples of fishes that display sympatric habitat partitioning include African cichlids (Kornfield and Smith 2000) and congeneric blennies (*Acanthemblemaria* spp.; Clarke et al*.* 2009).

Habitat partitioning may occur for a variety of reasons, including physical adaptations such as body shape that may be linked to a species' ability to live in different habitats (Clarke et al*.* 2009). Although bonefish species are morphologically very similar, differences between some sympatric species have been observed. For example, *A. virgata* and *A. glossodonta* display differences in depth preference and mouth structure, which may influence their diet regimes (Donovan et al. 2015). Additional sampling at different islands and at different depths is required to further investigate habitat partitioning between *A. oligolepis* and *A. glossodonta* (Kamikawa et al. 2015; Williams et al. 2020). Moreover, bonefish species' catch records in the SWIO need to be validated with both morphological and genetic methods.

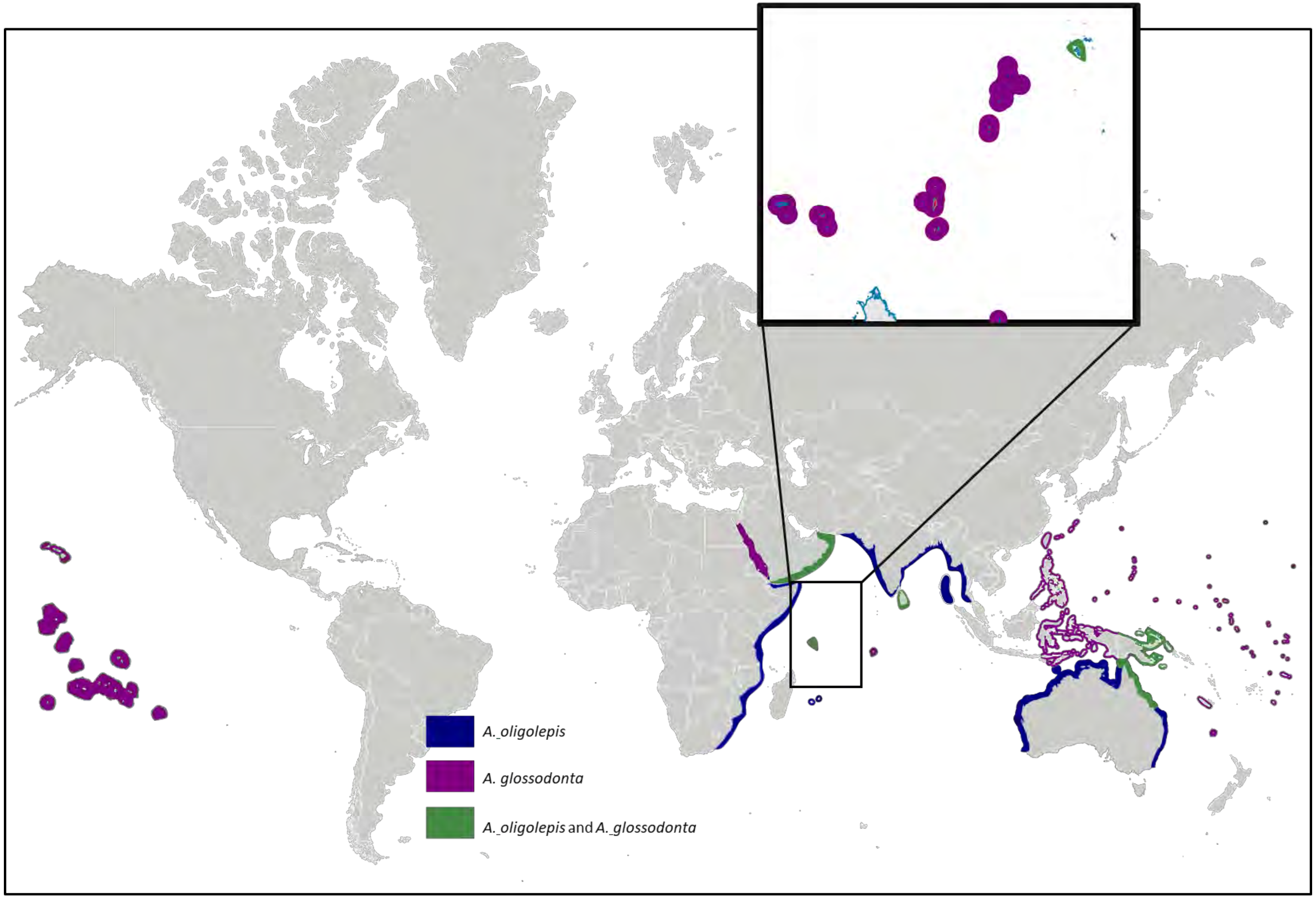


Figure 4.3 The worldwide occurrence of *A. glossodonta* and *A. oligolepis* (adapted from Pickett et al. 2020; Williams et al. 2020)

4.5 MANAGEMENT IMPLICATIONS AND FUTURE RESEARCH NEEDS

All countries in the SWIO have coastal populations that are highly dependent on fisheries. The targeted species and the methods employed by each fishery tend to be regionally dependent on species availability, as well as the type of fishing industry (e.g., commercial, artisanal, recreational). Economically important species like tunas (Scombridae), groupers (Serranidae) and snappers (Lutjanidae) are widely exploited throughout the SWIO for local and global markets, and unsustainable fishing practices for these species have led to population declines (Grandcourt *et al*. 2008; Purcell *et al.* 2017).

Unfortunately, many SWIO states with the highest reliance on fisheries (e.g., Seychelles, Comoros and Madagascar) also have the lowest capacity for research, management and fisheries enforcement (Mora *et al*. 2009). For example, research within the Seychelles has been limited, with an estimated 267 peer-reviewed papers in the field of ocean science published from the 1960's to the 2010's (Blamey *et al.* unpublished data). In comparison, countries such as the United States, Canada, Australia and France produced an estimated 15,000 to 100,000 papers relating to the marine sciences between 2010 – 2014 alone (UNESCO 2017). Developed nations have the financial resources to conduct more research on fisheries and marine science than countries such as the Seychelles, which does not allocate any of its GDP to research (UNESCO 2017). Studies on recreational fisheries have been widely neglected within the SWIO, which is the only ocean region surrounded entirely by developing countries. The Seychelles Fishing Authority and Ministry of Fisheries and Blue Economy have focused the bulk of fisheries research efforts on species of consumer importance, such as tunas (Scombridae), lobsters (Nephropsidae) or sea cucumbers (Holothuridae; SFA 2018). It is imperative that large ocean states diversify their research efforts for the betterment of their own economic and environmental prosperity. Recently, a new research initiative, the Seychelles Climate and Adaptation Trust, was established in Seychelles to empower Seychelles nationals with grants and loans to conduct marine research, including research on recreational fisheries. Other programs, such as the South West Indian Ocean Fisheries Governance and Shared Growth Project under the World Bank (SWIOFish3 2021), are aimed at improving fisheries management and knowledge within areas gazetted as important for sustainable use zones (including areas popular for recreational fisheries) as well as no take zones (SMSP 2021, SWIOFish3 2021). Such initiatives will assist with developing Seychelle's Blue Economy through science-based and sustainable practices (https://seyccat.org/; Ernesta 2020). To better manage recreational fishery species such as bonefishes, more research should be prioritised to close the extensive knowledge gaps on the biology, dispersal, and fishing mortality of recreational fishes in the SWIO.

An extensive research undertaking is needed to evaluate the economic value of bonefishes – and recreational fisheries more broadly – in the SWIO. Recreational fisheries provide large economic benefits due to the high spending power (Wallace 2015; Moxham 2019; Glass et al. 2020, Kadagi et al. 2020). Only one SWIO nation, South Africa, has engaged deeply in characterising and evaluating recreational fisheries and their economic benefits. In South Africa, the value of the recreational fishing industry is estimated at 36 billion ZAR (approximately US$ 2.4 billion) and to create over 94,000 jobs (Saayam et al. 2017). The value of the recreational bonefish fishery in the SWIO has not been evaluated, but studies estimating the value of bonefish fisheries in Florida (Fedler 2013), the Bahamas (Fedler 2010) and Belize (Fedler et al. 2008) have indicated they are multi-million dollar industries. These studies can be used as templates for similar work in the Seychelles and other SWIO nations and to identify the main stakeholders within the recreational fishery sector. Emphasis should be made on understanding how the income generated from recreational bonefish fisheries infiltrates local economies.

Although recreational fishing, especially fly fishing, is a growing venture in Seychelles, the fly fishing guides are comprised mainly of foreign nationals, and very few Seychellois are employed as guides within the industry. This is largely attributed to the fact that fly fishing is not traditionally engrained in Seychellois culture. However, the Seychelles has a Maritime Academy focused on fisheries and seafaring, and the introduction of fly fishing as part of their technical course might empower more Seychellois and youth to take on fly fishing as a career, leading to increased economic opportunities for Seychellois citizens.

The businesses that operate recreational fishing ventures in Seychelles work under a catch-and-release remit; therefore, the industry appears to be sustainable. However, post-release survival of *A. glossodonta* in Seychelles can be very low (Moxham et al. 2019). Hence, awareness in terms of best handling practices (e.g., Butler et al. 2020) within this sector is important, especially with ventures that may take place within the IIG where bonefish species' habitats are less prevalent and more disturbed by humans. In addition, there are no restrictions such as bag or size limits imposed on recreational fisheries in Seychelles, hence recreational fishers are not obliged to practice catch-and-release, which may ultimately compromise livelihoods of artisanal fishers (Kadagi et al. 2020). Research focused on the socio-economic aspects of recreational fisheries in Seychelles would assist in conflict resolution and effective management. In addition, such economic incentives may facilitate research funds needed to better understand the biology and distribution of important recreational fishery species in Seychelles and the SWIO more broadly.

My study demonstrated that *A. glossodonta* is the dominant species of bonefish that sustains the bonefish fly fishing industry in Seychelles and Mauritius in the SWIO. Though it is possible that more than two species of bonefishes occur in the SWIO, further research would require more intensive sampling to understand species occurrence and distribution. Similar studies have been conducted in the Atlantic, where the bulk of research on bonefishes has occurred (Wallace 2014, Wallace and Tringali 2016, Brownscombe et al. 2019). Species identification and occurrence need to be coupled with basic life history studies such as spawning habits and spawning seasonality. Fishes in general have been reported to be more susceptible to the negative effects of fishing pressures during spawning seasons (Cooke and Suki 2005). Therefore, bonefishes could be afforded protection through a formal closed season. In addition, the SWIO is subject to two distinct seasons linked to the NW and SE Monsoons. The SE Monsoon, giving way to strong winds and unpleasant fly-fishing conditions within most of the SWIO, dictates the open and closed seasons for fishing occurring on coralline flat habitat in Seychelles and Mauritius. If the spawning season of *A. glossodonta* occurs during the SE Monsoon, official closed seasons could be declared with minimal effort and maximum buy-in from stakeholders. Whilst this strategy could effectively be used in parts of the SWIO, in areas such as the inner islands of Seychelles where bonefishes are caught as bycatch in the mackerel fishery, other management strategies would need to address conflict between fishing and spawning time. Such strategies could include enforcement of net mesh sizes and the regulation of net-setting seasons, which would reduce the likelihood that reproductively ripe, aggregating bonefishes are caught as bycatch in the mackerel fishery.

In addition to spawning, there is no information on the larval recruitment behaviour of bonefishes in the SWIO. Future studies should aim to improve spatial and temporal modelling of currents in the SWIO, which can be tied to larval distribution maps, as have been developed for bonefishes in the Atlantic Ocean (Zeng et al. 2019). These models could also benefit fisheries managers in identifying important habitat features and locations for bonefish recruitment. Specifically, source-sink dynamics of larvae are vital for designing effective management strategies that will ensure bonefish populations remain connected.

In light of extensive Knowledge gaps, I propose four recommendations for improved bonefish fishery management in the Seychelles with the information at hand:

1. **Conduct socio-economic studies of recreational fisheries:** Socio-economic studies focused on quantifying the economic value of recreational fisheries will strengthen the investment from stakeholders and governments and inform a much-needed recreational fishery management plan. The management plan must also recognise the occurrence of a multi-species fishery that is even more complex when dealing with cryptic species like bonefishes.
2. **Conduct a comprehensive fishery-independent survey for bonefishes in Seychelles to quantify species distributions and estimate abundances:** The bonefishes of Seychelles, *A.*

glossodonta and *A. oligolepis,* may require different management plans, including depth- or habitat-based fishing restrictions specific to each species' behaviour and ecology.

3. **Review effectiveness of MPA's for recreational fishery species:** Seychelles has achieved a milestone by gazetting 30% of its EEZ as Marine Protected Areas with allowable activities. Under this new framework many of the outer islands such as Aldabra Atoll and some of the Alphonse and Amirantes Islands have been legally spared from detrimental effects such as unregulated, extractive fishing as well as habitat destruction and degradation. However, the inner island group of Seychelles has few marine parks which are experiencing management issues (e.g., Saint Anne Marine National Park; Cockerell and Jones 2020). Marine parks in the Inner Island Group need to be re-evaluated, specifically since the IIG is the only region in Seychelles where two species of bonefishes have been recorded.
4. **Focus efforts on generating data for top recreational fishery targets:** Whilst the Seychelles Marine Spatial Plan affords complete protection to some islands, others are listed as sustainable use zones and remain open to high-value industries such as tourism and catch-and-release fisheries. However, the effects of catch-and-release on target recreational fishery species remain unknown (Moxham 2019). In addition to catch-and-release mortality, future studies should focus on identifying spawning areas and the distribution of recreationally important species, especially in the context of future protected and non-protected areas.

SWIO countries such as Seychelles have recently suffered the crippling effects of a crashing tourism industry due to Covid-19 induced travel restrictions. As SWIO nations develop economic recovery plans, rebuilding their economies inclusive of sustainable fishing practices and improved recreational fishery regulations may provide a glimmer of hope for enticing recreational fishers to return to these destinations, while simultaneously establishing long-term sustainable fishing practices for generations to come.

5 REFERENCES

Adamack AT, Gruber B. 2014. PopGenReport : Simplifying basic population genetic analyses in R. Methods in Ecology and Evolution 5: 384–387.

Adams AJ, Wolfe RK, Tringali MD, Wallace EM, Kellison GT. 2007. Rethinking the status of Albula spp. biology in the Caribbean and Western Atlantic. In: Ault JS (ed), Biology and management of the world tarpon and bonefish fisheries. Boca Raton, Florida: CRC Press. pp 203–214.

Adams AJ, Horodysky AZ, McBride RS, Guindon K, Shenker J, MacDonald TC et al. 2014. Global conservation status and research needs for tarpons (Megalopidae), ladyfishes (Elopidae) and bonefishes (Albulidae). Fish and Fisheries 15: 280–311.

Als TD, Hansen MM, Maes GE, Castonguay M, Riemann L, Aarestrup K et al. 2011. All roads lead to home: Panmixia of European eel in the Sargasso Sea. Molecular Ecology 20: 1333–46.

Andrews KR, Luikart G. 2014. Recent novel approaches for population genomics data analysis. Molecular Ecology 23: 1661–1667.

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. Nature Reviews Genetics 17: 81–92.

Andriamahefazafy M, Kull CA, Campling L. 2019. Connected by sea, disconnected by tuna? Challenges to regionalism in the Southwest Indian Ocean. Journal of the Indian Ocean Region 15: 58–77.

Arnold B, Corbett-Detig RB, Hartl D, Bomblies K. 2013. RADseq underestimates diversity and introduces genealogical biases due to non-random haplotype sampling. Molecular Ecology 22: 3179–3190.

Attwood CG, Bennett BA. 1995. Modelling the effect of marine reserves on the recreational shore-fishery of the South-Western Cape, South Africa. South African Journal of Marine Science 16: 227–240.

Ault JS (ed). 2007. Biology and management of the world tarpon and bonefish fisheries. Boca Raton: CRC Press

Babin C, Gagnaire P-A, Pavey SA, Bernatchez L. 2017. RAD-Seq reveals patterns of additive polygenic variation caused by spatially-varying selection in the American eel (*Anguilla rostrata*). Genome Biology and Evolution 9: 2974–2986.

Badal RM, Rughooputh SDD V, Rydberg L, Robinson IIS, Pattiaratchi C. 2009. Eddy formation around South West Mascarene Plateau (Indian Ocean) as evidenced by satellite 'global ocean colour' data. Western Indian Ocean Journal of Marine Science 8: 139–145.

Baird NA, Etter PD, Atwood TS, Currey M C, Shiver AL, Lewis ZA et al. 2008. Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS ONE 3: e3376.

Basto MP, Santos-Reis M, Simões L, Grilo C, Cardoso L, Cortes H et al. 2016. Assessing Genetic structure in common but ecologically distinct carnivores: the stone marten and red fox. PLOS ONE 11: e0145165.

Beaumont MA, Balding DJ. 2004. Identifying adaptive genetic divergence among populations from genome scans. Molecular Ecology 13: 969–980.

Benestan L, Quinn BK, Maaroufi H, Laporte M, Clark FK, Greenwood et al. 2016. Seascape genomics provides evidence for thermal adaptation and current-mediated population structure in American lobster (Homarus americanus). Molecular Ecology 25: 5073–5092.

Benny PN. 2002. Variability of Western Indian Ocean currents. Western Indian Ocean Journal of Marine Science 1: 81–90.

Billington N. 2003. Mitochondrial DNA. In: Hallerman EM (ed.), Population genetics: principles and applications for Fisheries Scientists. Maryland: American Fisheries Soceity. pp 59–100.

Bivand R. 2007. Spdep. Spatial dependence: weighting schemes, statistics and models. R package version 0.4–9. Available: http://cran.r-project.org/web/packages/spdep/index.html [accessed 10 October 2017]

Blamey LC, Talma SCAL, Bodin N, Walton R, Bristol U, Shamlaye C et al. 2018. Past, present, future: an overview of marine research and biodiversity status in Seychelles. Paper presented at the Seychelles Ocean to Health Conference, Victoria, 8-10 November 2018. (unpublished)

Boistol L, Harper S, Booth S, Zeller D. 2011. Reconstruction of marine fisheries catches for Mauritius and its outer islands, 1950-2008. In: Harper S, Zeller D. (eds). Fisheries catch reconstructions: Islands, Part II. Vancouver: University of British Columbia. pp. 39-61.

Bonhommeau S, Blanke B, Tréguier A-M, Grima N, Rivot E. 2009. How fast can the European eel (*Anguilla anguilla*) larvae cross the Atlantic Ocean? Fisheries Oceanography 18: 371–85.

Bonhommeau S, Castonguay M, Rivot E, Sabatié R, Le Pape O. 2010. The duration of migration of Atlantic Anguilla larvae. Fish and Fisheries 11: 289–306.

Boucek RE, Lewis JP, Stewart BD, Jud ZR, Carey E, Adams AJ. 2019. Measuring site fidelity and homesite-to-pre-spawning site connectivity of bonefish (*Albula vulpes*): using mark-recapture to inform habitat conservation. Environmental Biology of Fishes 102: 185–195.

Breuil C, Grima D. 2014. Fisheries in the ESA-IO region: profile and trends country review 2014 Seychelles. Rome: FAO Fisheries Department.

Brownscombe JW, Danylchuk AJ, Adams AJ, Black B, Boucek R, Power M et al. 2019. Bonefish in South Florida: status, threats and research needs. Environmental Biology of Fishes 102: 329–348.

Butler G, Rogerson C. 2016. Inclusive local tourism development in South Africa: Evidence from Dullstrrom. The Journal of the local economy policy unit 31: 264–281.

Butler EC, Childs A-R, Parkinson MC, Potts WM. 2017. An assessment of the health and survival of fishes caught-and-released in high-energy surf zones during a South African competitive angling event. Fisheries Research 195: 152–168.

Butler EC, Childs AR, Duncan MI, Potts WM. 2020. Understanding the effects of recreational catch-and-release angling on an increasingly important foreign fishing tourism species, the giant African threadfin *Polydactylus quadrifilis* (Cuvier). Fisheries Management and Ecology 27: 603–614.

Campbell B, Hanich Q. 2014. Fish for the future: Fisheries development and food security for Kiribati in an era of global climate change. Penang: World Fish.

Campling L, Confiance H, Purvis M-T (eds). 2011. Social policies in Seychelles. London: Commonwealth Secretariat.

Cariou M, Duret L, Charlat S. 2016. How and how much does RAD-seq bias genetic diversity estimates?” BMC Evolutionary Biology 16: 1-8.

Cattell RB. 1966. The scree test for the number of factors. Multivariate Behavioral Research 1: 245-276.

Chang Y-LK, Miller MJ, Tsukamoto K, Miyazawa Y. 2018. Effect of larval swimming in the western North Pacific subtropical gyre on the recruitment success of the Japanese eel. PLOS ONE 13: e0208704.

Chattopadhyay B, Garg KM, Ramakrishnan U. 2014. Effect of diversity and missing data on genetic assignment with RAD-Seq markers. Biomedical Research Notes 7: 1-3.

Chen J-N, López JA, Lavoué S, Miya M, Chen W-J. 2014. Phylogeny of the Elopomorpha (Teleostei): Evidence from Six Nuclear and Mitochondrial Markers. Molecular Phylogenetics and Evolution 70: 152–61.

Christie IT, Crompton DE. 2003. Republic of Madagascar: tourism sector study. Africa Region working paper series no. 63. Washington, DC: World Bank.

Clarke JR, Buxton CD. 1989. A survey of the recreational rock-angling fishery at Port Elizabeth, on the south-east coast of South Africa. South African Journal of Marine Science 8: 183–194.

Clarke RD, Finelli CM, Buskey EJ. 2009. Water flow controls distribution and feeding behavior of two co-occurring coral reef fishes: II. Laboratory experiments. Coral Reefs 28: 475–488.

Cockcroft AC, Mackenzie AJ. 1997. The recreational fishery for West Coast rock lobster *Jasus lalandii* in South Africa. South African Journal of Marine Science 18: 75–84.

Cockerell LM, Jones PJS. 2020. Governance Analysis of St Anne Marine National Park, Seychelles. Marine Policy 103912.

Colborn J, Crabtree RE, Shaklee JB, Pfeiler E, Bowen BW. 2001. The evolutionary enigma of bonefishes (Albula spp.): cryptic species and ancient separations in a globally distributed shorefish. Evolution 55: 807–820.

Cooke SJ, Suski CD. 2005. Do we need species-specific guidelines for catch-and-release recreational angling to conserve diverse fishery resources? Biodiversity Conserservation 14: 1195-1209.

Cooke SJ, Suski CD, Danylchuk SE, Danylchuk AJ, Donaldson MR, Pullen C et al. 2008. Effects of different capture techniques on the physiological condition of bonefish *Albula vulpes* evaluated using field diagnostic tools. Journal of Fish Biology 73: 1351–75.

Crochelet E, Roberts J, Lagabrielle E, Obura D, Petit M, Chabanet P. 2016. A model-based assessment of reef larvae dispersal in the Western Indian Ocean reveals regional connectivity patterns — Potential implications for conservation policies. Regional Studies in Marine Science 7: 159–167.

Danylchuk SE, Danylchuk AJ, Cooke SJ, Goldberg TL, Koppelman J, Philipp DP. 2007. Effects of recreational angling on the post-release behavior and predation of bonefish (*Albula vulpes*): The role of equilibrium status at the time of release. Journal of Experimental Marine Biology and Ecology 346: 127–133.

Danylchuk AJ, Danylchuk SE, Cooke SJ, Goldberg TL, Koppelman J, Philipp DP. 2008. Ecology and management of bonefish (Albula spp) in the Bahamian Archipelago. In: Ault JS (ed), Biology and management of the world tarpon and bonefish fisheries. Boca Raton, USA. pp 79–92.

Danylchuk AJ, Cooke SJ, Goldberg TL, Suski CD, Murchie KJ, Danylchuk SE et al. 2011. Aggregations and offshore movements as indicators of spawning activity of bonefish (*Albula vulpes*) in the Bahamas. Marine Biology 158: 1981–1999.

Davey JW, Blaxter ML. 2010. RADSeq: Next-generation population genetics. Briefings in Functional Genomics 9: 416–423.

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML. 2011. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics 12: 499–510.

Davies MTT. 2002. The development of flyfishing as a recreational sport in Southern Africa. In: Coleman APM (ed), Proceedings of the 3rd World Recreational Fishing Conference, 21-24 may. Northern Territory, Australia. Fisheries Group, Department of Business, Industry and Resource Development. pp 209-211.

De Lestang. 2005. Report on existing and potential employment opportunities in the fisheries sector in Seychelles. Report prepared for the Ministry of Fisheries and Agriculture. Seychelles Fishing Authority, Victoria.

De Young C (ed). 2006. Review of the state of the World marine capture fisheries management: Indian Ocean. Rome: FAO.

Debrot D, Posada JM. 2005. A brief description of the bonefish recreational fishery in Los Roques Archipelago National Park, Venezuela. Marine Science 37: 61–65.

DeFaveri J, Shikano T, Shimada Y, Merila J. 2013. High degree of genetic differentiation in marine three-spined sticklebacks (*Gasterosteus aculeatus*). Molecular Ecology 22: 4811–4828.

Díaz-Viloria N, Sánchez-Velasco L, Perez-Enriquez R, Zárate-Villafranco A, Miller MJ, Jiménez-Rosenberg SPA. 2017. Morphological description of genetically identified Cortez bonefish (*Albula gilberti*, Pfeiler and van der Heiden 2011) leptocephali from the southern Gulf of California. Mitochondrial DNA Part A 28: 717-724.

DiBattista JD, Travers MJ, Moore GI, Evans RD, Newman SJ, Feng M et al. 2017. Seascape genomics reveals fine-scale patterns of dispersal for a reef fish along the ecologically divergent coast of Northwestern Australia. Molecular Ecology 26: 6206–6223.

Donohue KA, Toole JM. 2003. A near-synoptic survey of the Southwest Indian Ocean. Deep-Sea Research Part II: Topical Studies in Oceanography 50: 1893–1931.

Donovan S, Frank P, Yongjiu C, Brian L. 2012. Phylogeography of Anguilla Marmorata (Teleostei: Anguilliformes) from the Eastern Caroline Islands. Ichthyological Research 59: 70–76.

Donovan MK, Friedlander AM, Harding KK, Schemmel EM, Filous A, Kamikawa K, Torkelson N. 2015. Ecology and niche specialization of two bonefish species in Hawaiʻi. Environmental Biology of Fishes 98: 2159–2171.

Dray S, Dufour AB. 2007. The ade4 package: Implementing the duality diagram for ecologists. Journal of Statistic Software 22: 1–20.

Drummond A, Ashton B, Buxton S, Cheung M, AC. 2010. Geneious version 6.1. Available at http://www.geneious.com/ [accessed 18 March 2017].

Du Preez M, Lee DE. 2010. The contribution of trout fly fishing to the economy of Rhodes, North Eastern Cape, South Africa. Development Southern Africa 27: 241–53.

Du Preez M, Hosking ST. 2011. The value of the trout fishery at Rhodes, North Eastern Cape, South Africa: a travel cost analysis using count data models. Journal of Environmental Planning and Management 54: 267–82.

Duforet-Frebourg N, Blum M. 2014. User manual of PCAdapt Version 1.6. Grenoble, France. Accessible at: http://membres-timc.imag.fr/Michael.Blum/PCAdapt/Man.pdf [accessed 09 September 2019].

Duncan M. 2013. The genetic stock structure and distribution of Chrysoblephus. MSc Thesis, Rhodes University, South Africa.

Eakins BW, Sharman GF. 2010. Volumes of the World's Oceans from ETOPO1, NCEI. Available at https://www.ngdc.noaa.gov/mgg/global/etopo1_ocean_volumes.html [12 September 2020].

Eaton NR, Krueger RF, South SC, Gruenewald TL, Seeman TE, Roberts BW. 2012. Genes, environments, personality, and successful aging: toward a comprehensive developmental model in later life. The Journals of Gerontology 67: 480–488.

Eaton DAR. 2014. PyRAD: Assembly of de novo RADseq loci for phylogenetic analyses. Bioinformatics 30: 1844–1849.

Eaton DAR, Spriggs EL, Park B, Donoghue MJ. 2017. Misconceptions on missing data in RAD-seq phylogenetics with a deep-scale example from flowering plants. Systematic Biology 66: 399–412.

Edgar RC. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32: 1792–1797.

Engelhardt BE, Stephens M. 2010. Analysis of population structure: A unifying framework and novel methods based on sparse factor analysis. PLOS Genetics. 6: e1001117.

Ernesta, Sharon. 2020. $700,000 available for local projects supporting sustainable management and conservation in Seychelles. Seychelles News Agency. Available at http://www.seychellesnewsagency.com/articles/13275/%2C+available+for+local+projects+supporting+sustainable+management+and+conservation+in+Seychelles. [accessed 10 December 2020]

Ewens WJ. 1972. The sampling theory of selectively neutral alleles. Theoretical population biology 3: 87–112.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human Mitochondrial DNA restriction data. Genetics 479–491.

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

FAO (Food and Agriculture Organization of the United Nations). 2006. Review of the state of world marine capture fisheries management. Rome: FAO Fisheries Department.

FAO (Food and Agriculture Organization of the United Nations). 2017. Globefish highlights a quarterly update on world seafood markets. Rome: FAO Fisheries Department.

FAO (Food and Agriculture Organization of the United Nations). 2018. The state of world fisheries and aquaculture: Meeting the sustainable development goals. Rome: FAO Fisheries Department.

Fedler AJ, Hayes C, Consulting H, Hayes C. 2008. Economic impact of recreational fishing for bonefish, permit and tarpon in Belize for 2007. Report prepared for Turneffe Atoll Trust Gainesville, Florida. Available at http://www. turneffeatoll.org/tat-action-plan/economic-study [accessed 01 February 2020]

Fedler T. 2010. The Economic Impact of Flats Fishing in The Bahamas. Report prepared for the Everglades Foundation. Palmetto Bay, Florida. Available at https://www.bonefishtarpontrust.org/downloads/researchreports/stories/bahamas-flats-economic-impact-report.pdf [accessed 14 June 2018].

Fedler T. 2013. Economic Impact of the Florida Keys Flats Fishery. Report prepared for the Bonefish and Tarpon Trust. Gainesville, Florida. Available at https://www.bonefishtarpontrust.org/downloads/researchreports/stories/BTT%20-%20Keys%20Economic%20 Report.pdf [accessed 10 April 2018]

Filleul A, Lavoué S. 2001. Basal teleosts and the question of elopomorph monophyly. Morphological and molecular approaches. Life Sciences 324: 393–399.

Filous A, Lennox RJ, Eric EG, Danylchuk C, Danylchuk AJ. 2019a. Fisheries selectivity and annual exploitation of the principal species harvested in a data-limited artisanal fishery at a remote atoll in French Polynesia. Ocean and Coastal Management 178: 1-13.

Filous A, Lennox RJ, Coleman RR, Friedlander AM, Clua EEGG, Danylchuk AJ. 2019b. Life-history characteristics of an exploited bonefish *Albula glossodonta* population in a remote South Pacific atoll. Journal of Fish Biology 95: 562–574.

Foll M, Gaggiotti O. 2008. A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A bayesian perspective. Genetics 180: 977–993.

Foll, M. 2012. BayeScan v2.1 user manual. Ecology 20: 1450-1462.

Frezza PE, Clem SE. 2015. Using local fishers' knowledge to characterize historical trends in the Florida Bay bonefish population and fishery. Environmental Biology of Fishes 98: 2187–2202.

Frichot E, Mathieu F, Trouillon T, Bouchard G, François O. 2014. Fast and efficient estimation of individual ancestry coefficients. Genetics 196: 973–83.

Frichot E, François O. 2015. LEA : An R package for landscape and ecological association studies. Methods in Ecology and Evolution 6: 925–929.

Fricke H and Hissmann K. 2000. Feeding ecology and evolutionary survival of the living coelacanth *Latimeria chalumnae*. Marine Biology 136:379-386.

Friedlander A, Caselle J, Beets J, Lowe C, Bowen B, Ogawa T et al. 2007. Biology and ecology of the recreational bonefish fishery at Palmyra Atoll National Wildlife Refuge with comparisons to other Pacific Islands. In: Ault JS (ed), Biology and Management of the World Tarpon and Bonefish Fisheries. Florida: CRC Press Taylor & Francis Group. pp 27–56.

Fu YX, Li WH. 1993. Statistical tests of neutrality of mutations. Genetics 133: 693-709.

Fu YX. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915–25.

Gagnaire P-A, Minegishi Y, Zenboudji S, Valade P, Aoyama J, Berrebi P. 2011. Within-population structure highlighted by differential introgression across semipermeable barriers to gene flow in *Anguilla marmorata*. Evolution 65: 3413–3427.

Gagnaire P-A, Normandeau E, Côté C, Hansen MM, Bernatchez L. 2012. The genetic consequences of spatially varying selection in the panmictic American eel (*Anguilla rostrata*). Genetics 190: 725–736.

Gagnaire P-A, Broquet T, Aurelle D, Viard F, Souissi A, Bonhomme F et al. 2015. Using neutral, selected, and hitchhiker loci to assess connectivity of marine populations in the genomic era. Evolutionary Applications 8 : 769–86.

Galaiduk R, Radford BT, Saunders BJ, Newman SJ, Harvey ES. 2017. Characterizing ontogenetic habitat shifts in marine fishes: advancing nascent methods for marine spatial management. Ecological Applications 27: 1776–88.

Gamoyo M, Obura D, Chris JC. Reason C. 2019. Estimating connectivity through larval dispersal in the Western Indian Ocean. Journal of Geophysical Research: Biogeosciences 124: 2446–59.

Gaut BS, Long AD. 2003. The lowdown on linkage disequilibrium. The Plant cell 15: 1502–1506.

Gautier M, Gharbi K, Cezard T, Foucaud J, Kerdelhué C, Pudlo P et al. 2013. The effect of RAD allele dropout on the estimation of genetic variation within and between populations. Molecular Ecology 22: 3165–3178.

Glass J, Daly R Cowley P, Post DM. 2020. Spatial trophic variability of a coastal apex predator, the giant trevally *Caranx ignobilis*, in the Western Indian Ocean. Marine Ecology Progress Series 641: 195-208.

Gomes I, Collins A, Lonjou C, Thomas NS, Wilkinson J, Watson M, Morton N. 1999. Hardy – Weinberg quality control. Annual Human Genetics 63: 535–538.

Gosselin T. 2017. Radiator: RADseq data exploration, manipulation and visualization using R. Available at https://github.com/thierrygosselin/radiator [accessed 11 May 2018]

Goudet J. 2005. Hierfstat, a package for R to compute and test hierarchical F-statistics. Molecular Ecology Notes 5: 184–186.

Grandcourt EM, Hecht T, Booth AJ, Robinson Grandcourt J. 2008. Retrospective stock assessment of the Emperor red snapper (*Lutjanus sebae*) on the Seychelles Bank between 1977 and 2006. ICES Journal of Marine Science 65: 889 – 898.

Grant WS, Bowen BW. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. Journal of Heredity 89: 415–426.

Grewe F, Huang J-P, Leavitt SD, Lumbsch HT. 2017. Reference-based RADseq resolves robust relationships among closely related species of lichen-forming fungi using metagenomic DNA. Scientific Reports 7: 9884.

Griffiths CL, Robinson TB, Lange L, Mead A. 2010. Marine biodiversity in South Africa: An evaluation of current states of knowledge. PLoS ONE 5: e12008.

Griffiths CL, Robinson TB. 2016. Use and usefulness of measures of marine endemicity in South Africa. South African Journal of Science 112: 1–7.

Groeneveld JC, Koranteng KA (eds). 2017. The RV Dr Fridtjof Nansen in the Western Indian Ocean: Voyages of marine research and capacity development. Rome: Food and Agricultural Organisation.

Gruber B, Unmack PJ, Berry OF, Georges A. 2018. DartR: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. Molecular Ecology Resources 18 : 691 – 699.

Gubili C, Schabetsberger C, Poellabauer B, Bates RM. Wagstaff LM, Woodward US et al. 2019. High genetic diversity and lack of pronounced population structure in five species of sympatric pacific eels. Fisheries Management and Ecology 26: 31–41.

Guo X, Liu S, Liu Y. 2006 Evidence for recombination of mitochondrial DNA in triploid crucian carp. Genetics. 172:1745-9.

Haak CR, Power M, Cowles GW, Danylchuk AJ. 2019. Hydrodynamic and isotopic niche differentiation between juveniles of two sympatric cryptic bonefishes, *Albula vulpes* and *Albula goreensis*. Environmental Biology of Fishes 102: 129–145.

Hallier JP, Fonteneau A. 2015. Tuna aggregation and movement from tagging data: A tuna "hub" in the Indian Ocean. Fisheries Research 163: 34-45.

Hamblin MT, Mitchell SE, White GM, Gallego J, Kukatla R, Wing RA et al. 2004. Comparative population genetics of the panicoid grasses: sequence polymorphism, linkage disequilibrium and selection in a diverse sample of *Sorghum bicolor*. Genetics 167: 471- 183.

Hamylton S, Hagan A, Bunbury N, Fleischer-Dogley F, Spencer T. 2018. Mapping the lagoon at Aldabra Atoll, Western Indian Ocean. Atoll Research Bulletin 619: 45–59.

Han YS, Chia LH, Yi FL, Tzeng WN. 2010. Population genetic structure of the Japanese eel *Anguilla japonica*: panmixia at spatial and temporal scales. Marine Ecology Progress Series 401: 221–32.

Hardisty F, Robinson A. 2011. The GeoViz toolkit: using component-oriented coordination methods for geographic visualization and analysis. International journal of geographical information science 25: 191-210.

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

Harrison RG. 1989. Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. Trends in Ecology and Evolution 4: 6-11

Healey AJE, Gouws G, Fennessy ST, Kuguru B, Sauer WHH, Shaw PW, McKeown NJ. 2018. Genetic analysis reveals harvested *Lethrinus nebulosus* in the Southwest Indian Ocean comprise two cryptic species. ICES Journal of Marine Science 75: 1465–1472.

Hebert PDN, Stoeckle MY, Zemlak TS, Francis CM. 2004. Identification of birds through DNA barcodes. PLoS Biology 2: e312.

Helyar SJ, hemmer-hansen J, bekkevold J, Taylor MI, Ogden MI, Limborg MT et al. 2011. Application of SNPs for population genetics of nonmodel organisms: New opportunities and challenges. Molecular Ecology Resources 11: 123–36.

Hidaka K, Yukio A, Ae I, Randall JE. 2008. A review of the Indo-Pacific bonefishes of the *Albula argentea* complex, with a description of a new species. Ichthyological Research 55: 53–64.

Hidaka K, Tsukamoto Y, Iwatsuki Y. 2017. Nemoossis, a new genus for the eastern Atlantic long-fin bonefish *Pterothrissus belloci* Cadenat 1937 and a redescription of *P. gissu* Hilgendorf 1877 from the north-western Pacific. Ichthyological Research 64: 45–53.

Hilton ZM, Wellenreuther M, Clements KD. 2008. Physiology underpins habitat partitioning in a sympatric sister-species pair of intertidal fishes. Functional Ecology 22: 1108–1117

Hoogendoorn G. 2014. Mapping fly-fishing tourism in Southern Africa. African Journal of Hospitality, Tourism and Leisure 3: 1–13.

Hoogendoorn G. 2017. Fly-fishing as ecotourism in South Africa: a case study. Journal of Ecotourism 16: 152–68.

Hsu HY, Chen SW, Han Y-S. 2019. Habitat partitioning and its possible genetic background between two sympatrically distributed eel species in Taiwan. Zoological studies. 58: 1-13

Huang H, Knowles LL. 2014. Unforeseen consequences of excluding missing data from next-generation sequences: Simulation study of RAD Sequences. Systematic Biology 65: 357–365.

Hudson ME. 2008. Sequencing breakthroughs for genomic ecology and evolutionary biology. Molecular Ecology Resources 1: 3-17.

Humston R, Ault J, Larkin M, Luo J. 2005. Movements and site fidelity of the bonefish *Albula vulpes* in Northern Florida Keys determined by acoustic telemetry. Marine Ecology Progress Series 291: 237–248.

Hurst GDD, Jiggins FM. 2005. Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: The effects of inherited symbionts. Proceedings of the Royal Society B: Biological Sciences 272: 1525–1534

Huxley J. 1938. Clines an auxilliary taxonomic principle. Nature 142: 291-220.

Inoue JG, Miya M, Tsukamoto K, Nishida M. 2004. Mitogenomic evidence for the monophyly of elopomorph fishes (Teleostei) and the evolutionary origin of the leptocephalus larva. Molecular Phylogenetics Evolution 32: 274–286

Janžekovič F, Novak T. 2012. PCA – a powerful method for analyzing ecological niches. In: Sanguansat P. (ed), Principal Component Analysis. Rijeka: IntechOpen. Pp 127-142.

Johannes RE, Freeman MMR, Hamilton RJ 2008. Ignore fishers' knowledge and miss the boat. Fish and Fisheries 1: 257–271.

Jombart T. 2008. adegenet: An R package for the multivariate analysis of genetic markers. Bioinformatics: 24: 1403–1405.

Jombart T, Devillard S, Dufour A-B, Pontier D. 2008. Revealing cryptic spatial patterns in genetic variability by a new multivariate method. Heredity 10134: 92–103.

Jombart T, Pontier D, Dufour A-B. 2009. Genetic markers in the playground of multivariate analysis. Heredity 330–341.

Jombart T, Devillard S, Balloux F. 2010. Discriminant Analysis of Principal Components: a new method for the analysis of genetically structured populations. BMC Genetics 11: 94.

Jombart T 2012a. A tutorial for Discriminant Analysis of Principal Components (DAPC) using adegenet 1 . 3-4. Rvignette 1–37. Available at http://adegenet.r-forge.r-project.org/files/tutorial-dapc.pdf [accessed 05 February 2018].

Jombart T. 2012b. A tutorial for the spatial Analysis of Principal Components using adegenet 1.3-4. 1–50. Available at http://adegenet.r-forge.r-project.org/files/tutorial-spca.pdf [accessed 06 February 2018].

Jombart T. 2017. An introduction to adegenet 1.3-4. Rvignette 1–67. Available at https://adegenet.r-forge.r-project.org/files/tutorial-basics.pdf [accessed 10 May 2018].

Jordan DS, Jordan EK. 1922. A list of the fishes of Hawaii, with notes and descriptions of new species. Memoirs of the Carnegie Museum 10:1–92.

Kadagi NI, Wambiji N, Swisher ME. 2020. Potential for conflicts in recreational and artisanal billfish fisheries on the coast of Kenya. Marine Policy 117: 103960.

Kamikawa KT, Friedlander AM, Harding KK, Filous A, Donovan MK, Schemmel E. 2015. Bonefishes in Hawai'i and the importance of angler-based data to inform fisheries management. Environmental Biology of Fishes 98: 2147–2157.

Kamvar ZN, Tabima JF, Grünwald NJ. 2014. Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2: e281.

Kariuki J. 2005. Country review: Kenya. In: de Young, C (ed), Review of the state of the world marine capture fisheries management: Indian Ocean. Rome: FAO. pp. 349-364

Kimani EN, Okemwa GM, Kazungu JM. 2008. Fisheries in the Southwest Indian Ocean: trends and governance. In: Laipson E, Pandya A (eds), The Indian Ocean: resource and governance challenges. Washington: The Henry L. Stimson Center. pp 3–17.

Knutsen HM, Olsen PE, Jorde SH, Espeland CA, Stenseth NC. 2011. Are low but statistically significant levels of genetic differentiation in marine fishes 'biologically meaningful'? A case study of coastal Atlantic cod. Molecular Ecology 20: 768–83.

Kornfield I, Smith PF. 2000. African cichlid fishes: Model systems for evolutionary biology. Annual Review of Ecology and Systematics 31: 163–196.

Kudo K. 2001. Larval vertical-migration strategy of Japanese eel. Proceedings MTS/IEEE Oceans 2001: An Ocean Odyssey Conference5-8 November, Honolulu, Hawaii. Hawaii: The marine technology soceity. pp 870–875.

Kwun HJ, Kim JK. 2011. A new species of bonefish, *Albula koreana* (Albuliformes: Albulidae) from Korea and Taiwan. Zootaxa 2903: 57–63.

Langille BL, O'Leary KE, Whitney HG, Marshall HD. 2014. Mitochondrial DNA diversity and phylogeography of insular Newfoundland red foxes (*Vulpes deletrix*). Journal of Mammalogy 95: 772–780.

Laporte M, Pavey SA, Rougeux C, Pierron F, Lauzent M, Budzinski H et al. 2016. RAD sequencing reveals within-generation polygenic selection in response to anthropogenic organic and metal contamination in North Atlantic Eels. Molecular Ecology 25: 219–237.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Higgins DG. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-2948.

Larkin MF, Ault JS, Humston R, Luo J. 2010. A mail survey to estimate the fishery dynamics of southern Florida's bonefish charter fleet. Fisheries Management and Ecology 17: 254–261.

Larkin MF. 2011. Assessment of South Florida' s Bonefish Stock. PhD thesis, University of Miami, USA.

Lascelles B, Di Sciara GD, Agardy T, Cuttelod A, Eckert S, Glowka L et al. 2014. Migratory marine species: Their status, threats and conservation management needs. Aquatic Conservation: Marine and Freshwater Ecosystems 24: 111–127.

Lecomte M, Julien R, Yann L, Renaud L. 2017. Indian Ocean tuna fisheries: between development opportunities and sustainability issues. Available at https://www.iddri.org/en/publications-and-events/report/indian-ocean-tuna-fisheries-between-development-opportunities-and [accessed 10 November 2020].

Ledesma RD, Valero-Mora P, Macbeth G. 2015. The scree test and the number of factors: a dynamic graphics approach. Spanish Journal of Psychology. 18: 1-10.

Lever J, Krzywinski M, Altman N. 2017. Points of Significance: Principal component analysis. Nature Methods 14: 641–642.

Lewis L, Biersma E, Carey S, Holsinger K, McDaniel S, Rozzi R, Goffinet B. 2017. Resolving the northern hemisphere source region for the long-distance dispersal event that gave rise to the South American endemic dung moss *Tetraplodon fuegianus*. American Journal of Botany 104: 1–9

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452.

Linck EB, Battey CJ. 2019. Minor allele frequency thresholds strongly affect population structure inference with genomic datasets. Molecular Ecology Resources 19: 639–647.

Liu B-J, Li Y-L, Zhang B-D, Liu J-X. 2020. Genome-wide discovery of single-nucleotide polymorphisms and their application in population genetic studies in the endangered Japanese eel (*Anguilla Japonica*). Frontiers in Marine Science 6: 1-11.

Luu K, Bazin E, Blum MGB. 2017. pcadapt : an R package to perform genome scans for selection based on principal component analysis. Molecular Ecology Resources 17: 67–77.

Mann BQ, James NC, Beckley LE. 2011. An assessment of the recreational fishery in the St Lucia estuarine system, KwaZulu-Natal, South Africa. South African Journal of Marine Science 24: 263–279.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. Cancer Research 27:209–220.

Marchese C. 2015. Biodiversity hotspots: a shortcut for a more complicated concept. Global Ecology and Conservation 3: 297–309.

Marroni F, Pinosio S, Zaina G, Fogolari F, Felice N, Cattonaro F, Morgante M. 2011. Nucleotide diversity and linkage disequilibrium in *Populus nigra* cinnamyl alcohol dehydrogenase (CAD4) gene. Tree Genetics and Genomes 7: 1011–1023.

Martins H, Caye K, Luu K, Blum MGB, François O. 2016. Identifying outlier loci in admixed and in continuous populations using ancestral population differentiation statistics. Molecular Ecology 25: 5029–5042.

McCormack JE, Maley JM, Hird SM, Derryberry EP, Graves GR, Brumfield RT. 2012. Next-generation sequencing reveals phylogeographic structure and a species tree for recent bird divergences. Molecular Phylogenetics and Evolution 62: 397–406.

McKeown NJ, Arkhipkin AI, Shaw PW. 2016. Regional genetic population structure and fine scale genetic cohesion in the southern blue whiting *Micromesistius australis*. Fisheries Research 185: 176–184.

McKeown NJ, Hauser L, Shaw PW. 2017. Microsatellite genotyping of brown crab Cancer pagurus reveals fine scale selection and ‘non-chaotic’ genetic patchiness within a high gene flow system. Marine Ecology Progress Series 566: 91–103.

Meintjes P, Duran C, Kearse M, Moir R, Wilson A, Stones-Havas S, et al. 2012. Geneious Basic: An Integrated and Extendable Desktop Software Platform for the Organization and Analysis of Sequence Data. Bioinformatics 28:1647–49.

Miah G, Rafii MY, Ismail M, Puteh A, Rahim H. 2013. Microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. International Journal of Molecular Sciences 14: 22499-22528.

Miller MJ, Aoyama J, Mochioka N, Otake T, Castle PHJ, Minagawa G et al. 2006. Geographic variation in the assemblages of leptocephali in the western South Pacific. Deep-Sea Research Part I: Oceanographic Research Papers 53: 776–794.

Miller MJ, Wouthuyzen S, Feunteun E, Aoyama J, Watanabe S, Syahailatua A, Kuroki M, Robinet T, Hagihara S, Otake T et al. 2019. Contrasting biodiversity of eel larvae across the central Indian Ocean subtropical gyre. Deep Sea Research Part II: Tropical Studies in Oceanography 161: 120–131.

Minegishi Y, Gagnaire P, Aoyama J, Bosc P, Feunteun E, Tsukamoto K, Berrebi P. 2012. Present and past genetic connectivity of the Indo-Pacific tropical eel *Anguilla bicolor*. Journal of Biogeography 39: 408–420.

MMT (Mauritius Ministry of Tourism). 2019. Annual report on performance for the financial year 2018/2019. Mauritius Ministry of Tourism, Mauritius. Available at https://tourism.govmu.org/Documents/publication/Annual%20Report%20Performance1819.pdf [accessed 12 October 2020]

Mojica, R, Shenker, JM, Harnden, CW, Wagner, DE. 1995. Recruitment of bonefish, *Albula vulpes*, around Lee Stocking Island, Bahamas. Fishery Bulletin 93: 666–674.

Mora C, Myers RA, Coll M, Libralato S, Pitcher TJ, Sumaila RU et al. 2009. Management effectiveness of the world’s marine fisheries. PLoS Biology 7: e1000131.

Morin PA, Luikart G, Wayne RK. 2004. SNPs in ecology, evolution and conservation. Trends in Ecology and Evolution 19: 208–216.

Moritz C, Dowling TE, Brown WM. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. Annual Review of Ecology and Systematics 18: 269–292.

Moxham EJ. 2017. The spatial ecology of *Albula glossodonta* in the St. Joseph Atoll, Seychelles. MSc thesis. Rhodes University, South Africa.

Moxham EJ, Cowley PD, Bennett RH, Von Brandis RG. 2019. Movement and predation: a catch-and-release study on the acoustic tracking of bonefish in the Indian Ocean. Environmental Biology of Fishes 102: 365–81.

Mullins RB. 2017. Population genomics analysis of yellowfin tuna *Thunnus albacares* off South Africa reveals need for a shifted management boundary. MSc thesis. Rhodes University, South Africa.

Mullins, RB, McKeown NJ, Sauer WHH, and Shaw PW. 2018. Genomic analysis reveals multiple mismatches between biological and management units in yellowfin tuna (*Thunnus Albacares*). ICES Journal of Marine Science 75: 2145–52.

Muths D, Gouws G, Mwale M, Tessier E, Bourjea J. 2012. Genetic connectivity of the reef fish *Lutjanus kasmira* at the scale of the Western Indian Ocean. Canadian Journal of Fisheries and Aquatic Sciences 69: 842–853.

Mzingirwa FA, Mkare TK, Nyingi DW, Njiru J. 2019. Genetic diversity and spatial population structure of a deepwater snapper, *Pristipomoides filamentosus* in the South-West Indian Ocean. Molecular Biology Reports 46: 5079–5088.

Nei M. 1987. Molecular evolutionary genetics. New York: Columbia University Press

Nelson JS. 2006. Fishes of the world fourth edition. New Jersey: John Wiley and Sons. pp 601.

Obermiller LE, Pfeiler E. 2003. Phylogenetic relationships of elopomorph fishes inferred from mitochondrial ribosomal DNA sequences. Molecular Phylogenetics and Evolution 26: 202–214.

Olson DM, and Dinerstein E. 1998. The global 200: A representative approach to conserving the earth's distinctive ecoregions. Conservation Biology 12: 502-515.

Otwoma LM, Reuter H, Timm J, Meyer A. 2018. Genetic connectivity in a herbivorous coral reef fish (*Acanthurus leucosternon* Bennet, 1833) in the Eastern African Region." Hydrobiologia 806: 237–50.

Palm S, Dannewitz J, Prestegaard T, Wickström H. 2009. Panmixia in European eel revisited: No genetic difference between maturing adults from Southern and Northern Europe. Heredity 103 : 82–89.

Palumbi SR. 1994. Genetic divergence, reproductive isolation, and marine speciation. Annual Review of Ecology and Systematics 25: 547–572.

Panhuis TM, Swanson WJ. 2006. Molecular evolution and population genetic analysis of candidate female reproductive genes in Drosophila. Genetics 173: 2039–47.

Paula J (ed). 2015. The regional state of the coast report – a summary for policy makers: Western Indian Ocean. Nairobi: Nairobi Convention Secretariat.

Pereira M, Litulo C, Santos R, Leal MC, Fernandes R, Tibrica Y et al. 2014. Mozambique Marine Ecosystems Review. Final report submitted to Fondation Ensemble. Maputo: Biodinâmica/CTV.

Perez AU, Schmitter-Soto JJ, Adams AJ, Heyman WD. 2019. Connectivity mediated by seasonal bonefish (Albula vulpes) migration between the Caribbean Sea and a tropical estuary of Belize and Mexico. Environmental Biology of Fishes 102: 197–207.

Pérez-Portela R, Bumford A, Coffman B, Wedelich S, Davenport M, Fogg A, Swenarton MK, Coleman F, Johnston MA, Crawford DL et al. 2018. Genetic homogeneity of the invasive lionfish across the Northwestern Atlantic and the Gulf of Mexico based on Single Nucleotide Polymorphisms. Scientific Reports 8: 5062.

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE. 2012. Double digest RADseq: an inexpensive method for De Novo SNP discovery and genotyping in model and non-model species. PLoS ONE 7: e37135.

Peterson CB, Bogomolov M, Benjamini Y, Sabatti C. 2016. Many phenotypes without many false discoveries: error controlling strategies for multitrait association studies. Genetic Epidemiology 40: 45–56.

Pfeiler E. 1986. Towards an explanation of the developmental strategy in leptocephalous larvae of marine teleost fishes. Environmental Biology of Fishes 15: 3–13.

Pfeiler E. 1996. Allozyme differences in Caribbean and gulf of California populations of bonefishes (Albula). Copeia 1: 181–183

Pfeiler E. 1999. Developmental physiology of elopomorph leptocephali. Molecular and Integrative Physiology 123: 113–128.

Pfeiler E, Bitler BG, Ulloa RL 2006. Phylogenetic Relationships of the Shafted Bonefish *Albula nemoptera* (Albuliformes: Albulidae) from the Eastern Pacific Based on Cytochrome b Sequence Analyses. Copeia 4: 778–784.

Pfeiler E, Watts T, Pugh J, Van Der Heiden AM. 2008. Speciation and demographic history of the Cortez bonefish, Albula sp. A (Albuliformes: Albulidae), in the Gulf of California inferred from mitochondrial DNA. Journal of Fish Biology 73: 382–394.

Pickett BD, Wallace EM, Ridge PG, Kauwe JSKK. 2020. Lingering taxonomic challenges hinder conservation and management of global bonefishes. Fisheries 45: 347–358.

Pous S, Feunteun E, Ellien C. 2010. Investigation of tropical eel spawning area in the South-Western Indian Ocean: Influence of the oceanic circulation. Progress in Oceanography 86: 396–413.

Purcell SW, Ngaluafe P, Wang G, Lalavanua W. 2017. Market value of flower teatfish ("pentard"): A highly exploited Indian Ocean holothuroid. SPC Beche-de-mer Information Bulletin 37: 53-55.

Ramírez-Soriano A, Ramos-Onsins SE, Rozas J, Calafell F, Navarro A. 2008. Statistical power analysis of neutrality tests under demographic expansions, contractions and bottlenecks with recombination. Genetics 179: 555–567.

Ramos-Onsins SE, Rozas J. 2002. Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution 19: 2092–2100.

Randall JE, Bauchot M. 1999. Clarification of the two Indo-Pacific species of bonefishes, *Albula glossodonta* and *Albula forsteri*. Cybium 23: 79–83.

Rao CR. 1964. The use and interpretation of principal component analysis in applied research. Sankhya: The Indian Journal of Statistics 26: 329–359.

Rattle J. 2019. A case study on the management of yellowfin tuna by the Indian Ocean Tuna Commission Report No. IOTC-2019-S23-INF14. Blue Marine Foundation, London

Raymond M, Rousset F. 1995. Genepop (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248–249.

Reich D, Price AL, Patterson N. 2008. Principal component analysis of genetic data. Nature genetics 40: 491–492

Rennert JJ, Shenker JM, Angulo-Valdés JA, Adams AJ. 2019. Age, growth, and age at maturity of bonefish (Albula species) among Cuban habitats. Environmental Biology of Fishes 102:253–265.

Ridgway T, Sampayo EM. 2007. Population Genetic Status of the Western Indian Ocean: What do we Know? Western Indian Ocean Journal of Marine Science 4: 1–10.

Robinet T, Feunteun E, Keith P, Marquet G, Olivier JM, Réveillac E, Valade P. 2007. Eel community structure, fluvial recruitment of *Anguilla marmorata* and indication for a weak local production of spawners from rivers of Réunion and Mauritius islands. Environmental Biology of Fishes. 78: 93–105

Rogers AR, Harpending H. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9: 552–569.

Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sanchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34: 3299–3302.

Ryynänen HJ, Tonteri A, Vasemägi A, Primmer CR. 2007. A comparison of biallelic markers and microsatellites for the estimation of population and conservation genetic parameters in Atlantic salmon (*Salmo salar*). Journal of Heredity 98: 692–704.

Saayman M, Saayman AZ, Potts W, Mann B, Weyl O, Van der Merwe et al. 2017. Economic significance of recreational angling in South Africa 2017. Potchefstroom: Tourism Research in Economic Environs & Society. Available at: http://www.sacraa.co.za/wpcontent/uploads/2018/05/Recreational-fishing-report-2017-2.pdf [accessed 2 August 2018]

Sabarros PS, Romanov E V, Bach P. 2017. Movements and behaviour of yellowfin and bigeye tuna associated to oceanic structures in the Western Indian Ocean. Report IOTC–2017–WPTT19–25 prepared for IOTC. Indian Ocean Tuna Commission, Victoria, Seychelles.

Sambaouma HM, Resler M, Murray J, Quintal D, Lenzen M. 2016. A vision of ecotourism in the Comoros Islands. University of Sydney, Sydney. Available at https://isa.org.usyd.edu.au/publications/ComorosEcotourismVision.pdf [accessed 10 May 2018]

Santos RO, Rehage JS, Adams AJ, Black BD, Osborne J, Kroloff EKN. 2017. Quantitative assessment of a data-limited recreational bonefish fishery using a time-series of fishing guides reports. PLOS ONE 12: e0184776.

Santos RO, Schinbeckler R, Viadero N, Larkin MF, Rennert JJ, Shenker JM, Rehage JS. 2019. Linking bonefish (*Albula vulpes*) populations to nearshore estuarine habitats using an otolith microchemistry approach. Environmental Biology of Fish 102: 267–283.

SCAA (Seychelles Civil Aviation Authority). 2021. History of aviation in Seychelles. Available at https://www.scaa.sc/index.php/about-us/history-of-aviation-in-seychelles [accessed 10 March 2021].

Seyoum S, Wallace EM, Tringali MD. 2008. Twelve polymorphic microsatellite markers for the bonefish, Albula vulpes and two congeners. Molecular Ecology Resources 8: 354–356.

SFA (Seychelles Fishing Authority). 2018. Seychelles Fishing Authority Strategic Plan 2018-2020 Volume 1 Goals and strategies. Victoria: SFA Fisheries Department.

Shaklee JB, Tamaru CS. 1981. Biochemical and morphological evolution of Hawaiian bonefishes (Albula). Systematic Biology 30: 125–146.

Shinoda A, Aoyama J, Miller MJ, Otake T, Mochioka N, Watanabe S et al. 2011. Evaluation of the larval distribution and migration of the Japanese eel in the Western North Pacific. Reviews in Fish Biology and Fisheries 21: 591–611.

Shryock DF, Havrilla CA, DeFalco LA, Esque TC, Custer NA, Wood TE. 2017. Landscape genetic approaches to guide native plant restoration in the Mojave Desert. Ecological Applications 27: 429–445.

Simonsen KL, Churchill GL, Aquadro CF. 1995. Properties of statistical tests of neutrality for DNA polymorphism data. Genetics 141: 413-429.

Sinan H, Bailey M. 2020. Understanding barriers in Indian Ocean tuna commission allocation negotiations on fishing opportunities. Sustainability 12: 1-15.

Sjöstrand AE, Sjödin P, Jakobsson M. 2014. Private haplotypes can reveal local adaptation. BMC Genetics 15: 1-14.

Slatkin M. 1985. Gene flow in natural populations. Annual Review of Ecology Systematics 16: 393– 430.

Slatkin M, Hudson RR. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129: 555-62.

Smale MJ, Buxton CD. 1985. Aspects of the recreational ski-boat fishery off the Eastern Cape, South Africa. South African Journal of Marine Science 3: 131–144.

SMSP. 2021. Seychelles Marine Spatial Plan Initiative. Available at: https://seymsp.com/ [accessed 17 February 2021]

Sobhee SK. 2006. Fisheries biodiversity conservation and sustainable tourism in Mauritius. Ocean and Coastal Management 49: 413–420.

Song CB, Near TJ, Page LM. 1998. Phylogenetic relations among percid fishes as inferred from mitochondrial cytochrome b DNA sequence data. Molecular Phylogenetic Evolution. 10: 343–353.

Stevens JD. 1984. Life-History and Ecology of Sharks at Aldabra Atoll, Indian Ocean. Royal Sciences, Biological 222: 79–106.

Stinchcombe JR, Hoekstra HE. 2008. Combining population genomics and quantitative genetics: Finding the genes underlying ecologically important traits. Heredity 100: 158–170.

Stoddart ADR. 1968. The Conservation of Aldabra. The Geographical Journal 134: 471–485.

Stoeckle M. 2003. Taxonomy, DNA and the bar code of life. BioScience 53: 2–3

Storey JD, Bass AJ, Dabney A, Robinson D. 2020. Qvalue: Q-value estimation for false discovery rate control. R package version 2.22.0: Avilable at: http://github.com/jdstorey/qvalue [accessed 10 september 2020]

Strain J. 2014. Eating fish for two. Nutrition bulletin 39: 181–186.

SWIOFish3. 2021. World Bank Project : Third South West Indian Ocean Fisheries Governance and Shared Growth Project (SWIOFish3). Available at: https://projects.worldbank.org/en/projects-operations/project-detail/P155642 [accessed 16 February 2021].

Tajima F. 1983. Evolutionary relationship of DNA sequences in finite populations. Genetics 105: 437-460.

Tajima F. 1989. Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. Genetics 123: 585–595.

Teske PR, Von Der Heyden S, McQuaid CD, Barker NP. 2011. A review of marine phylogeography in Southern Africa. South African Journal of Science 107: 43–53.

Thrasher DJ, Butcher BG, Campagna L, Webster MS, Lovette IJ. 2018. Double-digest RAD sequencing outperforms microsatellite loci at assigning paternity and estimating relatedness: a proof of concept in a highly promiscuous bird. Molecular Ecology Resources 18: 953–65.

Tsaousis AD, Martin DP, Ladoukakis ED, Posada D, Zouros E. 2005. Widespread recombination in published animal mtDNA sequences. Molecular Biology and Evolution 22: 925–933.

Turner R, Freiermuth E. 2017. Travel and tourism economic impact 2017 Seychelles. London: World travel and tourism council. Available at http://www.databank.com.lb/docs/The%20Economic%20Impact%20of%20Travel%20and%20Tourism,%20March%202017,%20WTTC.pdf [accessed 01 May 2018]

UNESCO (The United Nations Educational, Scientific and Cultural Organization). 2017. Global Ocean Science Report: The Current Status of Ocean Science around the World – Executive Summary. Paris: UNESCO. Available at: https://en.unesco.org/gosr [accessed 10 March 2018]

Valenzuela-Quiñonez F. 2016. How fisheries management can benefit from genomics? Briefings in Functional Genomics 15: 352–357.

Van der Elst R, Everett B, Jiddawi N, Mwatha G, Afonso PS, Boulle D. 2005. Fish, fishers and fisheries of the Western Indian Ocean: Their diversity and status, a preliminary

Assessment. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences 363: 263–84.

Van der Elst RP, Groeneveld JC, Baloi AP, Marsac F, Katonda KI, Ruwa RK, Lane WL. 2009. Nine nations, one ocean: A benchmark appraisal of the South Western Indian Ocean Fisheries Project (2008-2012). Ocean and Coastal Management 52: 258–267.

Villacorta-Rath C, Ilyushkina I, Strugnell JM, Green BS, Murphy NP, Doyle SR et al. 2016. Outlier SNPs enable food traceability of the southern rock lobster, Jasus edwardsii. Marine Biology 163: 223.

Vinet, Luc, and Alexei Zhedanov. 2011. "A 'missing' Family of Classical Orthogonal Polynomials." Journal of Physics A: Mathematical and Theoretical 44 (8): 564–67.

Vitalis R, Dawson K, Boursot P. 2001. Interpretation of variation across marker loci as evidence of selection. Genetics 158: 1811-1823.

Wafar M, Venkataraman K, Ingole B, Khan SA, Lokabharathi P. 2011. State of knowledge of coastal and marine biodiversity of Indian ocean countries. PLoS ONE 6: e14613

Wagner CE, Keller I, Wittwer S, Selz OM, Mwaiko S, Greuter L et al. 2013. Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. Molecular Ecology 22: 787–798.

Wallace EM, Tringali MD. 2010. Identification of a novel member in the family Albulidae (bonefishes). Journal of Fish Biology 76: 1972–1983.

Wallace EM 2014. Assessing biodiversity, evolution, and biogeography in bonefishes (albuliformes): resolving relationships and aiding management. PhD thesis. University of Minnesota, USA.

Wallace EM. 2015. High intraspecific genetic connectivity in the Indo-Pacific bonefishes: Implications for conservation and management. Environmental Biology of Fishes 98: 2173–2186.

Wallace EM, Tringali MD. 2016. Fishery composition and evidence of population structure and hybridization in the Atlantic bonefish species complex (Albula spp.). Marine Biology 163: 142.

Wang CH, Tzeng WN. 2000. The timing of metamorphosis and growth rates of American and European eel leptocephali: a mechanism of larval segregative migration. Fisheries Research 46:191–205.

Waples RS. 1998. Separating the Wheat From the Chaff: Patterns of Genetic Differentiation in High Gene Flow Species. The American Genetic Association 438: 438–450.

Waples RS. 2015. Testing for Hardy–Weinberg Proportions: Have We Lost the Plot?. Journal of Heredity 106: 1–19.

Warnell LJ, Darrin HM, Pierce SJ. 2013. Threatened marine species in Mozambique: a summary of the conservation and legal status. Available at (PDF) Threatened marine species in Mozambique: A summary of the conservation and legal status. (researchgate.net) [accessed 01 October 2018].

Weigand H, Weiss M, Cai H, Li Y, Yu L, Zhang C, Leese F. 2017. Deciphering the origin of mito-nuclear discordance in two sibling caddisfly species. Molecular Ecology 26: 5705–5715.

Weir BS, Hill WG. 2002. Estimating F-statistics. Annual Review of Genetics 36: 721–750.

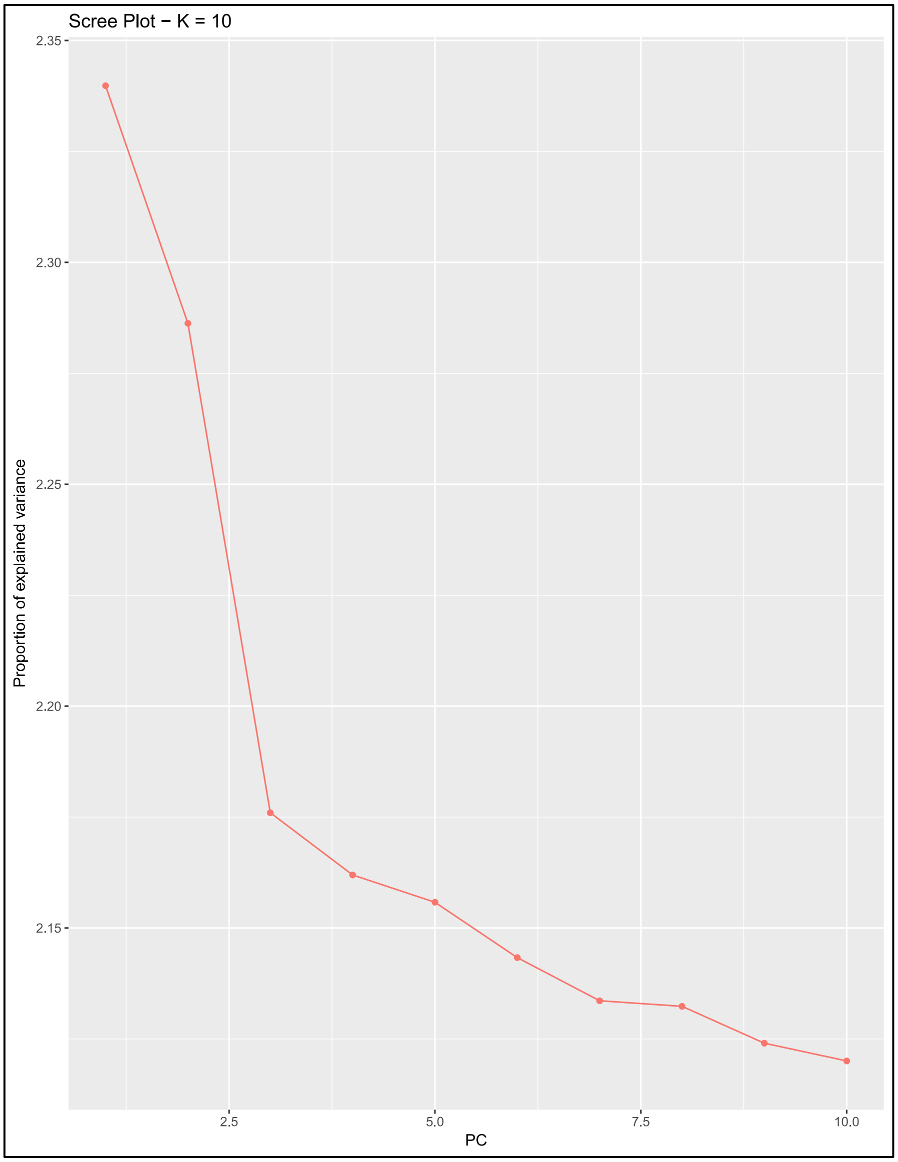
Weiss M, Weigand H, Weigand AM, Leese F. 2018. Genome-wide single-nucleotide polymorphism data reveal cryptic species within cryptic freshwater snail species—

The case of the *Ancylus fluviatilis* species complex. Ecology and Evolution 8: 1063–1072.

Whitehead PJP. 1986. The synonymy of Albula vulpes (Linnaeus, 1758) (Teleostei, Albulidae). Cybium 10: 211-230.

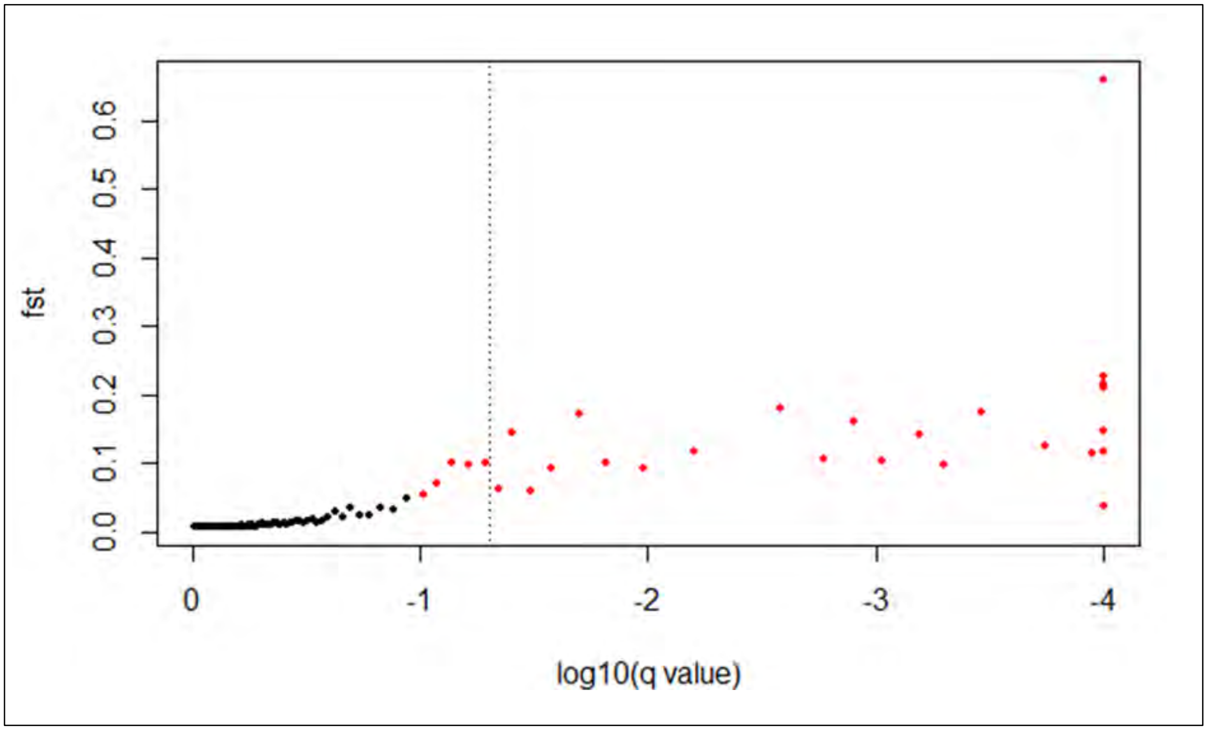
6 SUPPLEMENTARY

S.1. Testing for outliers using PCADAPT



Supplementary Figure 6.1 Scree plot of possible clusters chosen based on Cattell’s rule In this case and following the instructions of the PCADAPT package the K of choice was 2.

S.2. Testing for outliers using BayeScan



Supplementary Figure 6.2. Scatter Plot showing distribution of outlier Loci indicated by the red dots. These red samples show outliers with a q-value of 0.05.

S.3. Discriminant Principal Component Analysis using the neutral dataset.

Supplementary Table 6.1 Mean successful assignment (MSA) and root mean squared error (MSE) for increasing number of PC's for the two clusters without preassigned population. The number of PC's kept was based on the highest MSA and the lowest MSE (numbers showed in bold).

	1	5	**10**	15	20	25	30
MSA	0.915	0.924	**0.926**	0.912	0.796	0.738	0.710
MSE	0.206	0.193	**0.181**	0.203	0.314	0.361	0.382

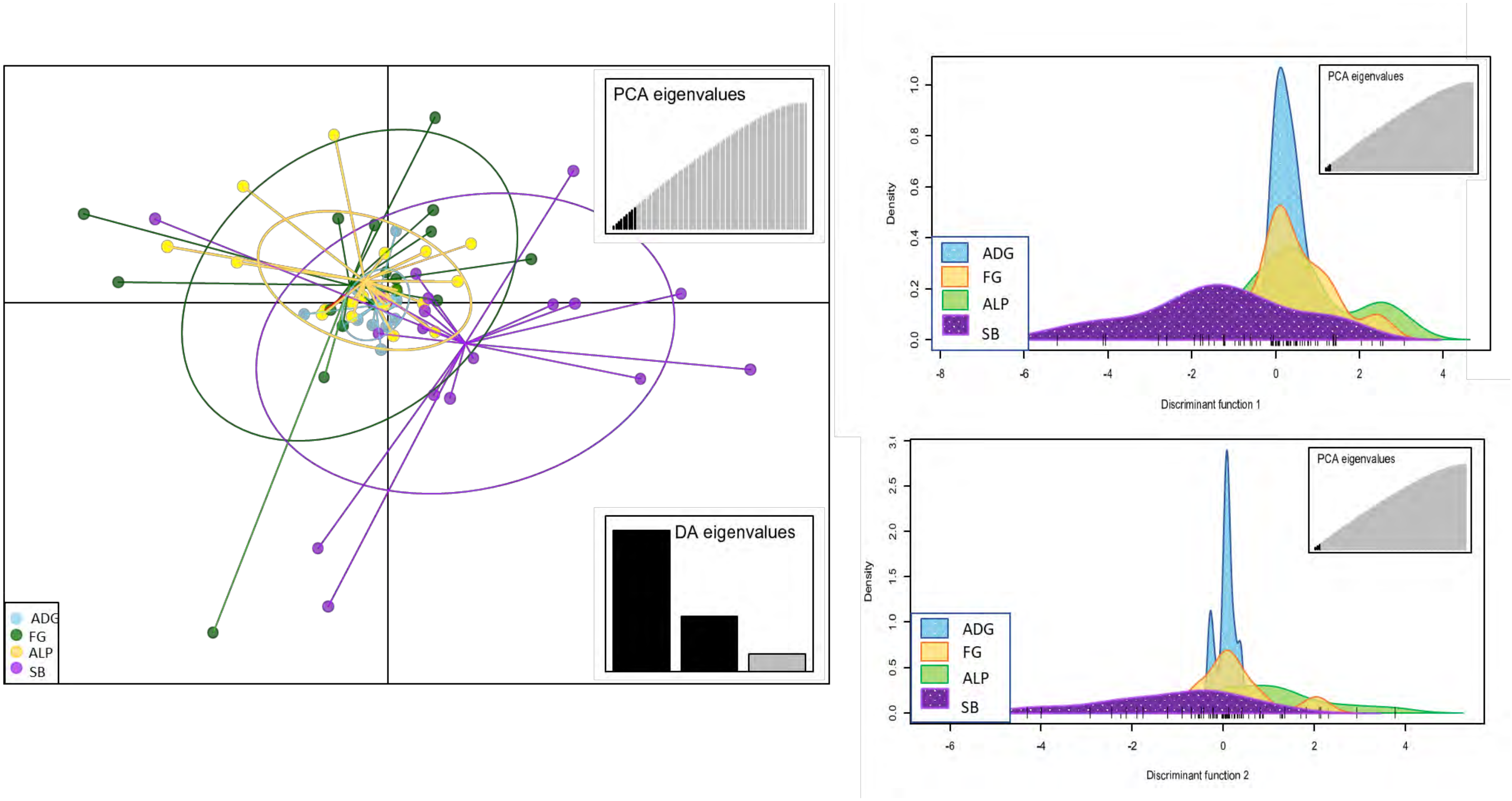
S.4. Discriminant Principal Component Analysis using the outlier dataset.

Supplementary Table 6.2 Mean successful assignment (MSA) and root mean squared error (MSE) for increasing number of PC's for the two clusters without preassigned population. The number of PC's kept was based on the highest MSA and the lowest MSE (numbers showed in bold).

	1	2	3	4	**5**	6	7	8	9	10
MSA	0.93	0.91	0.92	0.92	**0.98**	0.97	0.97	0.98	0.95	0.92
MSE	0.09	0.10	0.08	0.08	**0.09**	0.10	0.09	0.11	0.11	0.12

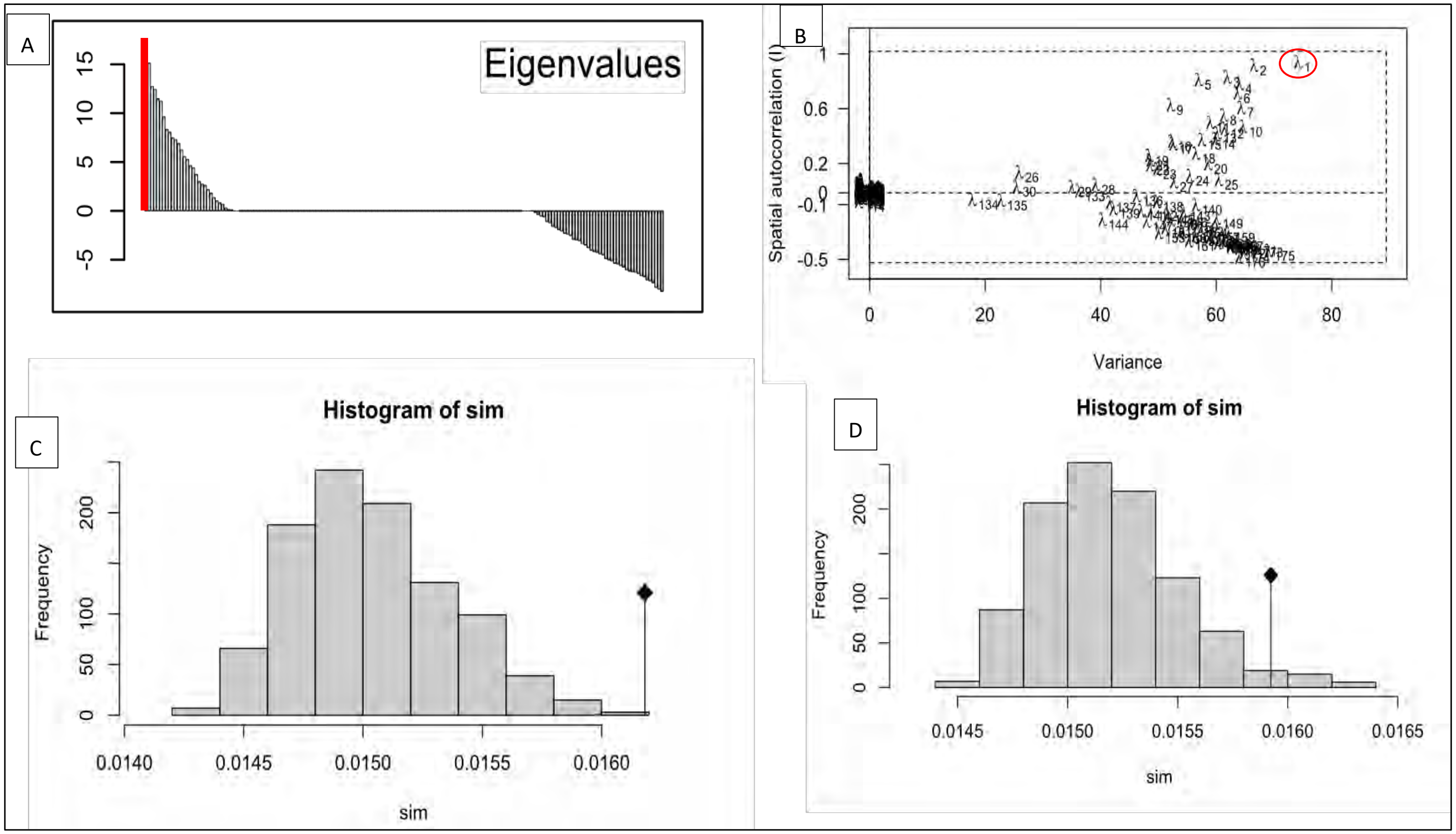
Supplementary Table 6.3. Mean successful assignment (MSA) and root mean squared error (MSE) for increasing number of PC's for the three clusters without preassigned population. The number of PC's kept was based on the highest MSA and the lowest MSE (numbers showed in bold).

	1	2	3	4	**5**	6	7	8	9	10
MSA	0.93	0.92	0.92	0.92	**0.97**	0.96	0.96	0.96	0.95	0.93
MSE	0.13	0.14	0.14	0.15	**0.08**	0.09	0.09	0.09	0.12	0.13



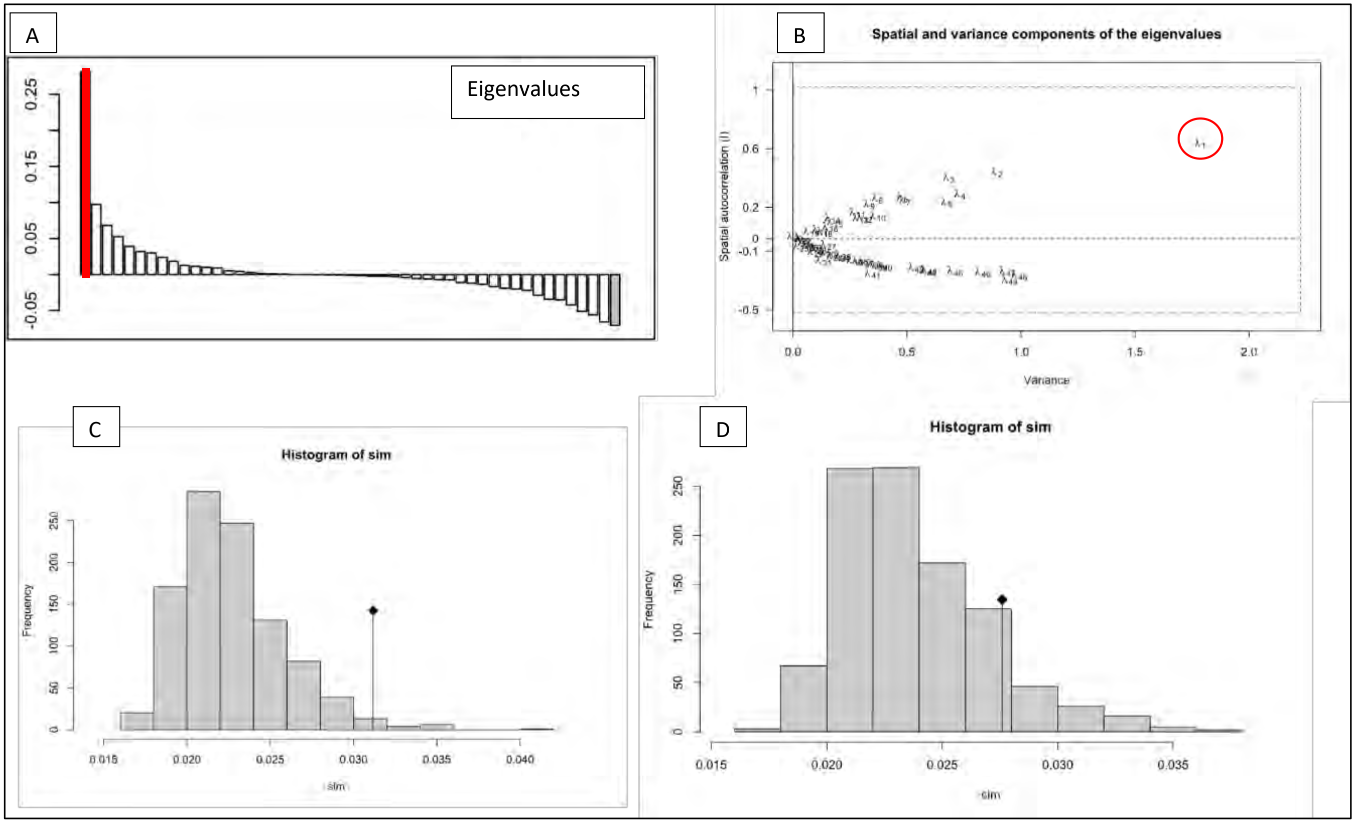
Supplementary Figure 6.3. A) DAPC exploring cluster *K* = 3 showing the DAPC along discriminant function 1. The plot densities (B and C) of individuals along the retained discriminant functions these are arranged along discriminant function DF1 (B) and DF2 (C), with the shaded areas showing individual densities for each of the island groups.

S.5. Choosing the most supported sPCA components of the neutral dataset



Supplementary Figure 6.4. sPCA components for the neutral dataset. A) Barplot of sPCA component eigenvalues. The positive eigenvalues are displayed on the left whereas the negative eigenvalues are displayed on the right, indicating local structure. The first global score highlighted in red is the only score that was interpreted. B) Scree plot displaying decomposed eigenvalues based on the eigenvalues variance and spatial autocorrelation. The eigenvalue represented as λ_1 (circled in red) is the highest positive eigenvalue and in this case the only eigenvalue to be interpreted. Histogram of permuted test statistics for the global C) and local D) structures. The observed test statistic displayed with a line and black diamond is larger than most simulated values in both the local and global tests. Therefore, the null hypothesis of spatial structure absence is rejected.

S.6 Choosing the most supported sPCA components of the outlier dataset



Supplementary Figure 6.5 sPCA components for the outlier dataset. A) A barplot of sPCA component eigenvalues, the positive eigenvalues are displayed on the left whereas the negative eigenvalues displayed on the right, indicating local structures. The first global score highlighted in red is the only score that was interpreted. B) Scree plot displaying decomposed eigenvalues based on the eigenvalues variance and spatial autocorrelation. The eigenvalue represented as λ_1 (in a red circle) is the highest positive eigenvalue and in this case the only eigenvalue to be interpreted. Histogram of permuted test statistics for the global C) and local D) structures. The observed test statistic displayed with a line and black diamond is larger than most simulated values in both the local and global tests. Therefore, the null hypothesis of spatial structure absence is rejected.