The evolutionary history of the genus *Seriola* and the phylogeography and genetic diversity of *S. lalandi* (yellowtail) across its distribution range

By

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Abstract

The genus *Seriola* includes several important commercial fish species, yet the phylogenetic relationships between species have not been fully investigated to date. This study reports the first molecular phylogeny for this genus based on two mitochondrial (*Cytb* and *COI*) and two nuclear gene (*RAG1* and *Rhod*) fragments for all extant *Seriola* species (nine species, n = 27). The phylogenetic patterns resolved three main lineages: a ((*S. fasciata* and *S. peruana*), *S. carpenteri*) clade, a (*S. dumerili* and *S. rivoliana*) clade and a (*S. lalandi* and *S. quinqueradiata*) clade. The closure of the Tethys Sea (12 - 20 MYA) coincides with divergence of the ((*S. fasciata* and *S. peruana*), *S. carpenteri*) clade from the rest of the *Seriola* species; while the uplifting of the Isthmus of Panama (± 3 MYA) played an important role in speciation between *S. fasciata* and *S. peruana*. The climate and water temperature fluctuation in the Pliocene played important roles during the divergence of the remainder of the *Seriola* species.

This study is also the first to describe the evolutionary history of the commercially important species *Seriola lalandi* across its distribution range. Global patterns of genetic variation within *S. lalandi* (n = 190) were examined using three genes fragments (mitochondrial DNA *COI*, *Cytb* and nuclear *RAG1*). Three distinct clades were identified, corresponding to three different geographic regions (North-western Pacific - Japan, North-eastern Pacific - USA, and the southern hemisphere clade). These groupings correspond with the previously identified subspecies of *S. lalandi* (North-western Pacific – *S. lalandi aureovittata*, North-eastern Pacific – *S. lalandi dorsalis*, and the southern hemisphere clade - *S. lalandi lalandi*). AMOVA results and pairwise F_{ST} values revealed significant population differentiation between these groups. The population subdivision between these clades in all probability is maintained by biogeographic or

oceanographic barriers (such as the equator and East Pacific Barrier) that disrupt gene flow. The southern hemisphere clade comprised of samples from the southern Pacific (AUS, NZL and Chile) and the southern Atlantic (SA). No haplotypes were shared between these areas in the southern hemisphere.

This southern hemisphere clade was further investigated with six microsatellite markers. The analyses revealed the South African populations as genetically distinct from populations of the South Pacific oceans (AMOVA, FCA and STRUCTURE results). In summary, the South African and southern Pacific grouping could be the result of recent vicariant events during the Pleistocene glacial / interglacial periods and / or contemporary oceanographic forces acting on these populations. Further population differentiation was found within the South African samples, but not in the South Pacific. In the southern Pacific clade this lack of population structure is the result of high gene flow (analysed with MIGRATE) between the sampling localities.

This is the first report on the genetic structure of this commercial important species for South African populations. Five sampling localities from the west- to the east coast of South Africa were sampled (n = 201). The microsatellite analyses revealed two potentially genetically distinct groups. AMOVA, F_{ST} and FCA results suggest small but significant differentiation between populations from the west coast and from the south- and east coast, suggesting a potential genetic break in the Cape Point region (BARRIER). However, the program STRUCTURE showed a high level of admixture along the South African coast and the migration results (MIGRATE and BAYESASS) also suggest a high degree of gene flow between these regions.

Opsomming

Die genus *Seriola* bevat verskeie kommersieel belangrike vis spesies, tog is filogenietiese verwantskap tussen species is nog nie ten volle bestudeer nie. Hierdie studie is die eeste molekulêre filogenietiese studie vir die genus gebaseer op twee mitochondriale DNA (*Cytb* en *COI*) en twee nukleêre DNA fragmente (*RAG1* en *Rhod*) vir al die bestaande *Seriola* spesies (nege spesies, n = 27). Drie beranglike filogenitiese patrone is geïdentifiseer: ‘n ((*S. fasciata* en *S. peruana*), *S. carpenteri*) groep, ‘n (*S. dumerili* en *S. rivoliana*) groep en ‘n (*S. lalandi* en *S. quinqueradiata*) groep. Die diversifikasie van die ((*S. fasciata* en *S. peruana*), *S. carpenteri*) groep van die ander *Seriola* spesies stem oor een met die sluiting van die Tethys See (12 - 20 MYA). Terwyl die opligging van die Isthmus van Panama (± 3 MYA) ‘n belangrike rol gespeel het in die spesiasie tussen *S. fasciata* en *S. Peruana*. Die veranderlike klimaat toestande en water temperature tydens die Pliocene help met die diversifikasie van die res van die *Seriola* spesies.

Hierdie studie is die eerste om verslag te doen op die evolusionêre geskiedenes van kommersiele belangrike vis spesies *Seriola lalandi* oor sy hele verspreidings gebied. Die globale patrone van genetiese variasie binne *S. lalandi* (n = 190) is ondersoek deur gebruik te maak van mitokondriale (*Cytb* en *COI*) en nukleêre (*RAG1*) DNA volgorde data. Drie groepe geassosieer met geografie is geïdentifiseer (Noord-westelike Stille Oseaan - Japan, Noord-oostelike Stille Oseaan - FSA en die suidelike hemisfeer groep). Hierdie groepeerings stem oor een met voorheen geïdentifiseerde subspesies van *S. lalandi* (Noord-westelike Stille Oseaan - *S. lalandi aureovittata*, Noord-oostelike Stille Oseaan - *S. lalandi dorsalis* en die suidelike hemisfeer groep

- *S. lalandi lalandi*). AMOVA en F_{ST} resultate bevestig die hoë graad van genetiese struktuur tussen groepe. Hierdie populasie groepeerings is moonlik die oorsaak van - en onderhou deur giogeografiese and oseaanografiese grense (soos the ewenaar en EPB) wat geen vloei ontwrig. Die suidelike hemisfeer groep kan verder verdeel word in ‘n suidelike Stille Oseaan (AUS, NZL en Chile) groep en ‘n suidelike Atlantiese groep (SA). Geen haplotiepes word gedeel deur hierdie twee groepe. Hiedie suidelike hemisfeer groep was verder ondersoek met ses mikrosatelliet merkers. Die analises bevestig dat die Suid Afrikaanse populasies geneties verskillend is van die suidelike Stille Oseaan groep (AMOVA, FCA and STRUCTURE). Verdere genetiese struktuur kan verkry word binne die Suid Afrikaanse monsters, maar nie in die suidelike Stille Oseaan nie. In die suidelike Stille Oseaan hiere gebrek aan populasie struktuur is as gevolg van die hoë geen vloei (MIGRATE) tussen die lokaliteite.

Hierdie is ook die eerste studie oor die genetiese structure van *S. lalandi* vir Suid Afrikaanse populasies. Monsters (n = 201) was geneem by vyf lokaliteite vanaf wes tot oos kus van Suid Afrika. Die mikrosatelliet analises dui op twee moontlike genetiese groepe. AMOVA, F_{ST} en FCA dui ‘n klein maar betekenisvolle differensiasie tussen populasies van die wes kus en die van die suid en oos kus. Dit dui aan op moontlike breek in die Kaap Punt omgewing (BARRIER). STRUCTURE analises toon egter ‘n bymengsel van twee groepe langs die Suid Afrikaanse kus en migrasie resultate (MIGRATE en BAYESASS) toon ook ‘n hoë graad van gene vloei tussen hierdie gebiede.

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Chapter 1: Introduction

1.1 Population differentiation in the marine environment

The marine environment is known for its species richness and endemism, with approximately 15 000 marine fish species identified globally (OBIS; http://www.iobis.org; FishBase, http://www.fishbase.org). South Africa, with its high diversity of ecosystems, have at least 11 000 marine animal species of which approximately 2 000 are fish species. Of these fish species 13 - 16% are endemic (Heemstra and Heemstra, 2004). Understanding the forces that promote population differentiation and eventually speciation in marine organisms provides insight into this extent of marine biodiversity. The four main evolutionary forces shaping population differentiation are: migration, mutation, random genetic drift, and selection. Predominantly, population differentiation is interpreted as reduced gene flow (migration) among populations (Cowen and Sponaugle, 2009; Pelc *et al*., 2009). However, genetic differentiation may not always be the result of the absence of gene flow alone, but may be influenced by other evolutionary forces (mutation, genetic drift and selection) as well as the interaction between these phenomena over time. Furthermore, changes in effective population sizes and differences in divergence times among populations can also result in genetic differentiation (Hart and Marko, 2010; Marko and Hart, 2011).

Typically, marine organisms are generally described to have high fecundities, extensive larval dispersal, high migratory capacities, large geographic ranges and large population and effective population sizes (Palumbi, 1994, 1997; Nielsen and Kenchington, 2001; Palumbi, 2004; Levin, 2006), characteristics which all tend to limit genetic differentiation. Several studies have shown

limited genetic differentiation in marine species due to some of these properties such as; the dispersal capacity of pelagic (living near the surface of the coastal- and ocean waters) larvae (Levin, 2006; González-Wangüemert *et al*., 2010), through migratory adults (Stopar *et al*., 2010; Wu *et al*., 2010) and large effective population sizes (Theisen *et al*., 2008; Díaz-Jaimes *et al*., 2010). Within South African coastal waters for instance, little population differentiation was found in some fish species with large dispersal potential of adults (spotted grunter, *Pomadasys commersonnii*; Klopper, 2005; dusky kob, *Argyrosomus japonicas*; Klopper, 2005; Cape stumpnose, *Rhabdosargus holubi*; Oosthuizen, 2007)]. This contrasts panmixia found in other South African coastal fish species including the red roman (*Chrysoblephus laticeps*; Teske *et al*., 2010) and the banded goby (*Caffrogobius caffer*; Neethling *et al*., 2008) with low adult dispersal potential.

An increasing number of studies suggest that the high dispersal potential of marine organisms (through larvae or adults) does not necessarily result in a lack of, or low levels of, population differentiation (Avise, 1998; Lundy *et al.*, 2000; Hutchinson *et al.*, 2001; Smith *et al.*, 2002; Luttikhuizen *et al.*, 2003; Chiang *et al*., 2008; Xu *et al*., 2009; Yue *et al*., 2009; Evans *et al.*, 2010). Significant genetic population differentiation has been found for a number of marine fishes including, Atlantic cod (*Gadus morhua*; Nielsen *et al.*, 2003, 2005; Poulsen *et al.*, 2006), Atlantic herring (*Clupea harengus*; Bekkevold *et al.*, 2005), the striped gray mullet (*Mugil cephalus*; Livi *et al*., 2011), Pacific Sierra mackerel (*Scomberomorus sierra*; López *et al*., 2010), dolphinfish (*Coryphaena hippurus*; Díaz-Jaimes *et al*., 2010) and European flounder (*Platichthys flesus*; Hemmer-Hansen *et al.*, 2007b). Such genetic differentiation found in species with high dispersal capabilities indicates that a combination of forces other than physical barriers must be

at work in limiting gene flow and thus promoting population differentiation and eventually speciation in the marine realm.

1.1.1 Forces influencing population differentiation in marine species

1.1.1.1 Isolation by distance

Isolation by distance (IBD) is a pattern of migration where a positive correlation between genetic distance and geographical distance can be found, therefore the tendency for populations that are in geographic proximity to be more genetically similar than populations that are located further apart (Wright, 1943). Thus gene flow is high between neighbouring populations and lower between more distant ones, where the more remote populations are connected via a series of intermediate 'stepping stones'. This pattern can clearly be observed in the genetic population structure of the eelpout (*Zoarces viviparous*; Kinitz *et al*., 2013) where populations are interconnected by step-wise migration among adjacent populations in the North Sea and the Baltic Sea.

The dispersal ability of a species is an important factor influencing migration rate. Individuals with limited dispersal capabilities can only move small distances; thus promoting IBD. On the other hand, it is believed that in some marine species, individuals can travel vast distances, with gene flow occurring throughout their entire distribution range, thereby promoting panmixia (Theisen *et al*., 2008; Chow *et al*., 2009). Palumbi (2003) has, however, shown through the use of simulations that IBD occurs in species with continuous distributions, regardless of the population dispersal patterns and oceanographic conditions. For example, IBD was found along a continuous coastline of 1 600km in the western North Atlantic for Atlantic cod (Pogson, 2001)

and along ~ 1 700km in the North-eastern Pacific Ocean for Pacific cod (*Gadus macrocephalus*; Cunningham *et al*., 2009). Genetic differentiation due to IBD on a micro-geographic scale can be the result of limited dispersal due to a patchy distribution of suitable habitats (Riginos and Nachman, 2001) as has been found in Atlantic cod (Knutsen *et al.*, 2003; Nielsen *et al*., 2003; Case *et al.*, 2005; Nielsen *et al*., 2005) and European flounder (Hemmer-Hansen *et al.*, 2007a). Although IBD plays an important role in the population structuring of many marine species, the correlation between genetic distance and other factors such as temporal (Hendry and Day, 2005) or longitudinal and latitudinal distance has also recently been described. For example, in the European flounder genetic structure was influenced by isolation by latitude rather than by IBD (Hemmer-Hansen *et al.*, 2007 b) and the population structure of the European eel (*Anguilla anguilla*) displayed significant isolation by time in yearly samples where gene flow was restricted because of differences in reproduction times between individuals (Maes *et al*., 2006).

1.1.1.2 Behavioural philopatry

Behavioural philopatry at specific stages in the life cycle of a species might also contribute to reducing gene flow and differences in population characteristics (Avise, 1998; Carvalho and Hauser, 1998). Philopatry is the tendency of an individual to return to, or stay in, a certain area, be it its home area, natal site, or another adopted locality (Mayr, 1963; Hueter *et al*., 2004). Two main patterns of philopatric behaviour are found: natal philopatry where individuals return to their natal nursery area and sex-specific philopatry, where one sex is more philopatric than the other. Several studies have found philopatry in marine organisms, including; Pacific bluefin tuna (*Thunnus orientalis*; Qiu *et al*., 2013), yellowfin tuna (*Thunnus albacares*; Qiu *et al*., 2013), sandbar sharks (*Carcharhinus plumbeus*; Portnoy *et al*., 2010), bull sharks (*Carcharhinus leucas*;

Tillett *et al*., 2012), blacktip reef sharks (*Carcharhinus melanopterus*; Mourier and Planes, 2013), sea turtles (Meylan *et al*., 1990; Fitzsimmons *et al*., 1997), European harbour seals (*Phoca vitulina vitulina*; Goodman, 1998), North American beluga whales (*Delphinapterus leucas*; Gladden *et al*., 1999), and sperm whales (*Physeter macrocephalus*; Lyrholm *et al*., 1999). Within the swordfish (*Xiphias gladius*) in the southwest Indian Ocean, for example, the population structure appeared to be sex-dependent with higher genetic differences among females than among males (Muths *et al*., 2009).

1.1.1.3 Clinal variation

Clinal variation is a gradual change in allele frequencies along a geographical or other environmental gradient (Storz, 2002). In oceans, clines can develop as the result of differential adaptation to conditions such as temperature, pH, salinity or depth (Koehn, 1969; Merritt, 1972; Koehn, 1980; Vasemägi, 2006), but it has also been suggested to result from stochastic processes acting upon fish populations (Kimura and Ohta, 1971). Secondary contact between two genetically distinct populations may also form clines (Barton and Hewitt, 1985; Durrett *et al.*, 2000). Regardless of how the clines form, the configuration of a cline usually results from the combined effect of spatially varying natural selection and gene flow, with the actual shape of a cline depending on a balance between these opposing forces (Maes and Volckaert, 2002). Clinal variation has been reported in several marine species (Koehn and Williams, 1978; Ropson *et al.*, 1990; Chan *et al.*, 1997; Cimmaruta *et al*., 2005; Chlaida *et al*., 2009; Bester-van der Merwe *et al*., 2011; Hasselman *et al*., 2013). For example, in the blue mussel, *Mytilus edulis*, the leucine aminopeptidase (*Lap*) locus display an allelic frequency that was strongly correlated with water salinity (Koehn *et al.*, 1980), while the Atlantic cod (Nielsen *et al.*, 2003), the turbot

(*Scophthalmus maximus*; Nielsen *et al*., 2004), and the killifish (*Fundulus heteroclitus*; Adams *et al*., 2006) all show clinal variation on a latitudinal gradient. Both these factors can be found in European hake (*Merluccius merluccius)* where the genetic pattern exhibited a strong correlation with salinity and the depth from surface to 320 m under the surface (Cimmaruta *et al*., 2005).

1.1.1.4 Genetic patchiness

For several marine species that have planktonic larvae, little is known of the distance and directionality of larval dispersal. For many of these species low genetic structuring can be found in the adult populations, however when the recruits from the same locality are repeatedly sampled over time, the different cohorts may, in some instances, be genetically different. This pattern is called genetic patchiness (Hellberg *et al*., 2002). Several studies have shown that the temporal variation in the genetic composition of recruits result in such genetic heterogeneity (David *et al.*, 1997; Li and Hedgecock, 1998; Planes and Lenfant, 2002; Gilbert-Horvath *et al.*, 2006; Pujolar *et al*., 2006; von der Heyden *et al*., 2007; Pujolar *et al*., 2011); indicating the importance of the population dynamics of pelagic larvae.

Several explanations have been put forth to explain temporal variation observed in marine larval recruits. The most common observation involves the variation in reproductive success of adults and the survival success of larvae. Although broadcast-spawning marine organisms may produce millions of gametes, the likelihood of these being fertilised and surviving pelagic dispersal, are remote (Pepin, 1991). Thus, only a few adults may contribute to the population in a given season and this random variance among recruits or cohorts over time, leads to the genetic patchiness. This is called sweepstake reproduction and the spatial as well as temporal changes in

oceanographic conditions within and between seasons play an important role in the parental contributions (Hedgecock, 1994). Another explanation is that the genetic patchiness results from larvae originating from different source populations, such as formerly isolated regions, with variable genetic composition (Kordos and Burton, 1983; Hare and Avise, 1996; Ruzzante *et al.*, 1996; Larson and Julian, 1999). Genetic patchiness has been shown in marine invertebrates including limpets (Johnson and Black, 1982, 1984, 1998), urchins (Watts *et al.*, 1990; Edmands *et al.*, 1996; Moberg and Burton, 2000), soft coral (Burnett *et al.*, 1994), bivalves (David *et al.*, 1997) and oysters (Li and Hedgecock, 1998). It has also been documented in marine fishes such as northern anchovy (*Engraulis rnovdax*; Hedgecock *et al.*, 1994), shortbelly rockfish (*Sebastes jordani*; Larson and Julian, 1999), sea bream (*Diplodus sargus*; Planes and Lenfant, 2002) and European eel (Pujolar *et al.*, 2006, 2011).

1.1.1.5 Biogeographical barriers

Biogeographical barriers can restrict gene flow, thus causing two neighbouring populations to become genetically distinct through stochastic processes; resulting in phylogeographical breaks. These breaks can result from contemporary physical barriers or historical events (Uthicke and Benzie, 2003).

1.1.1.5.1 Contemporary physical barriers

In the marine environment, contemporary barriers can influence species distributions by physically restricting gene flow (deep trenches and strong currents) or by limiting gene flow due to the distance between areas of suitable habitat such as the stretches of open ocean that divide the central and eastern Pacific (Robertson *et al*., 2004; Lessios and Robertson, 2006) and the

eastern and western Atlantic (Banford *et al*., 1999). Deep trenches and circular currents surrounding islands may act as a sufficient barrier to gene flow by limiting adult migration through the retention of early juvenile stages (Hansen and Østerhus, 2000). For example, the isolation between cod population from Iceland and those from Europe was probably caused by the deep water trenches between the islands and the European continent (Joensen *et al.*, 2000; Roman and Palumbi, 2004; Hoarau *et al.*, 2004).

Along continuous coastlines, contemporary physical barriers include ocean currents, river outflow, upwelling systems, temperature gradients / fronts and habitat instability. For instance the freshwater plume of the Amazon-Orinoco outflow may prevent gene flow of marine organisms between the Caribbean and Brazil (Rocha *et al*., 2002; Lessios *et al*., 2003) and cold-water upwelling off southwest Africa limits the dispersal of tropical and subtropical marine organisms from the Indian- to the Atlantic Ocean (Briggs, 1974; Rocha *et al*., 2005, 2007).

These phylogeographic breaks, which can exist across a wide geographical range, are also often consistent with the regional oceanography and are often seen as biogeographic provinces of a given coastline (Longhurst, 1998; Waters and Roy, 2003; Teske *et al.*, 2006; Zardi *et al.*, 2007; Griffiths *et al*., 2010; Teske *et al.*, 2011). In South Africa for example, four biogeographic provinces can be found along the coast: the tropical Delagoa Bioregion (extends southwards from Mozambique into KwaZulu-Natal); the subtropical Natal Bioregion (ranges from KwaZulu-Natal to east of Algoa Bay), the warm-temperate Agulhas Bioregion off the south coast (from Algoa Bay to Cape Point) and the cold-temperate Namaqua Bioregion of the west coast (from Cape Point to the Namibian border; Griffiths *et al*., 2010; Fig 1.1). These provinces differ

significantly in their abiotic and biotic make-up, with the main areas of transition generally accepted as being near Cape Point and Cape Agulhas and just south of Port St Johns (on the Wild coast) (Fig. 1.1).

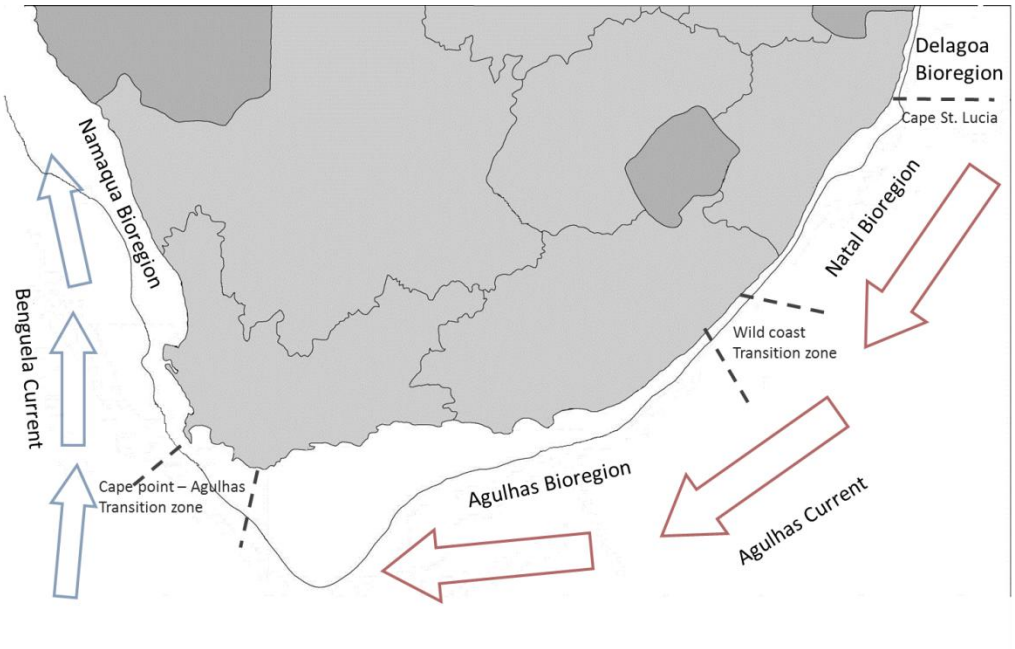


Fig. 1.1: Map of South Africa showing the major bioregions, potential genetic barriers (transition zones), major current systems, and position of the continental shelf break.

However, the pressures affecting the composition of these biogeographic provinces are dynamic, causing these regions to overlap. Furthermore, these barriers to gene flow have different effects on species along the southern Africa coastline (see von der Heyden *et al*., 2009; Teske *et al*., 2011; von der Heyden *et al*., 2011). For example, no genetic structure was found for various line fish species (Cape stumpnose, *Rhabdosargus holubi*; Oosthuizen, 2007; Cape hake species, *Merluccius capensis* and *M. paradoxus*; von der Heyden *et al*., 2007; kingklip, *Genypterus capensis*; Grant and Leslie, 2005) and species with a longer larval cycles (*Octopus vulgaris*;

Oosthuizen *et al*., 2004; spiny lobster, *Palinurus gilchristi*; Tolley *et al*., 2005; west coast rock lobster, *Jasus lalandi*; Matthee *et al*., 2007). In contrast, the population structure in other species was found to be defined (bluntnose klipfish, *Clinus cottoides*; von der Heyden *et al*., 2008; abalone, *Haliotis midae*; Evans *et al*., 2004; Bester-van der Merwe *et al*., 2011; round herring, *Gilchristella aestuaria*; Norton, 2005; three crustacean species; *Exosphaeroma hylecoetes*, *Upogebia africana*, *Iphinoe truncata*; Teske *et al*., 2006).

1.1.1.5.2 Paleo-oceanographic events

Vicariance (*i.e*. the historical separation of a continuously distributed population due to the formation of geographic barrier ensuing the differentiation of the original group into new varieties or species) also plays an important role in the diversification of marine species in particular during the Pliocene-Pleistocene glacial- and interglacial events (Widmer and Lexer, 2001; Olsen *et al*., 2004; Fraser and Bernatchez, 2005; Rabassa *et al*., 2005; Briggs and Bowen, 2010, 2013). These resonating climatic periods resulted in changes in sea levels, temperature, salinity, ocean currents, upwelling patterns and prey distribution, thus affecting the connectivity and isolation of populations (*e.g.* Costedoat *et al*., 2006; Harlin-Cognato *et al*., 2007; Briggs and Bowen, 2010, 2013). In particular, Pleistocene sea-level fluctuations (as low as 150 m below present) formed vicariant barriers such as the Indo-Pacific barrier between the Indian- and Pacific Ocean (Voris, 2000; Barber *et al*., 2006; Hobbs *et al*., 2009; Naish *et al*., 2009). This Indo-Pacific barrier enhanced genetic divergence between populations for many species (Lu *et al*., 2006; Menezes *et al*., 2006; Craig *et al*., 2007). The influences of Pleistocene glacial- and interglacial events on species and population diversification can be seen in a number of species [*e.g.* Pacific Sierra mackerel (*Scomberomorus sierra*; López *et al*., 2010); dolphinfish (Díaz-

Jaimes *et al*., 2010); broad-nosed pipefish (*Syngnathus typhle*; Wilson and Veraguth, 2010); peacock grouper (*Cephalopholis argus*; Gaither *et al*., 2011); Manila clam (*Ruditapes philippinarum*; Moa *et al*., 2011); *Atherina* species (Pujolar and Congiu, 2012)].

The forming and closure of seaways are also imported barriers to gene flow (Rögl and Steininger, 1983; Knowlton *et al*., 1993; White, 1994; Knowlton and Weigt, 1998; Marko, 2002; McCartney and Lessios, 2002; Kirby *et al*., 2008; Lessios, 2008; Stelbrink *et al*., 2010). Two major seaways have played an important role in the biogeographic history of many marine species: the closure of the Tethys Sea (18 *million years ago* [MYA]) and the forming of the Panamanian isthmus land bridge (3 - 5 MYA; Fig. 1.2). The closure of the Tethys Sea separated the Mediterranean Sea and the Indian Ocean influencing the evolutionary history of many marine species; for example *Trachurus* species (Cárdenas *et al*., 2005), needlefishes (Banford *et al*., 2004), and angel sharks (Stelbrink *et al*., 2010). The rising of the Panamanian isthmus land bridge effectively separated the Atlantic and Pacific oceans 3.5 - 2.9 MYA (Bermingham *et al*., 1997; Kirby *et al*., 2008). The evolutionary consequences of the Isthmus of Panama can be found in numerous marine species (*e.g.* Bermingham *et al.*, 1997; Knowlton and Weigt, 1998; Williams *et al.*, 2001; Arbogast *et al.*, 2002; McCartney and Lessios, 2002; Lessios, 2008; Hurt *et al.*, 2009; Miura *et al.*, 2010; Reece *et al*., 2010; Stelbrink *et al*., 2010).

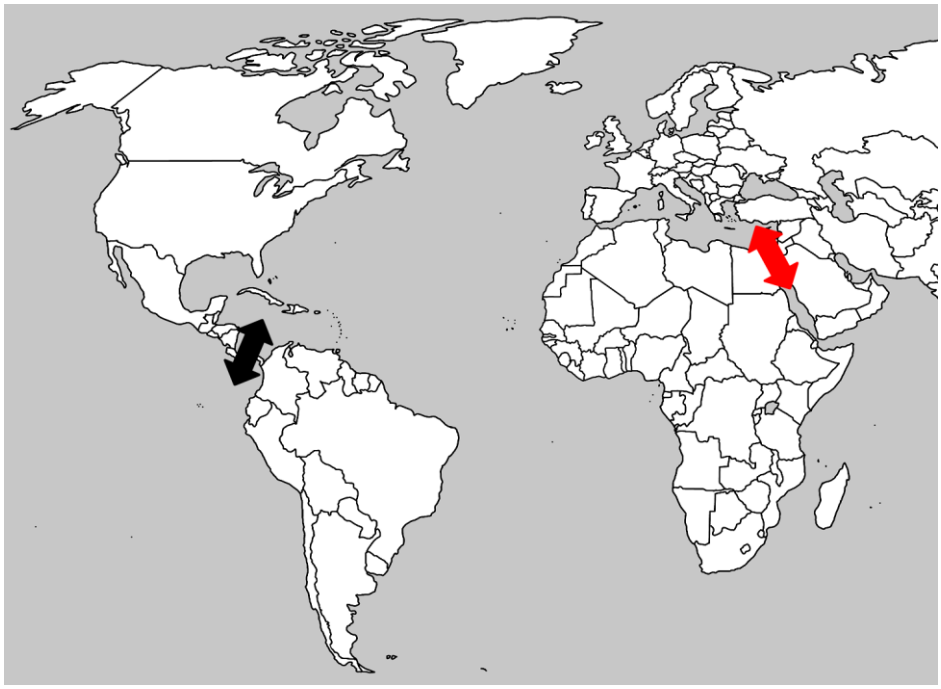


Fig. 1.2: The two major historical pathways for dispersal, the Tethys Sea (red arrow) and the Isthmus of Panama (black arrow).

1.2 Molecular genetic techniques

Molecular genetic methods such as phylogenetic reconstructions, phylogeography and population genetics are useful to resolve genetic variation at different taxonomic levels: species, populations and individuals.

1.2.1 Phylogenetic relationships in the marine environment

Traditionally taxonomists used phenotypic or ecological characters to group organisms. However, in recent years phylogenetic relationships between species have been mainly based on DNA sequence data (Ekrem *et al*., 2010; Lim *et al*., 2010; Liu *et al*., 2010).

When selecting genetic markers it is important to take into account the mutation rate of the DNA region being investigated. To identify recent evolutionary events, markers with higher mutation rates should be more informative, whereas markers with low mutation rates may prove more useful to detect more ancient divergence between lineages. In most species, mitochondrial DNA (mtDNA) has a higher mutation rate (up to 10x) than single copy nuclear DNA, providing estimates of genetic divergence over shorter evolutionary timescales (Wilson *et al.*, 1985; Ferris and Berg, 1987; Birky *et al.*, 1989; Meyer, 1993; Shaklee and Bentzen, 1998; Waples, 1998). However in order to fully understand the evolutionary relationships between organisms, a combination of markers are needed to detect recent and past events of speciation and therefore a multilocus / -gene approach is recommended to resolve complex lineages. Combining these datasets has been shown to result in higher resolution and reveal hidden support where relationships are in conflict among individual fragment analyses (Gatesy *et al*., 1999; Hrbek *et al*., 2007; Gray *et al*., 2009).

In phylogenetic analysis a phylogenetic tree is drawn to illustrate relationships between species. Several methods of inference can be used to construct these trees. Parsimony is a character-based method and the preferred phylogenetic hypothesis (tree) is the one with the minimum number of evolutionary steps (Swofford and Olsen, 1990). Maximum likelihood and Bayesian inference are model-based methods. Maximum likelihood estimates which tree topology is most likely to be observed given the available dataset and a specific substitution model of DNA sequence evolution (Felsenstein, 1981; Bensasson *et al*., 2001). Bayesian inference (BI), is a likelihood-based approach that aims at estimating the posterior probability distribution for a parameter (such as the tree), given the data, a substitution model of character evolution and prior

probabilities on parameters in the model (Huelsenbeck and Imennov, 2002). While phylogenetic analyses are relatively easy to perform it is essential to keep in mind that the constructed tree remains a hypothesis of the actual genetic relationships.

1.2.2 Population genetics and phylogeography in marine systems

The term "population" is one of the broadest concepts used in biology, because it may be interpreted in various contexts. For example, "population" from an ecological perspective is largely defined as the opportunity for individuals to interact demographically, whereas from an evolutionary perspective it is based on reproductive interactions among individuals and thereby based on a more genetic approach. In the current study Waples and Gaggiotti's (2006) definition for a population will be used: "*a group of interbreeding individuals that exist together in time and space having a higher probability of reproducing with each other than with individuals from other such groups*".

Since the introduction of PCR (Polymerase Chain Reaction) technology in the late 1980's two marker classes, microsatellites (nuclear DNA) and mtDNA have dominated estimations of population structure (Avise, 2000; Natoli *et al*., 2006; Cárdenas *et al*., 2009; Muths *et al*., 2009; Yamaguchi *et al*., 2010). These two marker classes were simultaneously developed for investigating different aspects of population genetics, because there is no 'one-marker-fits-all' scenario.

1.2.2.1 Markers for historical and contemporary processes in population genetics

The historical and contemporary processes that influence populations are reflected in their genetic structures (Balloux and Lugon-Moulin, 2002), however, teasing apart the relative effects of past vs. current processes can be challenging. Mitochondrial DNA predominantly provides information about historical processes for maternal intraspecific relationships but may also allow insights into ongoing gene flow (Avise, 2000). However, conclusions from mtDNA data alone suffer from the well-known drawback of single-locus gene trees. The smaller effective population size (fourfold) of the mitochondrial genome compared to the nuclear genome, increases the effects of genetic drift in segmented populations which in return results in more rapid fixation or loss of alleles. Mitochondrial DNA can detect strong population divisions but not subtle structure and is therefore most ideally suited in the identification of evolutionary lineages (Birky *et al*., 1983). However, while overall mutation rates tend to be higher for the mitochondrial- than nuclear genome (Brown *et al*., 1979), most regions in the mitochondrial genome are protein coding; thus potentially under selection (Ballard and Kreitman, 1995). The evolution of these regions may therefore not always occur rapidly enough to infer contemporary levels of gene flow (Angers and Bernatchez, 1998).

Over the last decade, microsatellite markers have become the most frequently used nuclear markers in population genetic studies. This can be attributed to the advantages microsatellites offer over mtDNA, including bi-parental inheritance, selective neutrality and rapid mutation rates (Balloux and Lugon-Moulin, 2002). The high information content of polymorphic microsatellites may reveal contemporary gene flow better than mtDNA, allozymes or single copy nuclear DNA (scnDNA) (Goudet *et al*., 1996; Buonaccorsi *et al*., 1999). These markers are

effective in population studies requiring allele frequency data, such as studies of population differentiation, and are ideal for fine-scale population structure investigations (Cárdenas *et al*., 2009; Yue *et al*., 2009; González-Wangüemert *et al*., 2010). In fish genetics, microsatellite markers are utilised in kinship- and population studies (Liu and Cordes, 2004; Castro *et al*., 2007; González-Wangüemert *et al*., 2010; Miller *et al*., 2011), analysing genetic diversity of wild- and cultured populations (Yue *et al*., 2004; Kanno *et al*., 2006; Burridge and Versace, 2007; Yue *et al*., 2009), reconstructing pedigrees (DeWoody *et al*., 2000; Norris *et al*., 2000; Sekino *et al*., 2003; Borrell *et al*., 2004; Novel *et al*., 2010), for construction of genetic linkage maps (Chistiakov *et al*., 2005; Wang *et al*., 2007; Ruan *et al*., 2010; Nomura *et al*., 2011), and mapping quantitative trait loci (QTLs) (Cnaani *et al*., 2003; Wang *et al*., 2008; Ruan *et al*., 2010).

However, no molecular markers are fundamentally better than another (Karl *et al*., 2012) and all types of markers are useful depending on the application. In some studies microsatellites proved to be more powerful than mtDNA in detecting population structure (Angers and Bernatchez, 1998; Wirth and Bernatchez, 2001; Johnson *et al*., 2003) but not in others (Pardini *et al*., 2001; Keeney *et al*., 2005), while largely similar patterns of population subdivision have also been discovered (*e.g.* Lehman *et al*., 1997; Allendorf and Seeb, 2000; Natoli *et al*., 2004). In cases where weaker population structure based on microsatellites than mitochondrial markers are found, the discrepancies are often attributed to male-biased dispersal (Karl *et al*., 1992; Castella *et al*., 2001; Eizerik *et al*., 2001; Keeney *et al*., 2005). However, Buonaccorsi *et al*. (2001) also showed that differences in population structure between nuclear- and mitochondrial markers could result from differences in effective population sizes and level of polymorphism and its

effects on F_{ST} estimates. Therefore the discrepancies in levels of population subdivision between nuclear and mitochondrial markers may be the result of the evolutionary characteristics and sampling properties of the marker classes (Birky *et al*., 1983; Buonaccorsi *et al*., 2001), such as mutation rates specific to each marker, modes of inheritance, and high variances in F_{ST} estimates (particularly for mtDNA) (Buonaccorsi *et al*., 2001). In addition, for microsatellite loci, allele size homoplasy (Estoup *et al*., 2002) may cause an underestimation in the detected amount of divergence between populations isolated over longer temporal and / or large spatial scales (Rousset, 1996; Estoup *et al*., 2002). Thus, by combining microsatellite- and mitochondrial markers, many of the abovementioned issues may be addressed.

1.3 Systematics of *Seriola*

The Carangidae is a diverse family that includes many species with ecological and economic importance, including the Jacks, Scads, Trevallies, Pompano, Amberjacks, and Queenfishes. This family is one of the largest families in the order Perciformes, and comprises of approximately 32 genera and 140 species (Nelson, 2006). Species of this family are found in the Atlantic, Indian and Pacific oceans, and are usually fast-swimming predatory fish hunting in the open sea, or in the waters above reefs. The fossil record of several carangid genera (especially *Caranx* and *Seriola*) are rather extensive and dates back to the early Paleogene (late Thanetian; Berg, 1947); having been identified from whole and incomplete specimens, skeletal fragments, and otoliths. Currently, within Carangidae there are four recognised tribes; the Scomberoidini, Trachinotini, Naucratini, and Carangini. For the current study the tribe Naucratini is of great importance because it consists of mostly one genus, *Seriola*.

Currently the genus *Seriola* consists of nine valid species that inhabit the tropical and temperate regions of the world's ocean. However, there is a great deal of confusion in distinguishing between some of the species, especially where the juveniles are concerned. Furthermore, all nine species have synonyms that are currently used in literature (Table 1.1). For example *Seriola dumerili* has 13 synonyms and *S. lalandi*, 16. In addition, some of these synonyms come from other valid species names, for example *S. carpenteri* is also known as *S. dumerili*, *S. fasciata* and *S. lalandi*. The current classification of *Seriola* is based mostly on external morphology and geography and the phylogenetic relationship of the species in the genus are currently poorly understood.

Within *Seriola* there are three principal market species; *S. quinqueradiata*, *S. seriola* and *S. dumerili*. Of these three, *S. lalandi* is one of the most valuable commercial and recreational species in southern Africa. *Seriola lalandi* has a non-equatorial distribution, thus it can be found in the temperate waters of the Pacific and Indian oceans, and off the coast of South Africa, Japan and the USA (McGregor, 1995) In South Africa, *S. lalandi* occurs from the west coast to southern KwaZulu-Natal, from the shore out to the continental edge (Penney, 1990). Interestingly, during an individual yellowtail's lifetime it can be both pelagic and demersal (living on or near the bottom of the ocean), which means they spend part of their lives roaming the oceans and the other part benthic (close to the ocean floor). Juveniles are generally found schooling in warmer offshore waters with adults preferring to live a solitary life, or in a small group, inside the continental shelf. According to the WWF (http://www.wwfsassi.co.za), yellowtail stocks are currently considered to be optimally exploited within South Africa. However, over the last two decades an increase in the number of catches was seen since the early

stages of South African fishery (Griffiths, 2000). Approximately 700 tons of *S. lalandi* was captured annually from 2008 to 2011 worldwide, and of this ± 340 tons was captured along the South African coast (FOA, 2012).

Table 1.1: Synonyms for the genus *Seriola* (compiled from http://www.fishbase.org).

Valid name	**Synonyms**
Seriola carpenteri	*Seriola dumerili, Seriola fasciata, Seriola lalandi*
Seriola dumerili	*Caranx dumerili, Regificola parilis, Seriola boscii, Seriola dumerilii, Seriola gigas, Seriola purpurascens, Seriola purpurescens, Seriola rhombica, Seriola simplex, Seriola tapeinometapon, Trachurus aliciolus, Trachurus fasciatus*
Seriola fasciata	*Scomber fasciatus, Seriola semicoronata*
Seriola hippos	*Naucratopsis excusabilis, Seriola gigas*
Seriola lalandi	*Halatractus dorsalis, Lichia pappei, Seriola aureovittata, Seriola banisteri, Seriola dorsalis, Seriola dumerili, Seriola dumerilii, Seriola foncki, Seriola fonki, Seriola grandis, Seriola lalandei, Seriola lalandei dorsalis, Seriola lalandii, Seriola mazatlana, Seriola pappei, Seriola zonata*
Seriola peruana	*Seriola mazatlana*
Seriola quinqueradiata	*Seriola sparna*
Seriola rivoliana	*Seriola bonariensis, Seriola bovinoculata, Seriola colburni, Seriola coronata, Seriola declivis, Seriola dubia, Seriola falcata, Seriola ligulata, Seriola proxima, Seriola ravoliana, Seriola rivolacea, Seriola rivolina, Seriola songoro*
Seriola zonata	*Scomber zonatus*

Although yellowtail is an important commercial species, it is profoundly understudied throughout its distribution range. The only large-scale genetic study published on *S. lalandi* found significant differentiation between populations from Japan and those from Australia –

New Zealand (Nugroho *et al.*, 2001). Furthermore, a fine-scale study along the Australian coast by Miller *et al*. (2011) found significant population differences between the west and east coast of Australia. To our knowledge, there is no information on the genetic structuring and -diversity of the species within southern Africa.

1.4 Management of exploited species

Marine organisms are not an unlimited resource, as can be seen in the collapse of most major fisheries (an entity, recognised by governments, engaging in harvesting fish usually from the wild) worldwide due to over-exploitation (Hutchings, 2000; Worm *et al*., 2006; Pinsky *et al*., 2011; Neubauer *et al*., 2013). Fisheries must employ management programs to assure long-term sustainable yield, prevent population crashes and assist in the recovery of depleted- or endangered species (Ward, 2000). For accurate management decisions regarding fisheries, knowledge of the population dynamics of the targeted species is essential. This is usually done by identifying, characterising and maintaining stocks of the focal species that can be managed separately.

Some form of discontinuous aggregations of individuals in space and / or time are found in most organisms. These aggregations have specific genetic characteristics that may be influenced by several evolutionary processes (see 1.1.1). For exploited marine species these aggregations are known as stocks (Shaklee and Bentzen, 1998). Various definitions for the term stock exist and are reviewed by Carvalho and Hauser (1994). Correctly identifying the number of stocks within fishing grounds are essential for the management of exploited species (Booke, 1999) because separate management strategies should be determined for each stock (Ward, 2000) to maintain

the genetic diversity in each stock. The preservation of genetic variation in species is essential for continually adaptations / responses to environmental changes and selective pressures (O'Connell and Wright, 1997).

Tagging surveys have traditionally been used to investigate interactions between stocks. Tagging surveys carried out on later life stages (juveniles and adults) usually show some movement between different geographical areas. Although, this movement (migration) does not necessarily translate into gene flow (Ferris and Berg, 1987; Waples, 1998), this information can be used as a baseline and, coupled with the life history parameters of a species, can be used for the initial recognition and description of geographically isolated stocks (Begg *et al*., 1999). However tagging has several downfalls in that it is costly, tagged animals have a reduced survival rate, loss of tags are common and the information obtained is limited to the lifetime of the tagged individual (Utter and Ryman, 1993).

Recently genetics has become a valuable tool in stock identification. Here the genetic structure of natural populations forms the basis for the identification of stocks from representative populations (Ward, 2006). The genetic variation between the stocks plays a major role in stock identification. When analysing the genetic structure of stocks, the failure to detect genetic variation indicates that (a) only one stock is present, (b) the question cannot be resolved by the specific marker used, (c) recent separation has taken place or (d) sampling was inadequate (Ward, 2000). Since even limited gene flow can result in genetic homogeneity among samples, it is important to determine the actual level of gene flow when identifying stocks (Dizon *et al*., 1992; Carvalho and Hauser, 1998; Ward, 2000). Genetic stock identification has played an

important role in the successful management of several threatened marine species *e.g.* corals (Van Oppen and Gates, 2006), loggerhead turtles (*Caretta caretta*; Bowen *et al*., 2005), and white abalone (*Haliotis sorenseni*; Gruenthal and Burton, 2005).

However it is widely recognised that an optimal management strategy uses a holistic approach, that includes genetic data, biological characteristics such as behaviour, migration and reproduction, as well as biogeographical patterns and oceanography (Begg and Waldman, 1999).

1.5 Thesis overview

The following chapters explore a broad scale of events driving evolution in *Seriola*. Firstly the evolutionary history of the nine *Seriola* species (Chapter 2) is examined and the first robust phylogeny of this genus using sequence data from two mtDNA (Cytochrome *b* [*Cytb*] and Cytochrome Oxidase I [*COI*]) and two nuclear fragments (recombination activating gene 1 [*RAG1*] and Rhodopsin [*Rhod*]) provided. The historical processes shaping speciation in this genus is also explored. The following two chapters investigate the genetic diversity and population structure of a specific *Seriola* species, *S. lalandi* on a global scale (Chapter 3) with DNA sequence data (*Cytb*, *COI*, and *RAG1*) and a regional scale (South Africa; Chapter 4) utilising six microsatellite markers. In these sections possible influences, such as historical- (Chapter 3) and demographic (Chapter 4) processes on genetic variation within *S. lalandi* populations are identified. The last chapter summarises the findings and explores how the results can be interpreted to assist with the effective management and sustainability of this important commercial species.

Chapter 2: Molecular Systematics of *Seriola* (Perciformes: Carangidae)

2.1 Introduction

The Carangidae is a diverse family that includes species of fish of ecological and economic importance, including the Jacks, Scads, Trevallies, Pampano, Amberjacks, and Queenfishes. Species of this family are found in the Atlantic, Indian and Pacific oceans, and are usually fast-swimming predatory fish hunting in the open sea, or in the waters just above reefs. The fossil record of several carangid genera (especially *Caranx* and *Seriola*) is rather extensive and dates back to the early Paleocene (50 - 55 MYA; Berg *et al*., 1947; Patterson and Owen, 1991); having been identified from whole and incomplete specimens, skeletal fragments, and otoliths. Currently, within Carangidae there are four recognised tribes; Scomberoidini, Trachinotini, Naucratini, and Carangini (Smith-Vaniz, 1984). Reed *et al*. (2002) has been the only study to date to address the phylogenetic relationships between these four tribes. A 700bp fragment of the mtDNA Cytochrome *b* gene was used for phylogenetic reconstruction of 50 species (18 genera) representative of the four tribes. The tribes Carangini, Naucratini and Trachinotini were found to be monophyletic. The fourth tribe, Scomberoidini was paraphyletic; including carangid- as well as outgroup species. The study also resolved a sister group relationship between Carangini and Naucratini. For the current study, the tribe Naucratini is of interest because it consists of a single genus *Seriola*. This genus is one of 50 genera found in the family Carangidae. Currently the genus *Seriola* comprises of nine recognised species (*S. carpenteri*, *S. dumerili*, *S. fasciata*, *S. hippos*, *S. lalandi*, *S. peruana*, *S. quinqueradiata*, *S. rivoliana*, *S. zonata*), that inhabit the tropical and temperate regions of the world's oceans.

Seriola carpenteri (Mather, 1971; Guinean amberjack) is predominantly found along the coast of West Africa ranging from Angola upward to Cape Verde, Dakar, while it has also been recorded in the Mediterranean Sea (Golani *et al*., 2002; Fig. 2.1). The adults of this species are benthopelagic (inhabiting the waters just above the bottom of the ocean) and occur in the coastal waters over the continental shelf in depths from 0 - 200m. This species is restricted by surface temperature and can usually be found in sea temperatures between 18 - 27°C, although temperatures higher than 25°C are preferred.

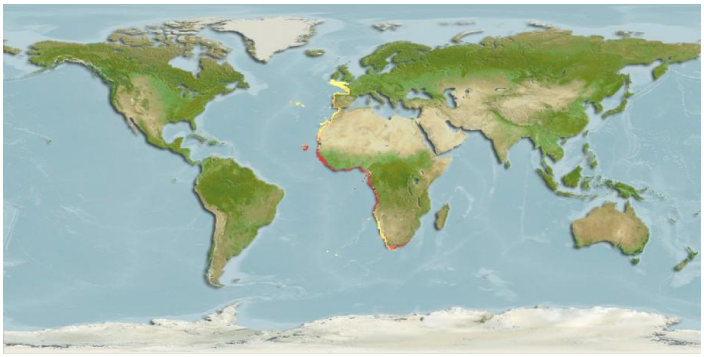


Fig. 2.1: Global distribution map for *Seriola carpenteri* (compiled and acquired from http://www.fishbase.org). The colours indicate the probabilities of occurrence in a particular area and range from yellow (low probability of occurrence) to red (high probability of occurrence).

Seriola dumerili (Risso, 1810; Greater amberjack) is the largest species in the genus and can reach a maximum size of 200 cm. This species is found in subtropical and temperate waters (Manooch and Potts, 1997) of the Mediterranean Sea, the Atlantic-, the Pacific- and the Indian Ocean (Fig. 2.2). This pelagic species is usually associated with deep water reefs.

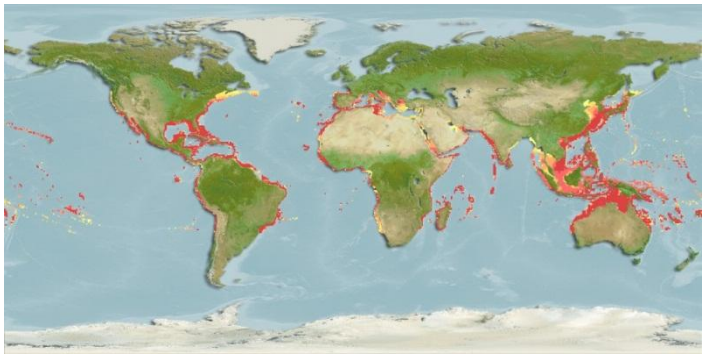


Fig. 2.2: Global distribution map for *Seriola dumerili* (compiled and acquired from http://www.fishbase.org). The colours indicate the probabilities of occurrence in a particular area and range from yellow (low probability of occurrence) to red (high probability of occurrence).

Seriola fasciata (Bloch, 1793; Lesser amberjack) can be found in the subtropical waters of the eastern- and western Atlantic Ocean and is also benthopelagic (Fig. 2.3).

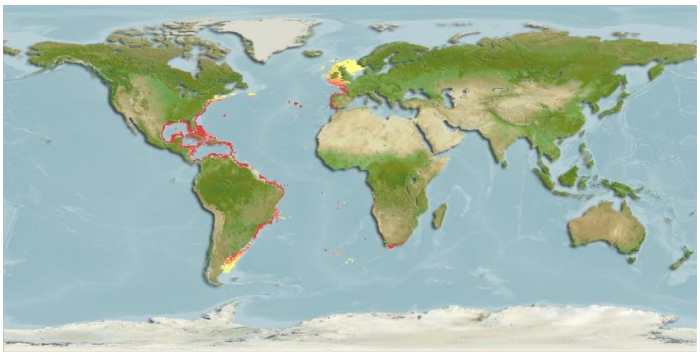


Fig. 2.3: Global distribution map for *Seriola fasciata* (compiled and acquired from http://www.fishbase.org). The colours indicate the probabilities of occurrence in a particular area and range from yellow (low probability of occurrence) to red (high probability of occurrence).

Seriola hippos (Günther, 1876; Samson fish) is endemic to Australia and New Zealand (Fig. 2.4). The species occurs close to the shoreline, often living near reefs, jetties and pylons (Kailola *et al*., 1993); sometimes even within estuaries (May and Maxwell, 1986). This species prefers

warmer water (18 - 24°C), although it does occasionally venture into cooler waters (Starling, 1988).

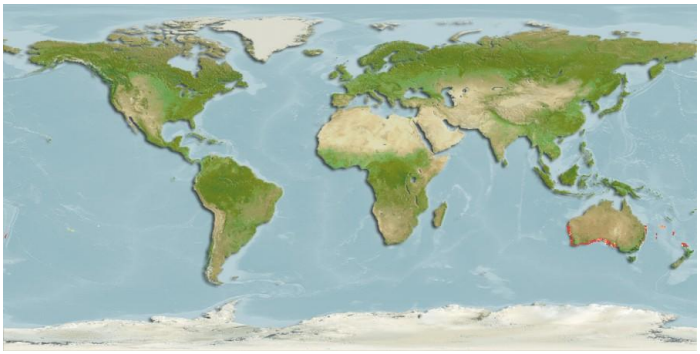


Fig. 2.4: Global distribution map for *Seriola hippos* (compiled and acquired from http://www.fishbase.org). The colours indicate the probabilities of occurrence in a particular area and range from yellow (low probability of occurrence) to red (high probability of occurrence).

Seriola lalandi (Valenciennes, 1833; yellowtail amberjack) is widespread along the temperate regions of the Indian- and Pacific Ocean and southern Africa (Fig. 2.5; Gillanders *et al*., 1997). This benthopelagic species can be found in coastal- and oceanic waters, close to kelp beds and rocky reefs (Eschmeyer *et al*., 1983).

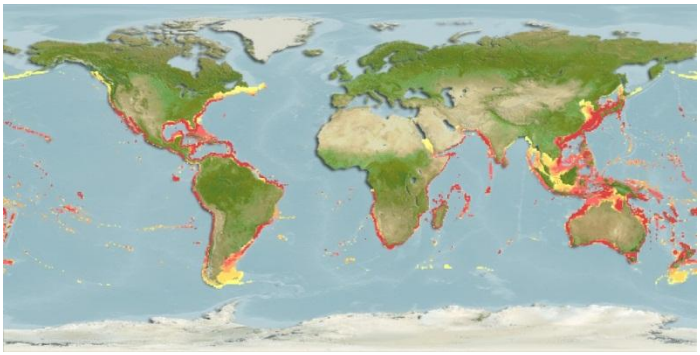


Fig. 2.5: Global distribution map for *Seriola lalandi* (compiled and acquired from http://www.fishbase.org). The colours indicate the probabilities of occurrence in a particular area and range from yellow (low probability of occurrence) to red (high probability of occurrence).

Seriola peruana (Steindachner, 1881; Fortune jack) can be found in the tropical coastal waters of the eastern Pacific Ocean (Mexico to Ecuador and the Galapagos Islands, Fig. 2.6). This species is pelagic and demersal.

Fig. 2.6: Global distribution map for *Seriola peruana* (compiled and acquired from http://www.fishbase.org). The colours indicate the probabilities of occurrence in a particular area and range from yellow (low probability of occurrence) to red (high probability of occurrence).

Seriola quinqueradiata (Temminck and Schlegel, 1845; Japanese amberjack) is endemic to the temperate waters of the north-west Pacific Ocean, from Japan to Hawaii (Fig. 2.7) and is demersal and exhibits shoaling behaviour (Frimodt, 1995).

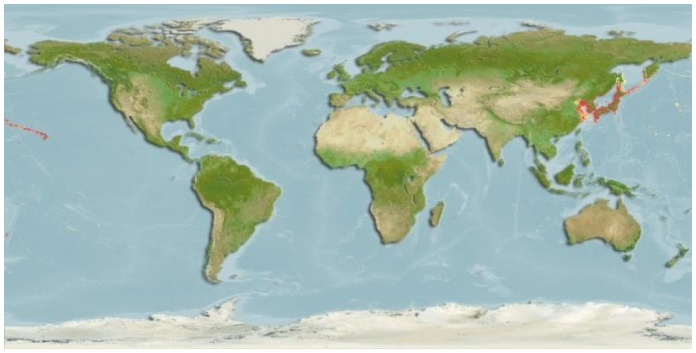


Fig. 2.7: Global distribution map for *Seriola quinqueradiata* (compiled and acquired from http://www.fishbase.org). The colours indicate the probabilities of occurrence in a particular area and range from yellow (low probability of occurrence) to red (high probability of occurrence).

Seriola rivoliana (Valenciennes, 1833; Almaco jack, Hawaiian yellowtail, kampachi) is a tropical species that can be found in the Indian-, Atlantic- and Pacific Ocean (Fig. 2.8; Quigley, 2007).

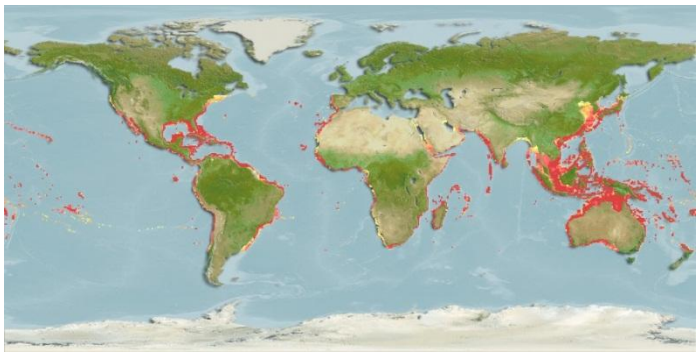


Fig. 2.8: Global distribution map for *Seriola rivoliana* (compiled and acquired from http://www.fishbase.org). The colours indicate the probabilities of occurrence in a particular area and range from yellow (low probability of occurrence) to red (high probability of occurrence).

Seriola zonata (Mitchill, 1815; Banded rudderfish) is found in subtropical waters around the world and the colder (temperate) waters of the North-western Atlantic Ocean (Fig. 2.9). This species is benthopelagic and is confined to the coastal waters over the continental shelf.

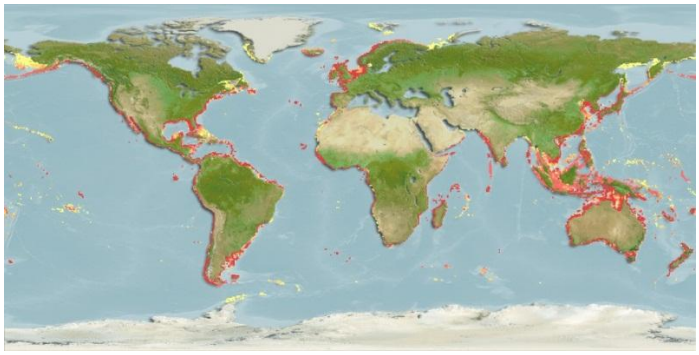


Fig. 2.9: Global distribution map for *Seriola zonata* (compiled and acquired from http://www.fishbase.org). The colours indicate the probabilities of occurrence in a particular area and range from yellow (low probability of occurrence) to red (high probability of occurrence).

The taxonomy and evolutionary relationships of these species is however not well-defined, with confusion in morphologically distinguishing between some members of the species, especially in regards to juveniles. All nine species have synonyms that are used in literature *e.g. S. dumerili* and *S. rivoliana* have 13 synonyms each. In addition, some of these synonyms come from other valid species names, for example *S. lalandi* is also known as *S. dumerili* and *S. zonata* (refer to Table 1.1).

Karyotyping studies in the genus are limited, but have found a similar diploid chromosome number (2n = 48) in four of the species (*S. dumerili*; Sola *et al*., 1997; *S. fasciata*; Tripathy and Das, 1988; *S. quinqueradiata;* Ida *et al*., 1978 and *S. lalandi*; Chai *et al*., 2009).

The current classification of *Seriola* is based mostly on external morphology and geographical distribution patterns. Although Reed *et al*. (2002) did include four *Seriola* species within their carangid study; the molecular phylogenetic relationships of all nine species in the genus are poorly understood. The present study aims to investigate the relationship between species in the genus *Seriola* based on mitochondrial (Cytochrome *b* [*Cytb*] and Cytochrome Oxidase I [*COI*]) and nuclear (recombination activating gene 1 [*RAG1*] and Rhodopsin [*Rhod*]) sequence data. This is the first study to construct a robust phylogeny for this genus that encompasses both mitochondrial- and nuclear genes and layed a better foundation for interpreting relationships within the genus.

2.2 Materials and methods

2.2.1 Samples and DNA extraction

Tissue samples were collected from individuals representative of the nine *Seriola* species (Table 2.1). For species with a large distributional range, samples were collected from localities throughout their respective ranges. Species were identified by the individual sample contributers. Samples were preserved in 95% (v/v) ethanol and stored at room temperature. Tissue samples of selected outgroup were also collected and stored in 95% ethanol. Outgroup species were closely related carangid species of the sister tribe Carangini (Reed *et al*., 2002; Table 2.1).

The hexadecyl-trimethylammoniumbromide (CTAB) protocol by Doyle and Doyle (1987) was used for total genomic DNA extraction with minor modification. Approximately 0.01g of tissue (fin clips or muscle) was incubated overnight at 60°C in 500µl extraction buffer (100mM Tris-HCl, 1.4mM NaCl, 20mM EDTA, 2% (m/v) CTAB), containing 5µl 0.5mg/ml proteinase K (Promega). The blades used for the tissue dissection were thoroughly cleaned with 95% ethanol between individual samples to avoid cross-contamination. Two chloroform-isoamyl alcohol (24:1) separation steps followed to remove proteins and lipoproteins. To precipitate the DNA, 300µl of ice-cold absolute ethanol (100%) was added to the aqueous liquid, followed by incubation at -20°C overnight. The solution was subsequently centrifuged (14 000rpm) for 20min. The DNA pellet was rinsed with 70% (v/v) ethanol, air dried, and suspended in 100µl ddH_2O.

Table 2.1: Taxa included in this study.

Species	Sample number	Locations
S. carpenteri	Scar1[c]	Angola, West Africa
	Scar2[c]	Angola, West Africa
S. dumerili	Sdu15[d]	Madeira Island, West Africa
	Sdu9[g]	Cape Lambert, Australia
	Sdu611[a]	Key Largo, Florida, USA
S. fasciata	Sfa3[a]	South Carolina, USA
	Sfa7[b]	South Carolina, USA
	Sfa10[c]	South Carolina, USA
S. hippos	Ship1[g]	Eyre Peninsula, Australia
	Ship2[g]	New South Wales, Australia
	Ship5[h]	Rottnest, Australia
S. lalandi	A1[k]	Southwest rift, South Africa
	Slala2[f]	New South Wales, Australia
	Slala3[g]	New South Wales, Australia
	Slala902[l]	Fukuoka, Kyushu, Sea of Japan
S. peruana	Sper1[f]	Peru
S. quinqueradiata	Squi1[e]	Japan
	Squi2[e]	Japan
	Squi21[i]	Japan
S. rivoliana	Sri3[a]	South Carolina, USA
	Sri36[j]	South Carolina, USA
	Sri37[b]	South Carolina, USA
Seriola sp.	Sri47[c]	Angola, West Africa
	Sri48[c]	Angola, West Africa
S. zonata	Szo2[a]	Panama City, Florida, USA
	Szo15[a]	Panama City, Florida, USA
	Szo20[b]	South Carolina, USA
Outgroups		
Coryphaena equiselis	Corhip[d]	West Africa
Pseudocaranx dentex	Pse[d]	West Africa
Trachinotus ovatus	Tra1[d]	West Africa
	Tra2[d]	West Africa

Samples were contributed by [a] Mark Renshaw, [b] Byron White, [c] Kent Carpenter, [d] Peter Wirtz, [e] Haruhisa Fukada, [f] HJ Walker, [g] Alastair Graham, [h] Kate Hudson, [i] T Masumoto, [j] Luca Castriota, [k] Sven Kerwath, and [l] Natalie Martinez-Takeshita**.**

2.2.2 Primers, PCR amplification and sequencing

Polymerase Chain Reaction (PCR) products for the two mitochondrial- (*Cytb* [800bp] and *COI* [400bp]) and two nuclear (*Rhod* [800bp] and *RAG1* [700bp]) fragments were amplified with the

primer sets listed in Table 2.2. Approximately 20ng of template DNA was used in a 25µl PCR reaction (0.1µM primer, 1X Go*Taq* buffer, 2.5mM $MgCl_2$, 0.5mM dNTPs and 0.5unit Go*Taq*; Promega). Amplification was conducted following an initial denaturing step at 94°C for 3min, 35 cycles of denaturation at 94°C for 30s, respective annealing temperature for 30s, extension at 72°C for 45s and a final extension step at 72°C for 5min.

Table 2.2: Primer sequences used in this study.

Gene	Primer name	Primer sequence (5'- 3')	T_a (°C)	Reference
COI	L6569	CCT GCA GGA GGA GGA GAT CC	48-55	Martin *et al*., 1992
	H7110	CCA GAG ATT AGA GGG AAT CAG TG		Martin *et al*., 1992
Cytb	L14724	TGA CTT GAA RAA CCA YCG TTG	55	Palumbi *et al*., 1991
	H15889	TGG RAC TGA GCT ACT AGT GC		Reed *et al*., 2002
Rhod	Rh193	CNT ATG AAT AYC CTC AGT ACT ACC	55	Chen *et al*., 2003
	Rh1073r	CCR CAG CAC ARC GTG GTG ATC ATG		Chen *et al*., 2003
RAG1	*RAG1*F1	CTG AGC TGC AGT CAG TAC CAT AAG ATG T	58	Lopez *et al*., 2004
	*RAG1*R1	CTG AGT CCT TGT GAG CTT CCA TRA AYT T		Lopez *et al*., 2004

T_a = annealing temperature

PCR products were visualised on 1% (m/v) agarose gels containing ethidium bromide. Amplicons were purified with SigmaSpin post-reaction clean-up columns (Sigma). Cycle sequencing of the purified products was performed only in the forward direction with the BigDye terminator kit v3.0 (Applied Biosystems) and analysed on a 3100 ABI automated sequencer at Stellenbosch University Central Analytical Facilities. Sequences were edited and manually aligned with BioEdit v7 (Hall, 1998) and subsequent alignments checked in ClustalX (Thompson *et al.*, 1997) to ensure optimal alignment.

2.2.3 Phylogenetic analysis

The evolutionary relationships in the genus *Seriola* were investigated with three phylogenetic reconstruction methods: Maximum parsimony (MP), Maximum likelihood (ML) and Bayesian inference (BI). MP analyses were performed in MEGA v4.02 (Tamura *et al.*, 2007) and were conducted using heuristic searches with 100 random addition (RA) replicates and tree bisection reconnection (TBR) branch swapping. Confidence in the resulting relationships was assessed with the nonparametric bootstrap procedure (Felsenstein, 1985) with 10 000 bootstrap replicates. The ML trees were constructed with PHYML v2.4.4 (Guindon and Gascuel, 2003). The analysis was started from a Neighbour-Joining tree and the TBR approach was used to search for tree topology. Bootstrap values for each dataset were acquired with 1 000 pseudoreplicates and all ML trees' node values are represented as the proportion of replicates in which that clade was recovered. MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) was used for Bayesian analysis. Four independent searches were performed each with four Markov chains for 1 000 000 generations, with trees saved every 100 generations. Bayesian posterior probabilities were estimated from the resulting 10 000 parameter point-estimates minus the first 25% (2 500) burn-in phase.

Model selection is an important component in phylogenetic inference. The most common method of model selection is the hierarchical likelihood ratio test (Posada and Crandall, 1998). However, this method may not be ideal due to its limitations regarding the nested model structure required for comparing hypotheses (Pol, 2004; Posada and Buckley, 2004). Other methods of model justification, such as the Akaike Information Criteria (AIC), are preferred because both nested- and non-nested sets of models can be evaluated, while allowing for model

averaging procedures and assessments of model selection uncertainties (Adachi and Hasegawa, 1996; Shimodaira and Hasegawa, 1999; Shimodaira, 2001). In the current study, model selection for ML and BI followed the procedures outlined by Posada and Buckley (2004) for AIC. ModelTest v3.6 (Posada and Crandall, 1998) was used to identify the model with the highest AIC weight; thus the evolutionary model that would best fit the datasets, for each gene individually, and for the combined dataset. The best-fit models identified under AIC for the *Cytb*, *COI* and *RAG1* datasets was the general time-reversible model with gamma-distributed rate heterogeneity parameter (GTR+G). The Hasegawa-Kishino-Yano plus gamma (HKY+G) model was selected for the *Rhod* dataset. These models were subsequently implemented in the ML and BI analysis for each separate dataset.

Two different strategies were used to analyse the combined dataset containing all four fragments. First, ML and MP were carried out on a single concatenated dataset and secondly, BI was performed on a partitioned dataset. ModelTest was employed to choose the best-fitting model, with GTR+G identified as the model to best fit the concatenated dataset. For BI, the dataset was partitioned by gene and a mixed-model performed, implementing the models and parameters previously identified by Modeltest for each individual gene. Uncorrected pairwise genetic distances (*p*-distance) among the *Seriola* species were calculated in MEGA.

Divergence times were estimated in BEAST v1.7.3 (Drummond *et al*., 2012) and BEAUti v1.7.3 was used to generate the input files. An uncorrelated log-normal relaxed molecular clock (Drummond *et al*., 2006) was used to estimate the node ages using the same partitioning strategy and models of evolution for the combined data set as implemented during the BI analysis. The

Yule speciation process (assuming a constant speciation rate per lineage) was selected as tree prior (Drummond and Rambaut, 2007). Two calibration points were used: 55.0 MYA (SD = 1.0) and 3.2 MYA (SD = 0.2). The first date reflects the first time the genus was documented in the fossil record (Berg, 1947; Patterson and Owen, 1991) and was used to calibrate the ancestral node in the root of the phylogenetic tree of the genus *Seriola.* The second date is a biogeographic calibration point for the speciation between *S. fasciata* and *S. peruana.* These two species are sister taxa and are separated by the Isthmus of Panama, a land bridge between the Indo-Pacific and Atlantic oceans. The most recently geological age of the uprising of the Panamanian isthmus is approximately 3.2 MYA (Duque and Aro, 1990; Bermingham *et al*., 1997; Marko, 2002; Lessios, 2008). Five independent runs with simulations of 10 million Monte Carlo Markov Chain (MCMC) steps with sampling every 1 000 generations and a burn-in of 10% were computed in BEAST. After the first run the program TRACER v1.5 (Rambaut and Drummond, 2007) was used to examine effective samples sizes (ESS) to assess if this parameter was above 200 and if the parameter space had been efficiently searched to reach convergence. After the five runs TRACER was used to examine and combine the log files.

2.3 Results

Separate phylogenetic data analyses were conducted for each gene region as well as the combined dataset. Partial sequences were obtained for *Cytb*, *COI*, *Rhod* and *RAG1* after trimming of the 3' end (Appendix A - D). Three inference methods were used to construct the phylogenetic trees: Maximum parsimony (MP), Maximum likelihood (ML) and Bayesian inference (BI). Phylogenies based on the individual gene regions differed somewhat depending on the fragment examined and inference method used. The relationship between the species will

be discussed in more detail in the combined analysis. For each region the ML phylogenetic tree is shown with bootstrap values (ML/MP) and posterior probabilities (BI) given for nodes.

2.3.1 Cytb

A total sequence length of 800bp was obtained for *Cytb* for all nine species (27 individuals) and three outgroup species. A total of 342 nucleotide sites were variable of which 277 positions were parsimony informative and 65 were singletons. Base composition analysis revealed a bias against guanine (A: 0.238; C: 0.320; G: 0.163; T: 0.279). The transition: transversion ratio (1.5/1) showed some bias towards transitions with a rate of $R = 5.971$. Parsimony analysis produced nine equally parsimonious trees of 649 steps (CI = 0.555; RI = 0.769). The three different analytical methods all indicated the monophyletic nature of the nine species within the genus *Seriola* (bootstrap values ≥ 80 and posterior probabilities = 1.00; Fig. 2.10).

2.3.2 COI

Despite various PCR optimisation efforts, only six of the nine species could be reliably amplified. *COI* sequences from these six species (18 individuals) and one outgroup species totalled 400bp in length.Of these characters, 104 were variable, of which 67 were parsimony informative. A bias against guanine (A: 0.239; C: 0.260; G: 0.199; T: 0.302) was again observed. For the *COI* sequences, a transversion bias was observed (transition: transversion ratio = 1/2.4; $R = 10.669$). The MP analysis resulted in 51 equally parsimonious trees with a length of 139 (CI = 0.799; RI = 0.885). Within this tree, the monophyly of the *Seriola* species is well-supported (bootstrap values ≥ 80 and posterior probabilities = 1.00; Fig. 2.11).

2.3.3 RAG1

The final *RAG1* alignment included all nine species (25 individuals) and two outgroup species. A total length of 700 characters was obtained of which 80 were variable and only 39 characters were parsimony informative. Base frequencies were A: 0.217; C: 0.251; G: 0.301; T: 0.231 and the transition: transversion ratio was 1.4/1 (R = 2.558). For *RAG1*, parsimony analysis produced 182 equally parsimonious trees with a length of 77 (CI = 0.896; RI = 0.929). Within this particular tree the monophyly of some of the species has weak support (*e.g. S. hippos* and *S. rivoliana*; bootstrap values ≤ 70 and posterior probabilities ≤ 0.95; Fig. 2.12).

2.3.4 Rhod

A length of 800bp (99 variable sites, 62 parsimony informative sites) was obtained for the *Rhod* sequences for all nine species (27 individuals) and three outgroup species. Base frequencies were A: 0.184; C: 0.288; G: 0.238; T: 0.289 and the transition: transversion ratio was 1.2/1 (R = 2.005). Parsimony analysis done on the *Rhod* dataset resulted in 482 equally parsimonious trees (length = 123; CI = 0.886; RI = 0.941). All three analytical methods indicated the monophyletic nature of five of the *Seriola* species (bootstrap values ≥ 80 and posterior probabilities = 1.00; Fig. 2.13). Two of the three phylogenetic methods (ML and MP) retrieved a single clade for *S. hippos* and *S. lalandi*, although support for these clades are low (bootstrap values between 43 and 74).

2.3.5 Combined dataset

The analyses of the combined mtDNA and nuclear DNA datasets resulted in phylogenetic hypotheses that were consistent among the three methods of phylogenetic inference (Fig. 2.14).

Parsimony analysis among the 27 taxa (2 700bp, 625 variable sites, 443 parsimoniously informative sites) produced six equally parsimonious trees of 782 steps (CI = 0.599; RI = 0.779). Trees constructed by the three analytical methods all indicated the monophyly of the *Seriola* species and this was strongly supported (bootstrap values ≥ 80 and posterior probabilities = 1.00; Fig. 2.14).

The combined dataset revealed three main groupings. Firstly, there is a close association between *S. fasciata* and *S. peruana*, with *S. carpenteri* as a sister taxon. This relationship can also be seen in the *Cytb*, *RAG1* and *Rhod* trees (Fig. 2.10, 2.12, 2.13, 2.14). The second noteworthy relationship is observed between *S. dumerili* and *S. rivoliana*; a trend also supported in the separate analysis of the *Cytb*, *RAG1* and *Rhod* regions (Fig. 2.10, 2.12, 2.13, 2.14). Lastly, the close relationship between *S. lalandi* and *S. quinqueradiata* can be seen in the *Cytb*, *COI*, *RAG1*, *Rhod* and the combined analyses (Fig. 2.10 - 2.14). Within the combined dataset, *S. zonata* forms a sister taxon to the *S. lalandi* and *S. quinqueradiata* grouping; a trend only found for the *RAG1* region (Fig. 2.12, 2.14). However, this species is basal in the clade containing the rest of the species excluding *S. lalandi* and *S. quinqueradiata* in the *Cytb* and *Rhod* phylogenies (Fig. 2.10, 2.13). The relationship between *S. hippos* and the rest of the *Seriola* species is still unresolved because only ML analyses showed some support for this grouping (bootstrap value = 61%). However, the *Cytb*, *RAG1* and *Rhod* trees show a relationship between *S. hippos* and the *S. dumerili* / *S. rivoliana* clade. The *Seriola* sp. samples show a close relationship to *S. carpenteri* (Fig. 2.10 - 2.14).

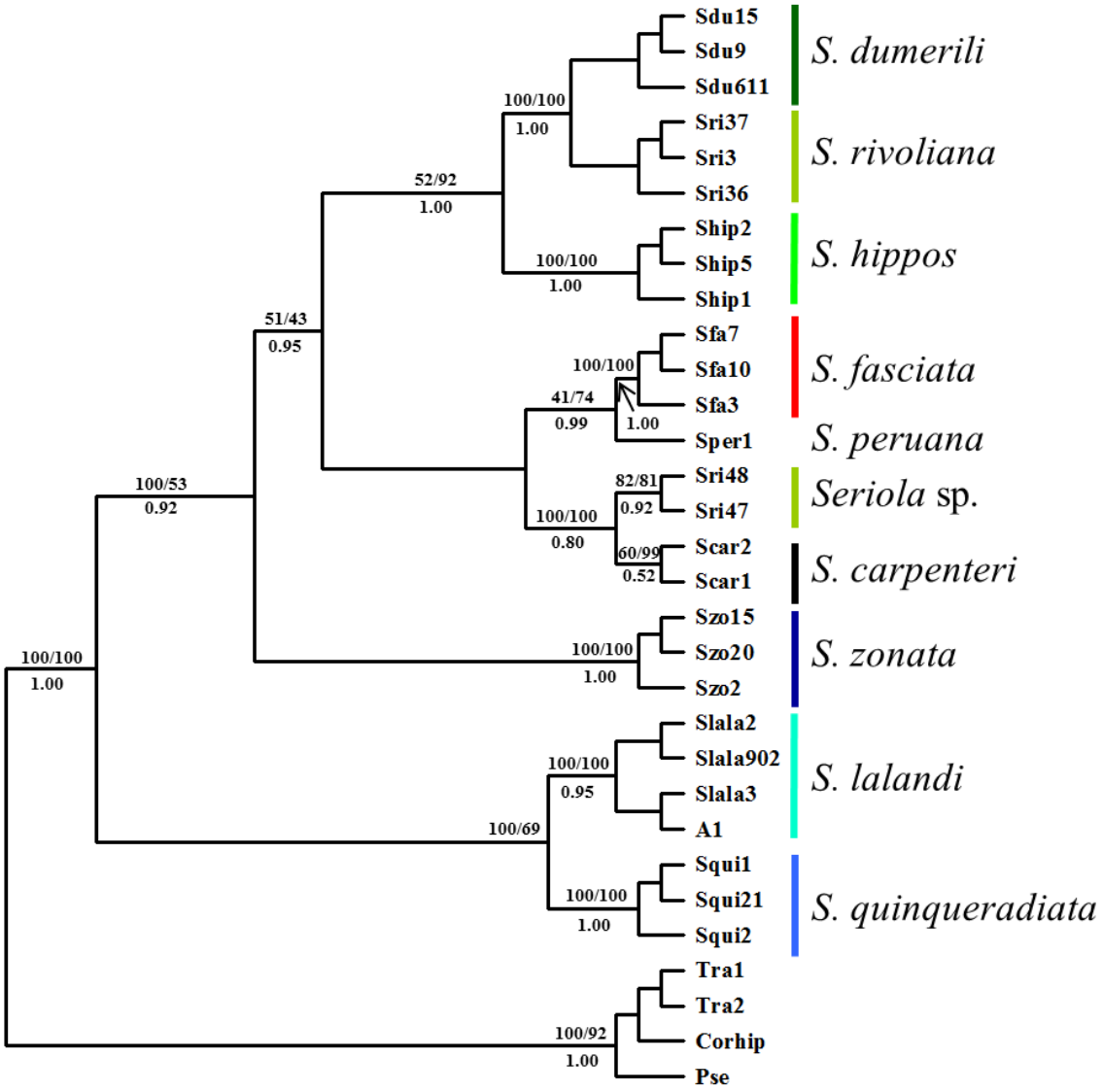


Fig. 2.10: The ML tree of the *Cytb* region for the genus *Seriola*. Numbers above branches to the left are MP bootstrap support values and to the right, ML bootstrap support values. Only bootstrap values greater than 50% are shown. Numbers below branches are Bayesian posterior probabilities. Arrow indicates branch to which support values refers to.

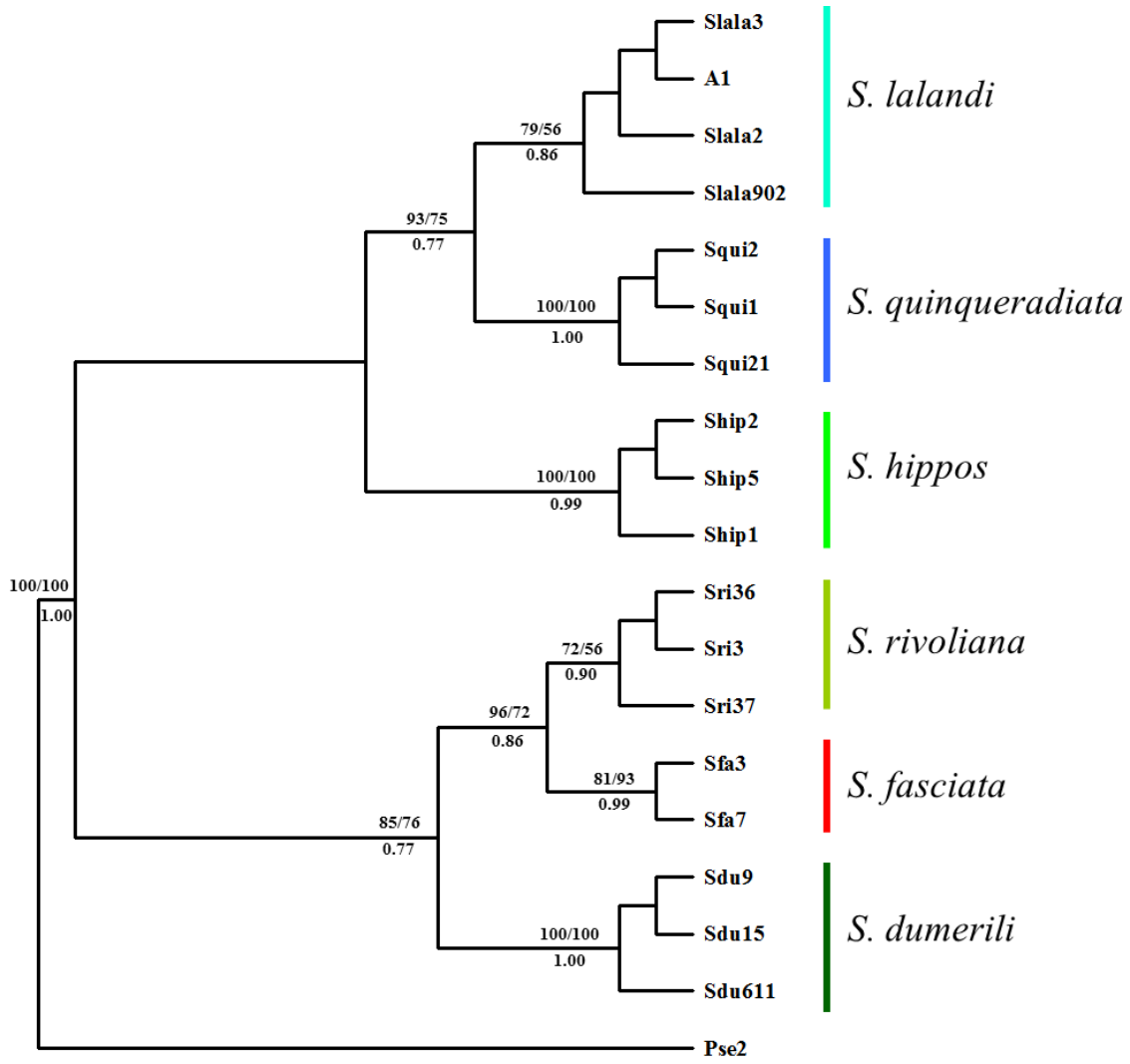


Fig. 2.11: The ML *COI* phylogeny of the genus *Seriola*. Numbers above branches to the left are MP bootstrap support values and to the right, ML bootstrap support values. Only bootstrap values greater than 50% are shown. Numbers below branches are Bayesian posterior probabilities.

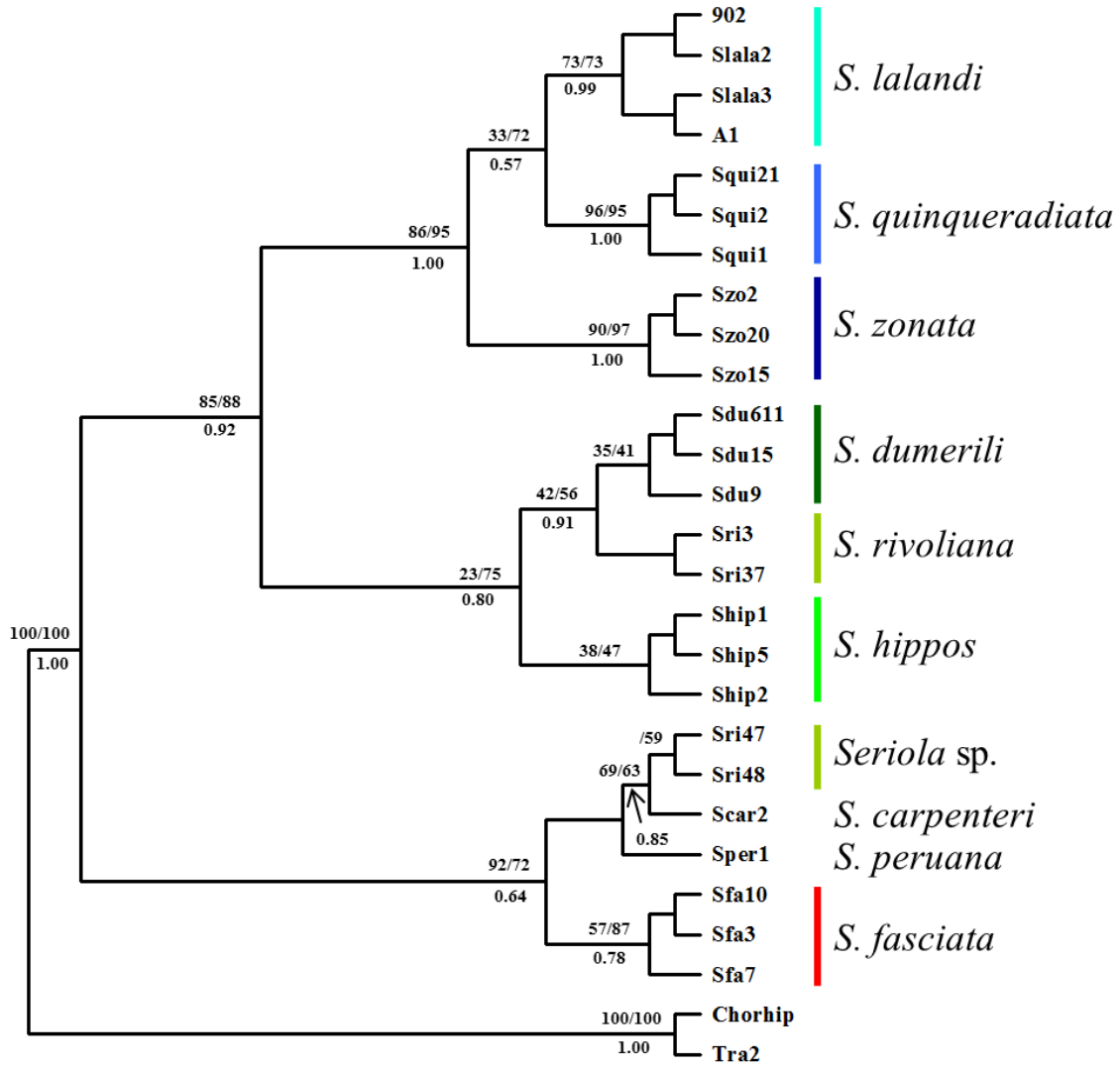


Fig. 2.12: The ML genealogy of the *RAG1* region for the genus *Seriola*. Numbers above branches to the left are MP bootstrap support values and to the right, ML bootstrap support values. Only bootstrap values greater than 50% are shown. Numbers below branches are Bayesian posterior probabilities. Arrow indicates branch to which support values refers to.

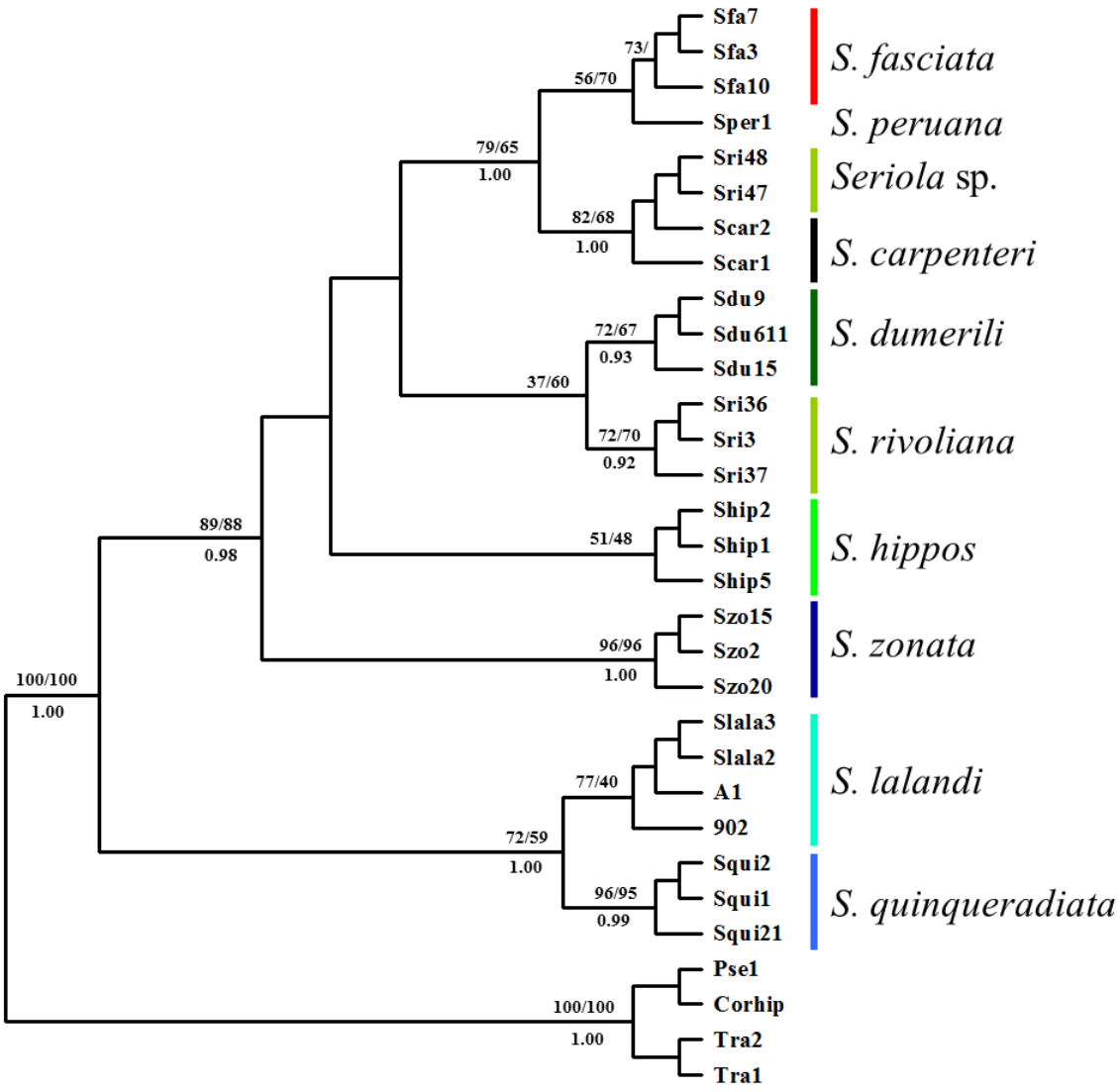


Fig. 2.13: The *Rhod* phylogeny constructed using ML for the genus *Seriola*. Numbers above branches to the left are MP bootstrap support values and to the right, ML bootstrap support values. Only bootstrap values greater than 50% are shown. Numbers below branches are Bayesian posterior probabilities.

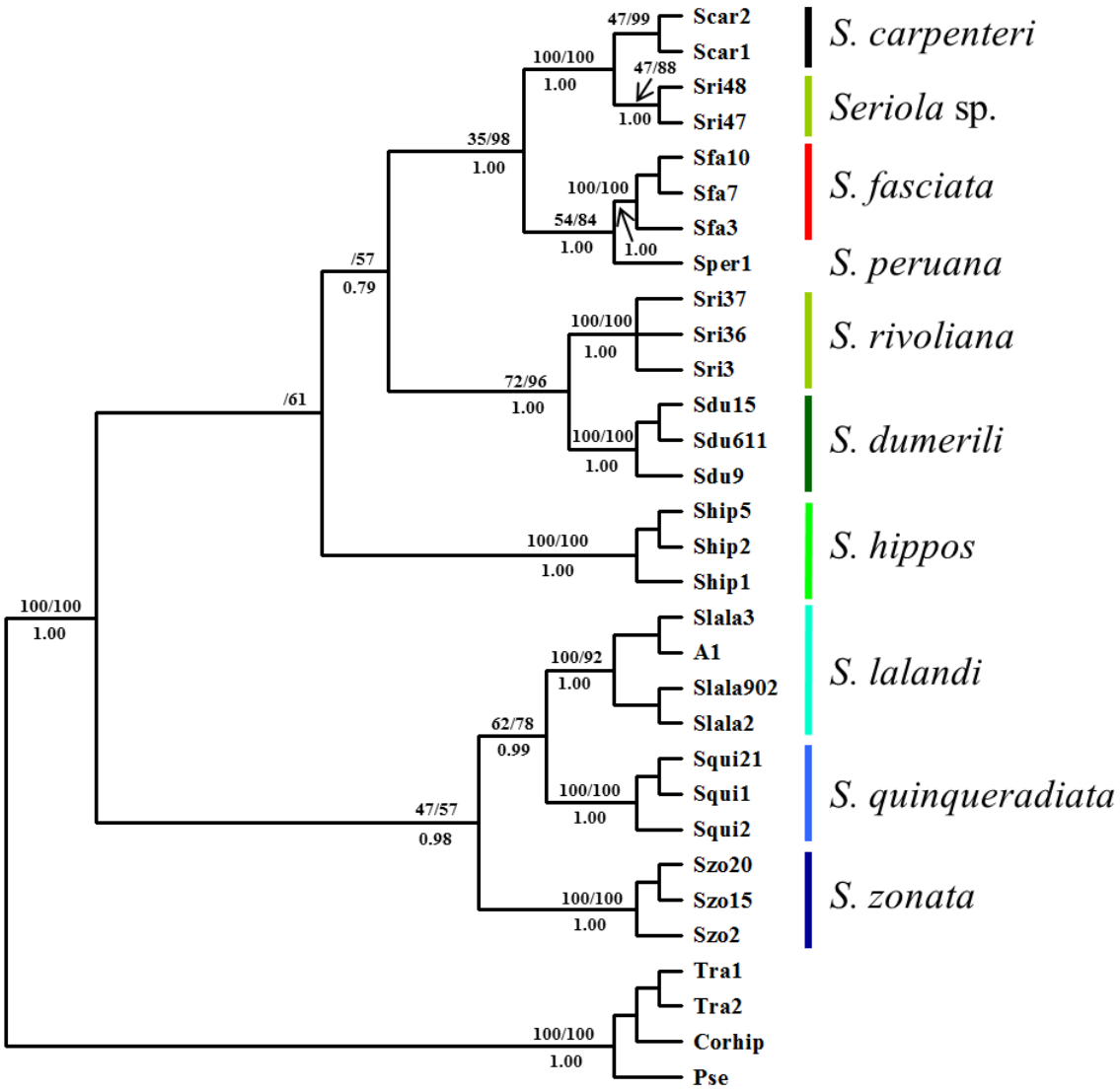


Fig. 2.14: The ML phylogeny of the combined dataset for the genus *Seriola*. Numbers above branches to the left are MP bootstrap support values and to the right, ML bootstrap support values. Only bootstrap values greater than 50% are shown. Numbers below branches are Bayesian posterior probabilities. Arrows indicate branches to which support values refer to.

2.3.6 Sequence divergence

Uncorrected percentage sequence divergence for *Cytb* (*p*-distances; Table 2.3) ranged from 7.1 - 14% among the *Seriola* species. The lowest sequence divergence was found between *S. lalandi* and S. *quinqueradiata* and the highest, between *S. rivoliana* and *S. fasciata.* The range of *p*-distances for *COI* between the *Seriola* taxa is 1 - 10.8% (Table 2.3). The range of *p*-distances between the species for *RAG1* is 0.3 - 2.1%, with the highest sequence divergence between *S. lalandi* and *S. carpenteri* (Table 2.4). For *Rhod* sequences the highest sequence divergence was found between *S. carpenteri* and *S. quinqueradiata* (1.3%), with the sequence divergence between all species ranging from 0.0 - 1.3% (Table 2.4).

Table 2.3: Uncorrected pairwise distances (*p*-distances) for the nine *Seriola* species for *Cytb* (below diagonal) and *COI* (above the diagonal).

	S. rivoliana	*S. fasciata*	*S. zonata*	*S. dumerili*	*S. carpenteri*	*S. peruana*	*S. quinqueradiata*	*S. hippos*	*S. lalandi*
S. rivoliana	-	0.010	N/A	0.053	N/A	N/A	0.099	0.065	0.085
S. fasciata	0.140	-	N/A	0.055	N/A	N/A	0.099	0.070	0.083
S. zonata	0.109	0.133	-	N/A	N/A	N/A	N/A	N/A	N/A
S. dumerili	0.071	0.122	0.113	-	N/A	N/A	0.108	0.078	0.080
S. carpenteri	0.109	0.118	0.112	0.110	-	N/A	N/A	N/A	N/A
S. peruana	0.132	0.128	0.119	0.125	0.123	-	N/A	N/A	N/A
S. quinqueradiata	0.108	0.130	0.099	0.113	0.122	0.131	-	0.094	0.058
S. hippos	0.104	0.130	0.114	0.093	0.120	0.133	0.107	-	0.085
S. lalandi	0.102	0.132	0.101	0.093	0.124	0.132	0.071	0.103	-
Seriola sp.	0.127	0.134	0.130	0.127	0.006	0.146	0.139	0.139	0.141

Table 2.4: Uncorrected pairwise distances (*p*-distances) for the nine species of the genus *Seriola* for *RAG1* (below diagonal) and *Rhod* (above diagonal).

	S. rivoliana	*S. fasciata*	*S. zonata*	*S. dumerili*	*S. carpenteri*	*S. peruana*	*S. quinqueradiata*	*S. hippos*	*S. lalandi*	*Seriola* sp.
S. rivoliana	-	0.005	0.008	0.003	0.008	0.005	0.011	0.003	0.008	0.008
S. fasciata	0.018	-	0.008	0.005	0.003	0.000	0.011	0.005	0.008	0.003
S. zonata	0.017	0.019	-	0.008	0.007	0.007	0.011	0.005	0.008	0.008
S. dumerili	0.009	0.014	0.010	-	0.008	0.005	0.011	0.003	0.008	0.008
S. carpenteri	0.019	0.004	0.020	0.015	-	0.003	0.013	0.007	0.010	0.001
S. peruana	0.018	0.003	0.019	0.014	0.004	-	0.011	0.005	0.008	0.003
S. quinqueradiata	0.017	0.019	0.009	0.010	0.020	0.019	-	0.009	0.005	0.015
S. hippos	0.010	0.012	0.008	0.003	0.013	0.012	0.008	-	0.006	0.008
S. lalandi	0.018	0.020	0.010	0.011	0.021	0.020	0.008	0.009	-	0.012
Seriola sp.	0.018	0.003	0.021	0.015	0.000	0.003	0.022	0.014	0.024	-

2.3.7 Divergence times

The calibrated molecular clock indicates an early Paleocene origin (54.79 MYA) for the genus *Seriola* (Table 2.5). Only divergence times (indicated as time to most recent common ancestor - TMRCA) for the three well-supported groupings found by the phylogenetic analyses are presented. All three main groupings originated approximately at the same time during the early Miocene (14.85 MYA for *S. carpenteri*, *S. fasciata* and *S. peruana*; 16.45 MYA for *S. rivoliana* and *S. dumerili*; 17.87 MYA for S. *lalandi* and *S. quinqueradiata*); with most of the species radiation within the Pliocene (1.32 - 5.47 MYA).

Table 2.5: Divergence times estimated in MYA and 95% confidence intervals (CI) in brackets for *Seriola* species.

Node description	TMRCA
Root age	54.79 (52.786 - 56.803)
S. carpenteri, *S. fasciata* and *S. peruana* clade	14.85 (3.982 - 24.082)
S. fasciata and *S. peruana* clade	3.28 (2.907 - 3.673)
S. rivoliana and *S. dumerili* clade	16.45 (4.140 - 29.910)
S. lalandi and *S. quinqueradiata* clade	17.87 (5.365 - 32.8554)
S. rivoliana clade	5.43 (0.608 - 12.021
S. fasciata clade	1.78 (0.845 - 2.730)
S. zonata clade	1.76 (0.028 - 5.485)
S. dumerili clade	3.79 (0.377 - 8.903)
S. carpenteri clade	3.05 (0.280 - 7.060)
S. quinqueradiata clade	1.32 (0.033 - 3.850)
S. hippos clade	1.90 (0.084 - 5.747)
S. lalandi clade	9.03 (2.631 - 17.476)

2.4 Discussion

This study represents the first phylogenetic analysis of the genus *Seriola*. The evolutionary relationships within *Seriola* were based on analyses of mitochondrial- (*Cytb* and *COI*) and nuclear DNA sequences (*RAG1* and *Rhod*), both separately and in combination. The mitochondrial- and nuclear phylogenies were largely congruent. The *Cytb* genealogy showed a higher resolution in terms of well-supported terminal clades compared to the nuclear fragments. The *RAG1* tree was more resolved than the tree based on the *Rhod* gene, which seemed to have a slower rate of molecular evolution and lower sequence divergence.

For most of the molecular phylogenies generated, three main groupings could be found: a grouping of *S. fasciata* and *S. peruana*, with *S. carpenteri* as sister grouping (*Cytb*, *RAG1* and *Rhod*), a *S. dumerili* and *S. rivoliana* grouping (*Cytb*, *RAG1* and *Rhod*) and a *S. lalandi* and *S.*

quinqueradiata clade (*Cytb*, *COI*, *RAG1* and *Rhod*). However, the relationship between these groupings differed between datasets. For example, the S. *lalandi* / *S. quinqueradiata* clade is the earliest diverging in the *Cytb* and *Rhod* trees, while in the *RAG1* and *COI* topologies this species cluster is most derived. This clade was also placed basal in the combined trees. More significant however, is the placement of *S. zonata* and *S. hippos* with respect to the other species in the separate datasets. Three of the five topologies (*Cytb*, *RAG1* and *Rhod*) show a relationship between *S. hippos* and the *S. dumerili* / *S. rivoliana* clade, but no support was found for this grouping except for the ML bootstrap value for *RAG1* (75%) and the MP bootstrap value for *Cytb* (67%). The relationship between *S. zonata* and the *S. lalandi* / *S. quinqueradiata* clade was only found in the combined- and *RAG1* topologies. Here the support values for this clade were high for *RAG1* (MP = 86%, ML = 88%, BI = 1.00), with the combined tree showing low bootstrap support values (MP = 47%, ML = 57%) but a high Bayesian posterior probability (0.98). In the combined dataset, nodal support was overall higher.

Several studies have used the four different markers employed in the current study to resolve the evolutionary relationships of marine fishes (Tang *et al*., 2010, 2011), which resulted in robust and well-resolved phylogenies and all the gene fragments used in this study resolved the relationship between the *Seriola* species to some extent. Within the current study, *Cytb* and *RAG1* seem to be the most informative regions, resulting in the most robust and best-supported trees. Cytochrome *b* has been the preferred DNA fragment in studies of the phylogenetic relationship between carangid genera and tribes (Reed *et al*., 2002) and for intraspecific relationships within carangid genera (*Selene*; Reed *et al*., 2001; *Trachurus*; Poulin, 2004; Cárdenas *et al*., 2005; Bektas and Belduz, 2008). Several phylogenetic studies on other marine

species also found *RAG1* to be highly informative (Chen *et al*., 2007; Vélez-Zuazo and Agnarsson, 2011). The phylogenetic analysis of the *Rhod* gene fragment did resoled the main *Seriola* groupings; however some of the support values (bootstrap and posterior probabilities) were very low. Rhodopsin (*Rhod*) is connected with light vision and in fish species it may be under selection because of the various light intensities within the aquatic environment. For example, in sand gobies (*Pomatoschistus minutus*) substantial intraspecific variation and local adaptation to light conditions was found in *Rhod*; thereby showing a strong positive selection to dim-light conditions (Larmuseau *et al*., 2009). The fact that *Rhod* is possibly under selection probably makes it a less ideal marker to use in phylogenetic studies; however several studies found this gene useful in resolving phylogenetic relationships (*e.g.* Acanthomorpha; Chen *et al*., 2002; Gerreidae; Chen *et al*., 2007; Liu *et al*., 2010; Lin and Hastings, 2011). In the current study there was a problem amplifying the *COI* fragment in three of the nine *Seriola* species. The phylogenies did support the monophyly of the species, however only the *S. lalandi* / *S. quinqueradiata* clade could be recovered. The effectiveness of *COI* was shown in several phylogenetic studies (*e.g. Channa* species; Larka *et al*., 2010; characid species; Javonillo *et al*., 2010; *Etmopteridae* species; Straube *et al*., 2010). Although the *Cytb* and *RAG1* datasets produced well-supported phylogenies, the combined dataset lead to increased support values for most *Seriola* relationships. Thus, uncertainties due to differences in resolving power in individual gene trees may be resolved when datasets are combined. Phylogenetic analyses of combined datasets have been shown to resolve conflicts among individual fragment analyses (Gatesy *et al*., 1999; Hrbek *et al*., 2007; Gray *et al*., 2009).

Genetic divergence values based on the four genes proved to be sufficient to distinguish between different *Seriola* species. These values were within the range reported for other teleost species for *Cytb* (0.26 - 26.3% in Carangidae species; Reed *et al*., 2002); *COI* (1.04 - 35.72% in Australian fishes; Ward *et al*., 2005); *RAG1* (0.5 - 2.7% in *Pseudoplatystoma*; Carvalho-Costa *et al*., 2011) and *Rhod* (0.5 - 2% in Atherinidae; Pujolar *et al*., 2012). The observed transition vs. transversion ratios in *Seriola* are also comparable to those of many other teleost species (Sciaenidae fish; Vinson *et al*., 2004; Australian fishes; Ward *et al*., 2005; *Trichiurus* species; Chakraborty *et al*., 2006). The bias against guanine found in all four DNA fragments has been frequently observed in several teleost species (*e.g.* Stromateoid fishes; Doiuchi and Nakabo, 2006; *Trachurus* species; Bektas and Belduz, 2008; *Macronectes* species; Techow *et al*., 2010).

2.4.1 Genetic relationships within the Genus Seriola

All phylogenetic analyses confirm the monophyly for each of the *Seriola* species, The two unidentified *Seriola* samples of (referred to as *Seriola* sp.) show a close relationship with *S. carpenteri*. Within most of the datasets, three main groupings can be found: a ((*S. fasciata* and *S. peruana*), *S. carpenteri*) grouping, a (*S. dumerili* and *S. rivoliana*) grouping and a (*S. lalandi* and *S. quinqueradiata*) clade. The close association between *S. fasciata* and *S. carpenteri* was also found by Reed *et al*. (2002) in a phylogenetic study of the family Carangidae.

The phylogenetic placement of *S. hippos* and *S. zonata* could not be determined in the current study. While *S. zonata* shows a close relationship to the *S. lalandi* / *S. quinqueradiata* grouping in the combined dataset, this was only supported in the *RAG1* analysis. Within the *Cytb* and

Rhod phylogenies, *S. zonata* appears to be basal to the group containing the rest of the *Seriola* species.

It is well-known that selection of appropriate outgroup taxa are imperative to resolve the relationships between groupings within phylogenetic analyses. Each of the outgroup species in the current study was from a different tribe in the Carangidae (Carangini - *Pseudocaranx dentex*; Trachinotini - *Trachinotus ovatus*; Scomberoidini - *Coryphaena equiselis*). An outgroup within the tribe Naucratini might have been more appropriate due to the closer relationship of species in this tribe to *Seriola* (Reed *et al*., 2002). However the Naucritini consists mostly of the genus *Seriola* with the other genera in the tribe, *Elagatis*, *Seriolina*, *Naucrates* and *Campogramma* (Smith-Vaniz, 1984) being monotypic (one species in the genus). Samples of these genera are consequently hard to find.

2.4.2 Biogeography of Seriola

Irrespective of the remaining phylogenetic ambiguities, several biogeographical patterns emerge from the current study. The first important pattern is the separation of the temperate species (*S. lalandi* / *S. quinqueradiata*) as one monophyletic group. A second is the sister relationship of two widespread pelagic species, *S. dumerili* and *S. rivoliana*. The observation of the sister group relationship within the ((*S. fasciata* and *S. peruana*), *S. carpenteri*) clade, may however be one of the most significant results of this study. Species of this clade are mainly found in the Atlantic Ocean with the exception of *S. peruana* which is restricted to the tropical waters off the coast of the western part of Central America. *Seriola carpenteri* has a limited distribution off the coast of

Africa, while *S. fasciata* can be found on both sides of the Atlantic. This suggests that this clade probably has an Atlantic origin.

There are several hypotheses as to the origin of the Atlantic species: 1) these species are Tethys relicts present prior to the Tethys Sea closure about 12 - 20 MYA (Dercourt *et al*., 1986), 2) these taxa had a West Atlantic–East Pacific origin prior to the rise of the Isthmus of Panama about 3.1 MYA (Coates and Obando, 1996), 3) these species radiated within the Atlantic, or 4) there was an Indo-Pacific invasion by means of southern Africa (Floeter *et al*., 2008; Bowen and Biggs, 2013). When considering the divergence time of the ((*S. fasciata* and *S. peruana*), *S. carpenteri*) clade (14.85 MYA) estimated in the current study, the closure of the Tethys Sea is however, suggested to have played the most important role in the origin of this clade.

The biogeographic history of many marine species originated when their ancestors, present in the Tethys Sea, were separated by the division of this single body of water into the present day Mediterranean Sea (western part of the Tethys) and the present day Indian Ocean (eastern part of the Tethys) during the Early Miocene (12 - 20 MYA; Dercourt *et al*., 1986). Therefore, the Atlantic Ocean most probably became separated from the Indian Ocean around this time (Steininger and Rögl, 1984; reviewed in Barber and Bellwood, 2005) and has not been connected until the construction of the Suez canal in 1869. The Tethys Sea closure is postulated as the first major event shaping the phylogenetic relationship in *Seriola*; thus representing an example of vicariance (*i.e.* the separation of a species into two or more fragments through the occurrence of a geographical barrier or barriers). The *S. fasciata* / *S. peruana* / *S. carpenteri* / *Seriola* sp. clade was estimated to have originated 13 MYA; which falls within the time period of the closing of

the Tethys Sea. In this vicariant setting, the ancestral *Seriola* populations for this clade became separated in the Mediterranean Sea, and could then subsequently disperse across the Atlantic Ocean and diverge into different species. Similar sister group relationships resulting from the Tethys Sea closure has been observed in other marine fish, *e.g. Trachurus* species (Cárdenas *et al*., 2005), needlefishes (Banford *et al*., 2004), and angel sharks (Stelbrink *et al*., 2010).

Within the Tethys Sea the original fauna was tropical, however during the Oligocene and Early Miocene (~ 23 - 18 MYA) water temperatures decreased (Berggren and Hollister, 1975). This cooling of the oceanic waters may have caused the ancestral *Seriola* populations to move southward along the coast of West Africa to warmer waters and westward across the Atlantic Ocean, thus giving rise to *S. carpenteri* and *S. fasciata*.

However, more noteworthy with regards to paleo- and biogeography for the *S. fasciata* / *S. peruana* / *S. carpenteri* clade is the sister group relationship of *S. peruana* and *S. fasciata*, which occur in the eastern central Pacific and in the Atlantic, respectively; thus separated by the Panama land bridge. Jordan (1908) was one of the first authors to point out the morphological similarities between teleost fish species on either side of the Panamanian isthmus, consequently these species can be regarded as identical species in the past. He also proposed that this relationship between the ‘‘geminate species” (twin species) might be a consequence of earlier geological events, particularly, the rise of the Isthmus of Panama during the Miocene (Jordan, 1908). Several studies on Central America geology (reviewed in Kirby *et al*., 2008) have shown that the rise of the Panamanian isthmus was a successive process beginning in the Middle Miocene (ca. 20 MYA) and ending in the Late Pliocene with the final rise of the Panamanian

isthmus ca. 3.5 - 2.9 MYA (Bermingham *et al*., 1997; Kirby *et al*., 2008). Therefore, the Panamanian isthmus was constantly rising or submerging within this period. During the submerging of the Panamanian isthmus several narrow straits including the Culebra Strait and Atrato Seaway might have provided the opportunity for gene flow between *Seriola* populations on both sides. However, gene flow was completely disrupted by the final rise of the Panamanian isthmus during the Late Pliocene (3.5 - 2.9 MYA). In this manner *S. peruana* could have been separated from the Atlantic species. The rise of the Panamanian isthmus has been found to have been a driving force for speciation in many marine genera (*e.g.* hake species; Grant and Leslie, 2001; *Trachurus* species; Cárdenas *et al*., 2005; Moray eels; Reece *et al*., 2010; angel sharks; Stelbrink *et al*., 2010; coastal marine snails; Miura *et al*., 2010; angelfishes; Alva-Campbell *et al*., 2010).

Divergence estimates in the current study suggest that the *S. rivoliana* and *S. dumerili* clade and the *S. lalandi* and *S. quinqueradiata* clade originated in the Miocene (~ 18 - 16 MYA). However, while *S. dumerili* and *S. rivoliana* are tropical / subtropical species, *S. lalandi* and *S. quinqueradiata* adapted to more temperate conditions. The last 22.5 MYA are marked by a long-term decline in global temperatures, with steep drops during the mid-Miocene (~ 16 - 11 MYA) and late Pliocene (~ 2.5 - 6 MYA; Zachos *et al*., 2001). The *S. lalandi* and *S. quinqueradiata* clade could have adapted to colder sea temperatures and became separated from the rest of the *Seriola* species in the North Pacific. The remainder of *Seriola* species (*S. rivoliana, S. dumerili, S. hippos* and *S. zonata*) probably evolved within the tropical waters of the Indo-Pacific. The North Pacific is a hot spot for speciation in numerous species (Briggs and Bowen, 2013). Speciation could have occurred during the glacial- or interglacial events during the Pliocene.

Seriola quinqueradiata probably became separated from an ancestral *S. lalandi* population during these periods of variable conditions on the western side of the North Pacific. The complex geological history of the western side of the North Pacific with periodic isolations of the Sea of Japan and the Okhotsk Sea played an important role in the diversification of numerous fish taxa (Cottidae, Zoarcidae and Liparididae, the genera *Oncorhynchus* and *Sebastes*; Hyde and Vetter, 2007; Briggs and Bowen, 2013). Interestingly, a subspecies of *S. lalandi* (*S. lalandi aureovittata;* Asian yellowtail) also became isolated in this area (see chapter 3). Therefore, these two species could have evolved sympatrically (speciation of two species in the same area with no obvious barrier) in the western North Pacific or more likely allopatrically by becoming isolated in different areas (western and eastern side) with re-colonisation of the area by *S. lalandi*. *Seriola lalandi* populations subsequently dispersed to the southern hemisphere. A number of cold-temperate southern hemisphere taxa originated in the North Pacific (Hyde and Vetter, 2007; Adey *et al*., 2008; Bolton, 2010). These species spread south through isothermic submergence, whereby species move beneath the tropics at great depth to maintain a suitable temperature or in the case of coastal temperate species, migrating south by using upwelling systems as stepping stones to cross the tropics (Lindberg, 1991).

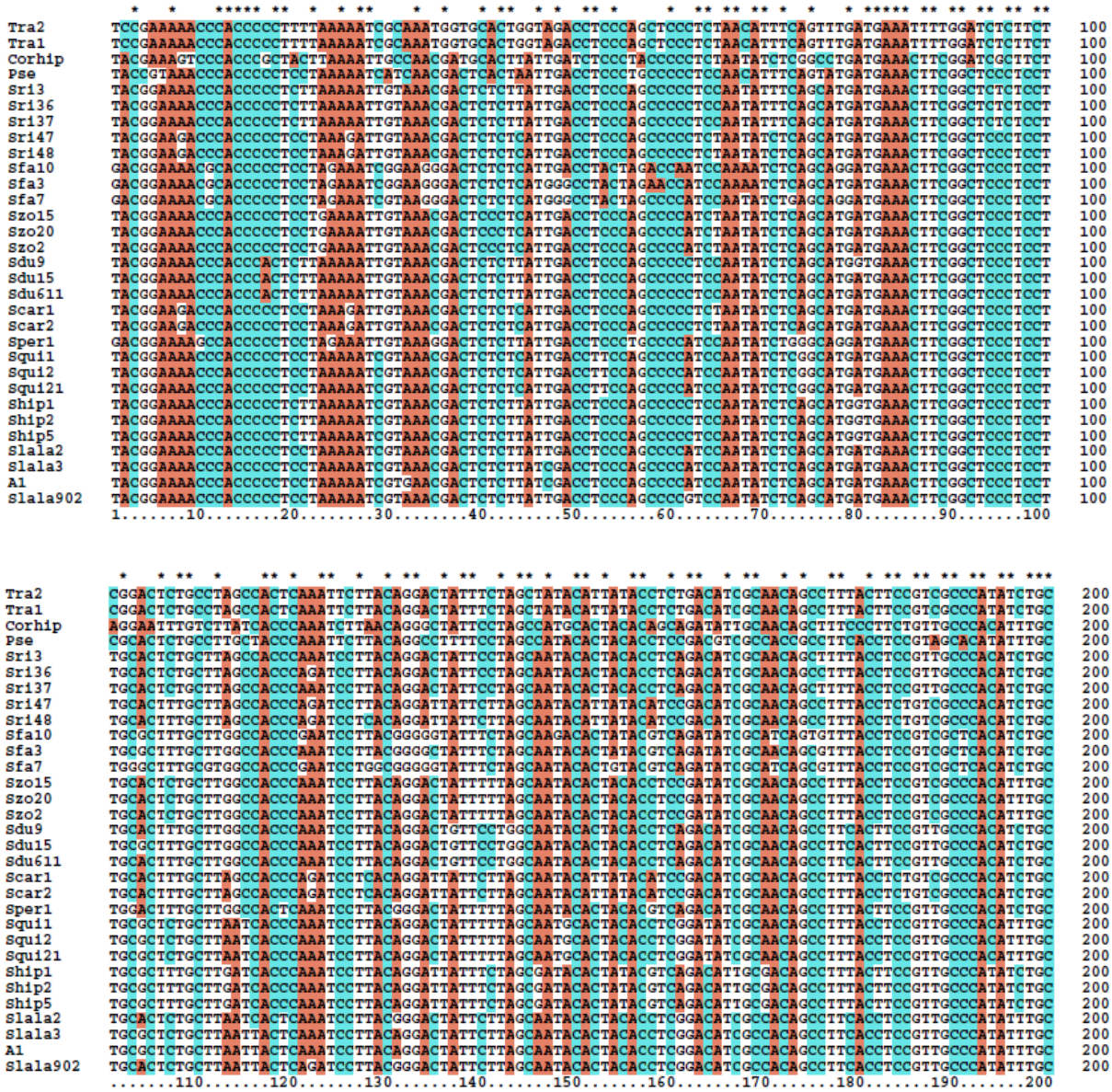
Most of the *Seriola* species are pelagic with great dispersal capabilities and have a circumglobal distribution (*S. lalandi*, *S. dumerili*, *S. rivoliana*, and *S. zonata*). These species radiated in the Pliocene and following range expansion with secondary contact of formerly allopatric clades may have resulted in this sympatric distribution of *Seriola* species. Dispersal into the Atlantic Ocean for all these species was via the southern African pathway. Although this pathway was

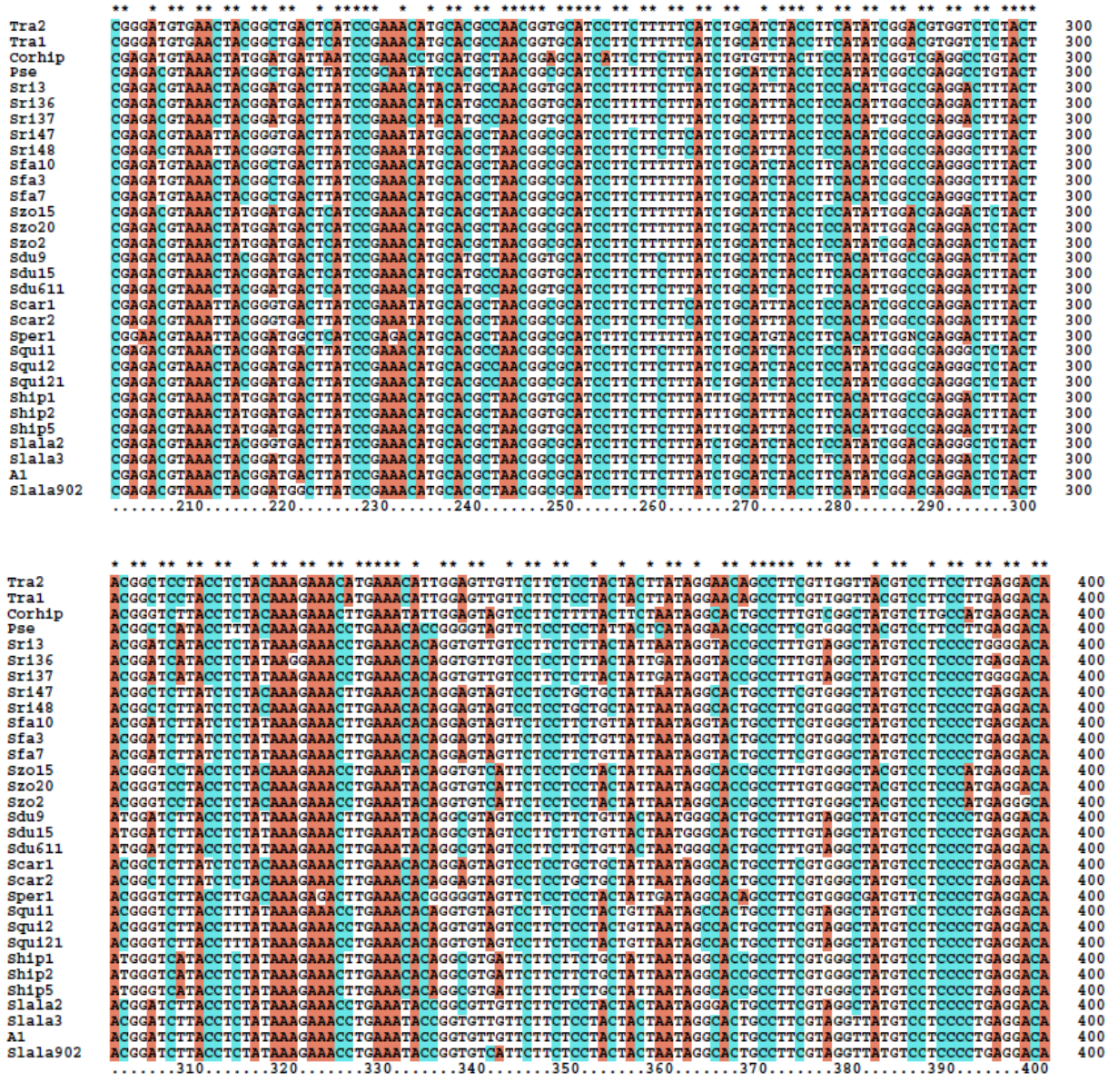
closed by the onset of cold-water (Benguela) upwelling about 2.5 MYA, it did periodically open at the end of each Pleistocene glacial cycle (Shannon, 1985; Peeters *et al*., 2004).

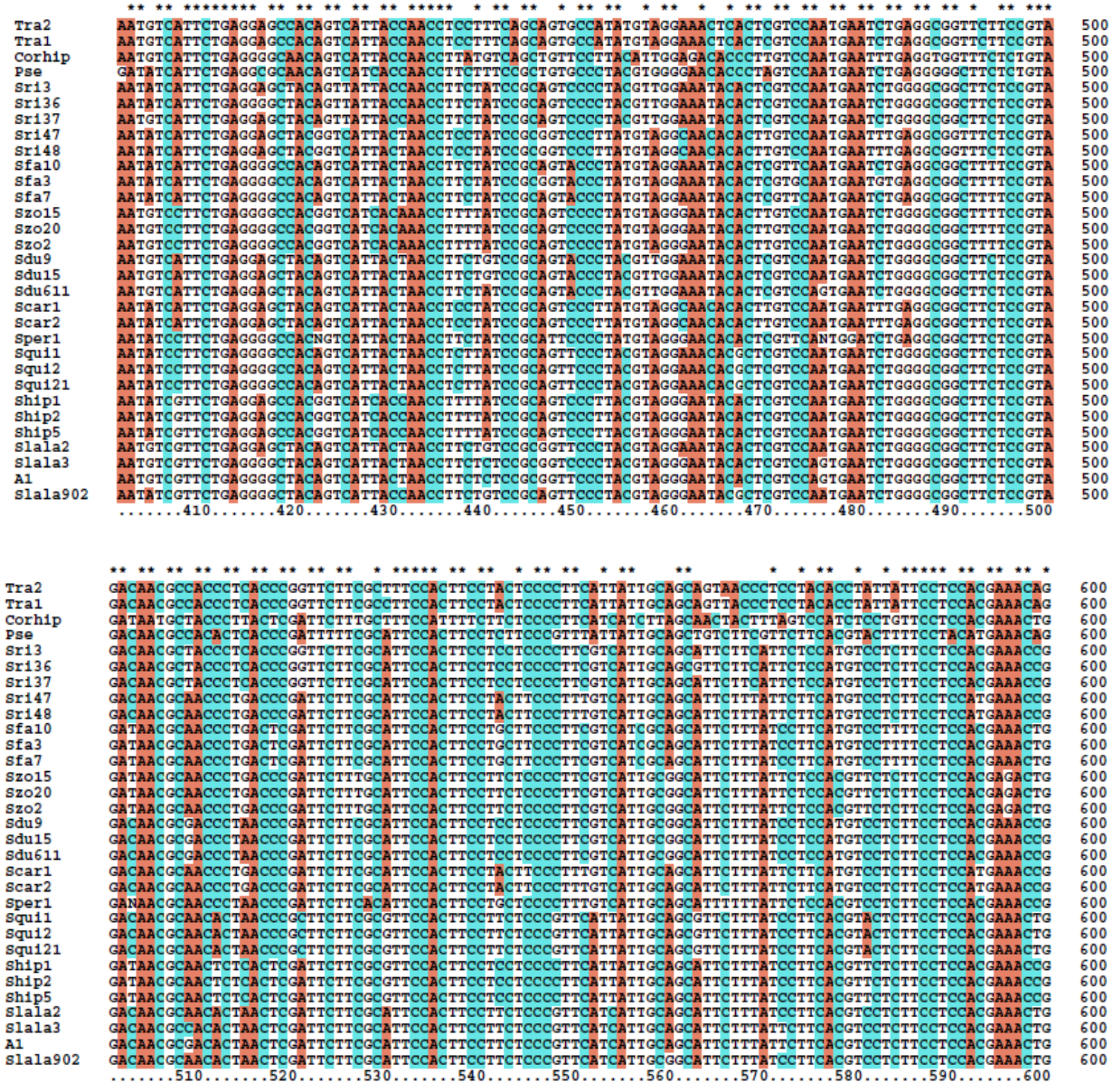
With the unclear placement in the phylogeny of the *S. dumerili* / *S. rivoliana*, *S. zonata* and *S. hippos* clades, the origin of these species can only be discussed in broad terms. *Seriola zonata* most likely evolved in conjunction with the *S. lalandi* / *S. quinqueradiata* clade. Validation for this is that this species forms a sister clade to the *S. lalandi* / *S. quinqueradiata* clade in the combined phylogeny and this species also occurs in temperate waters (although it is also found in the subtropical waters). *Seriola hippos* probably originated along the Australian coast from the Indo-Pacific ancestral group during these glacial- or interglacial cycles in the Pliocene.

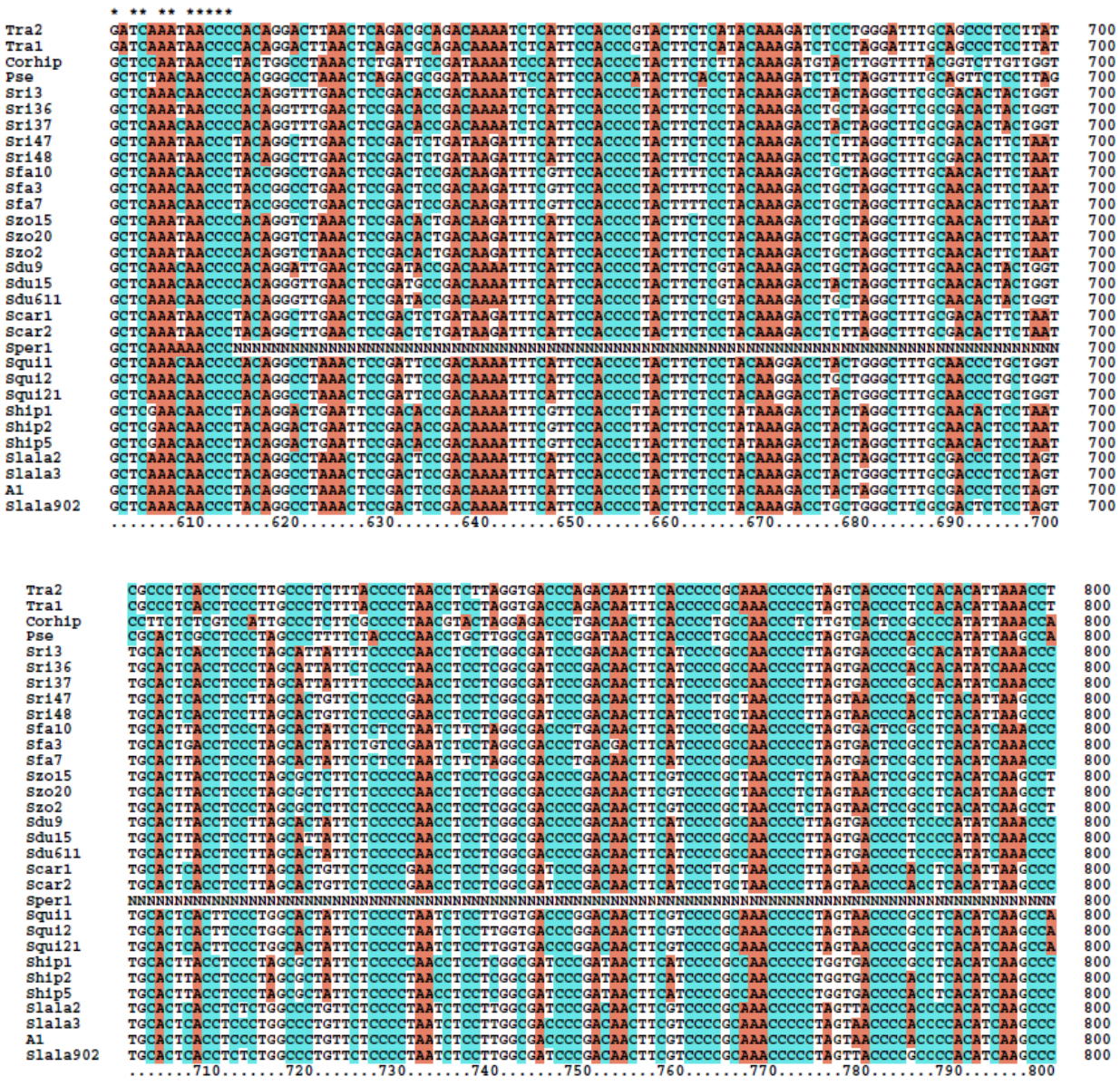
In conclusion, various paleo-oceanographic events have shaped the evolution of *Seriola* and examining how these may explain the phylogenetic relationshipsobserved in the current study have broadened our understanding of this genus.

Appendix A: *Cyt B* sequence alignments









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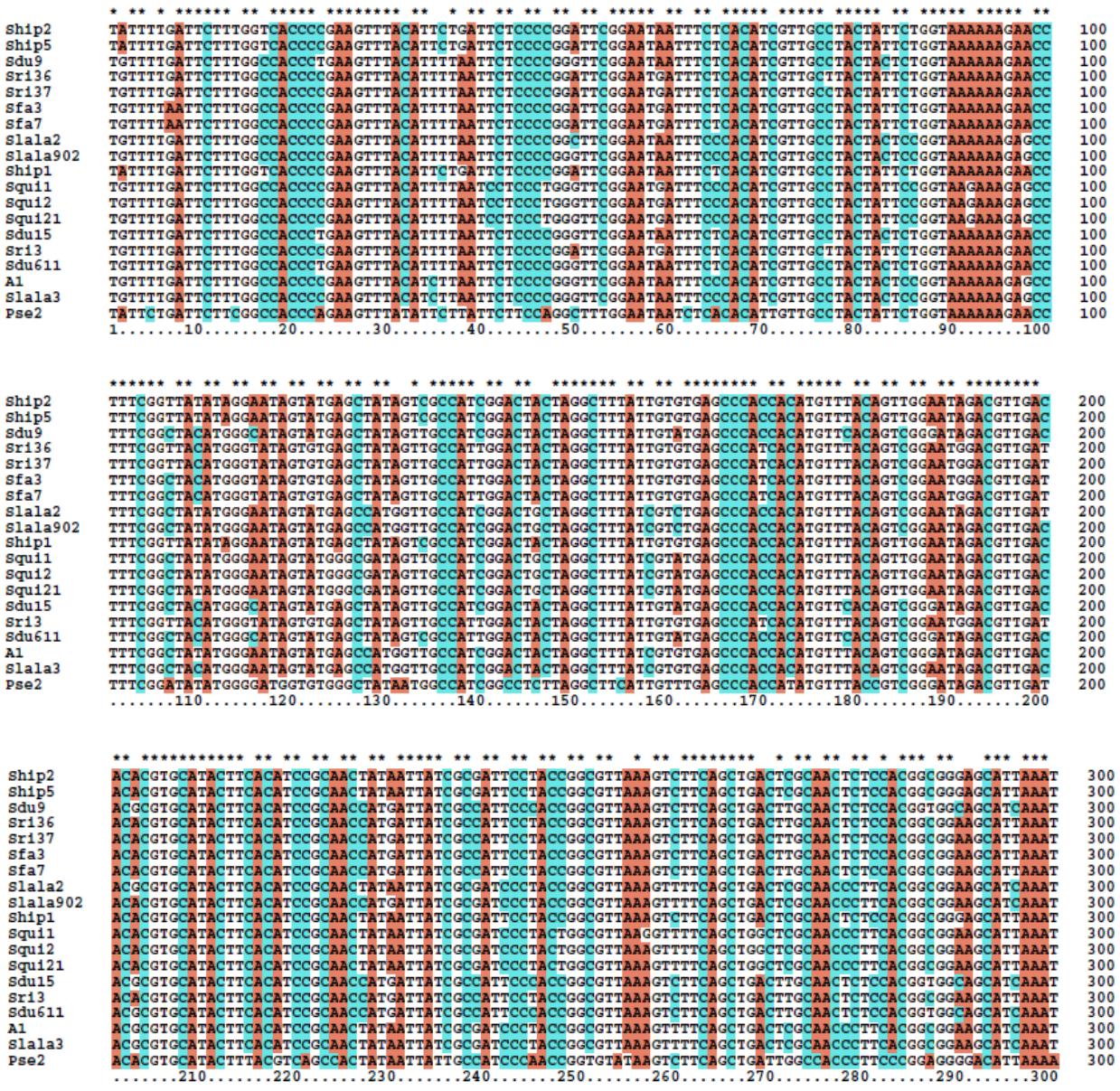

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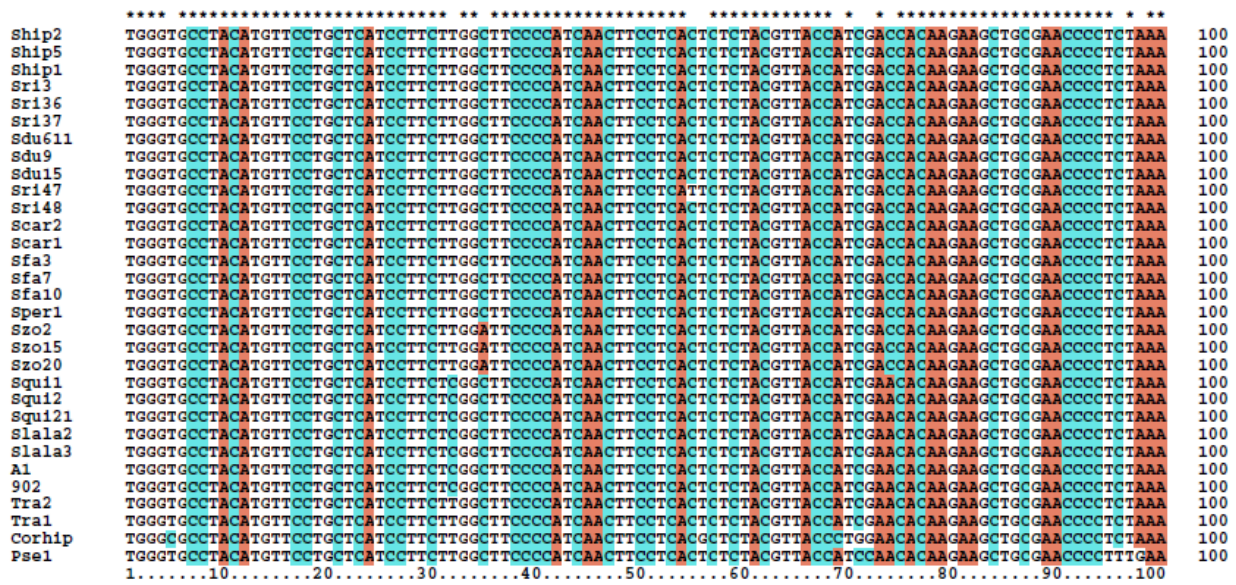
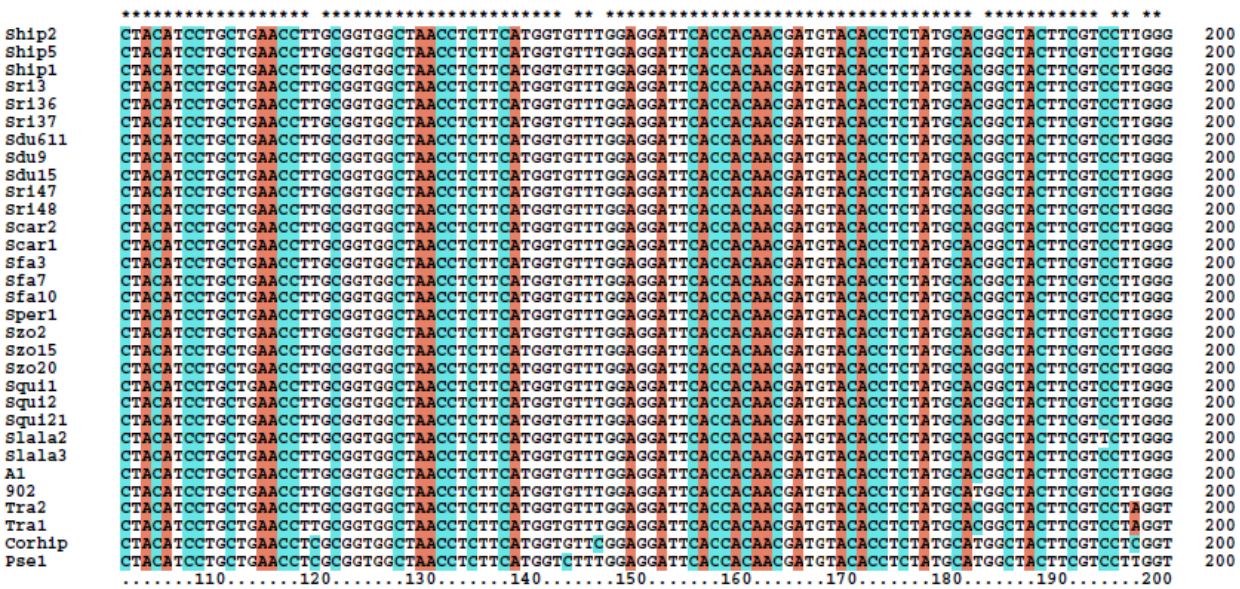
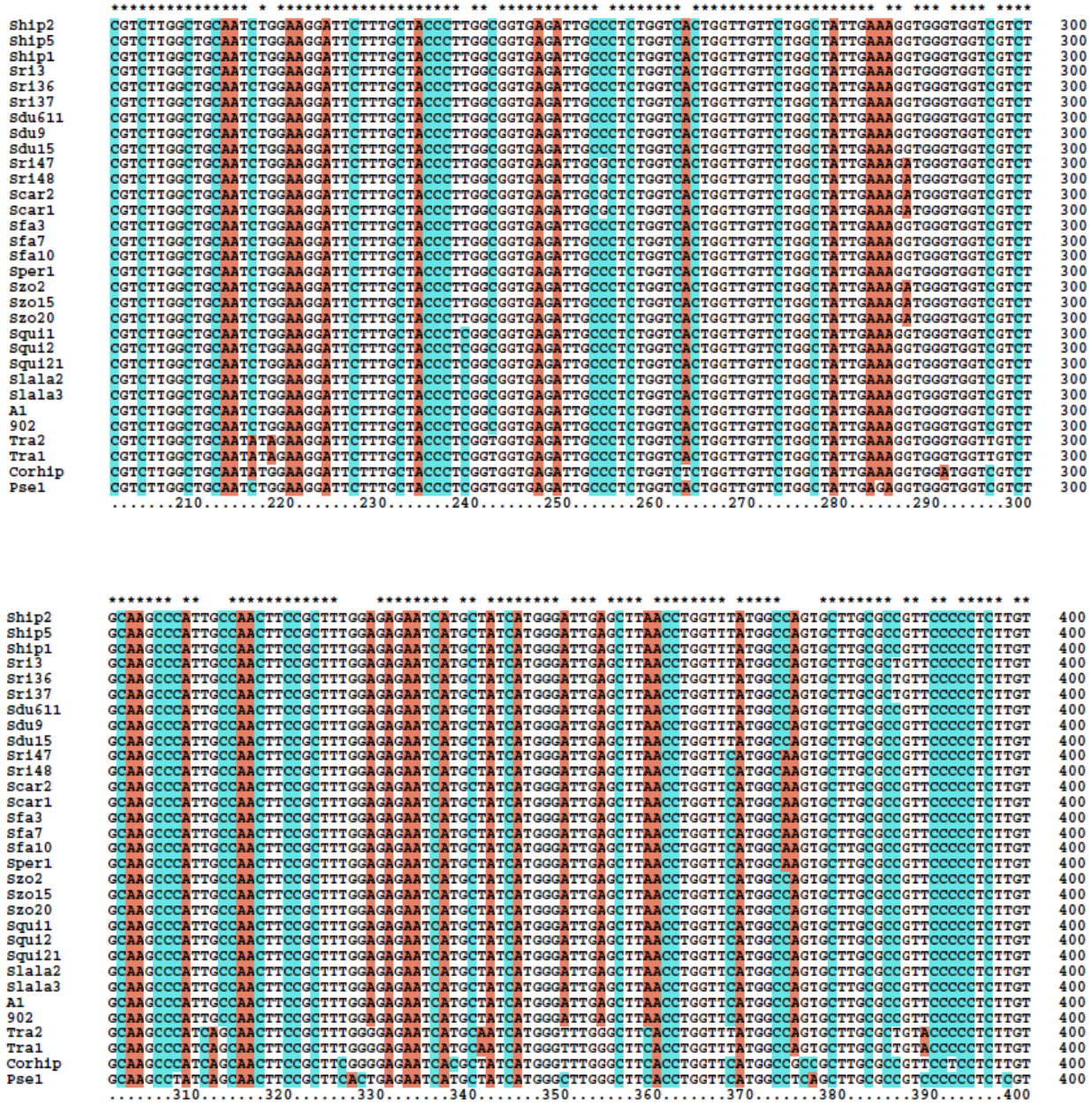
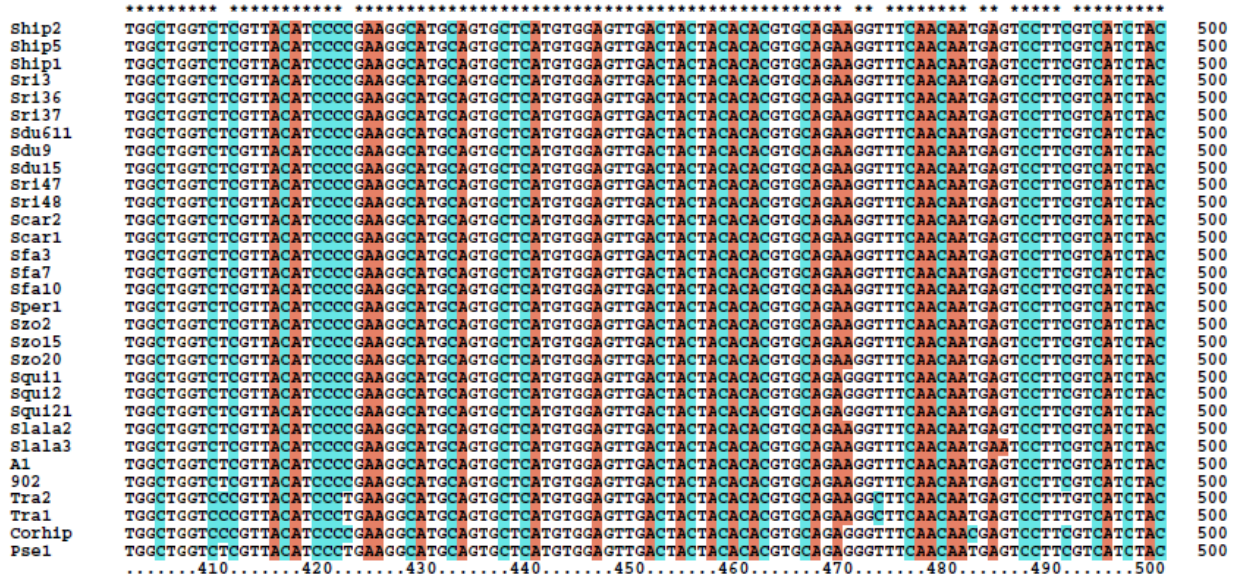
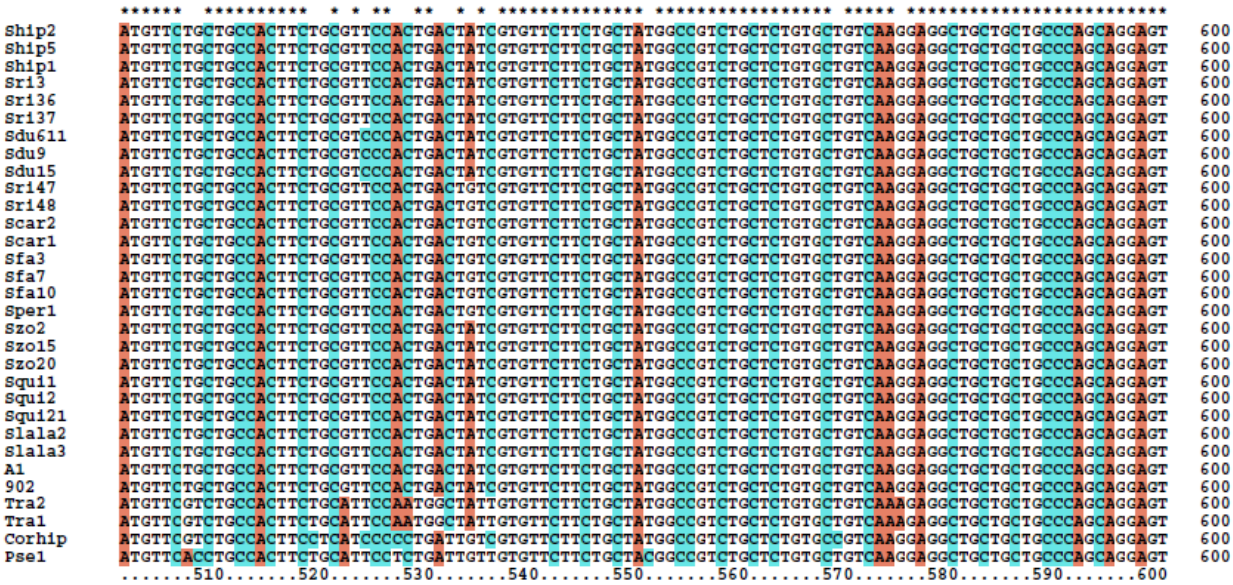
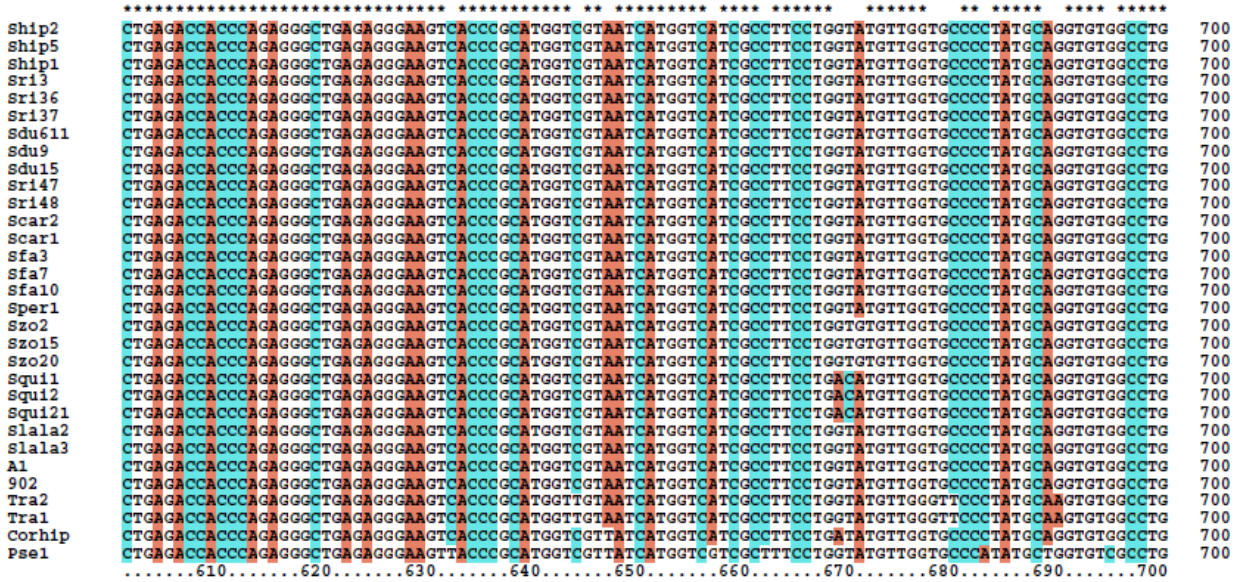


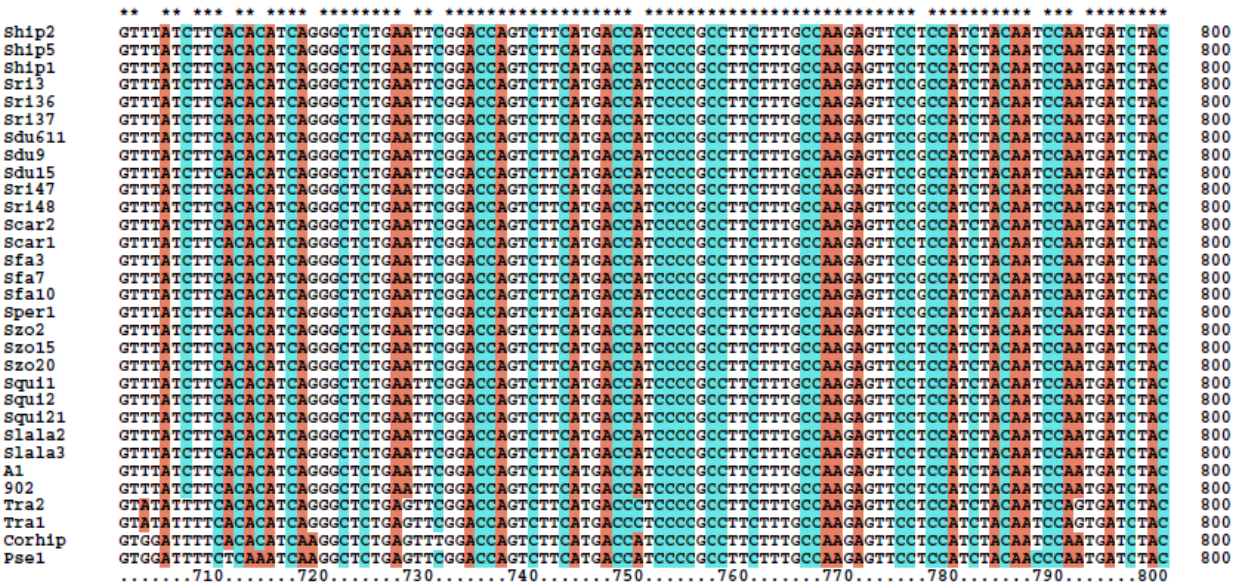




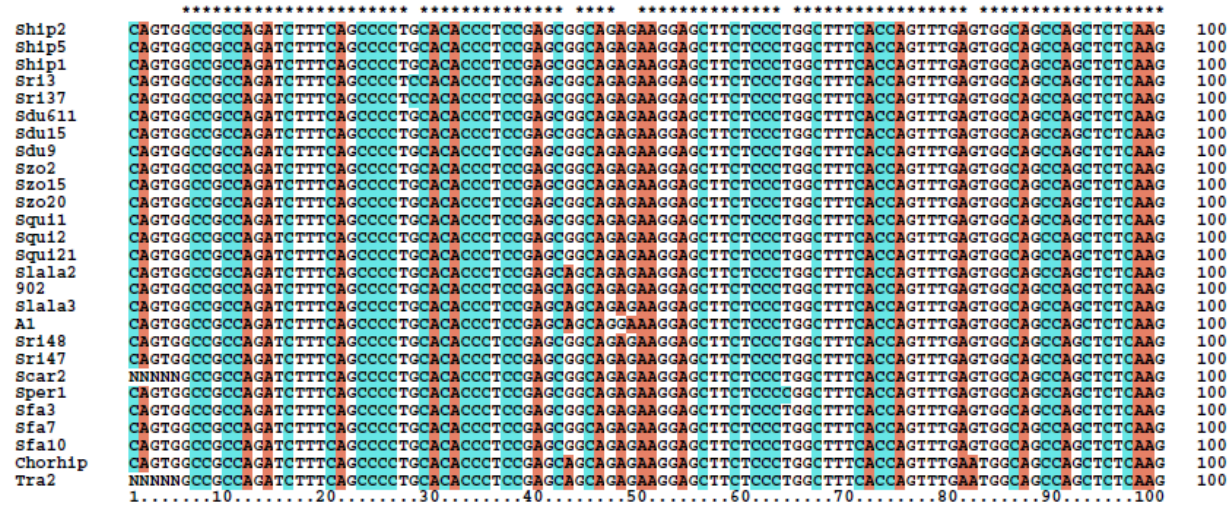


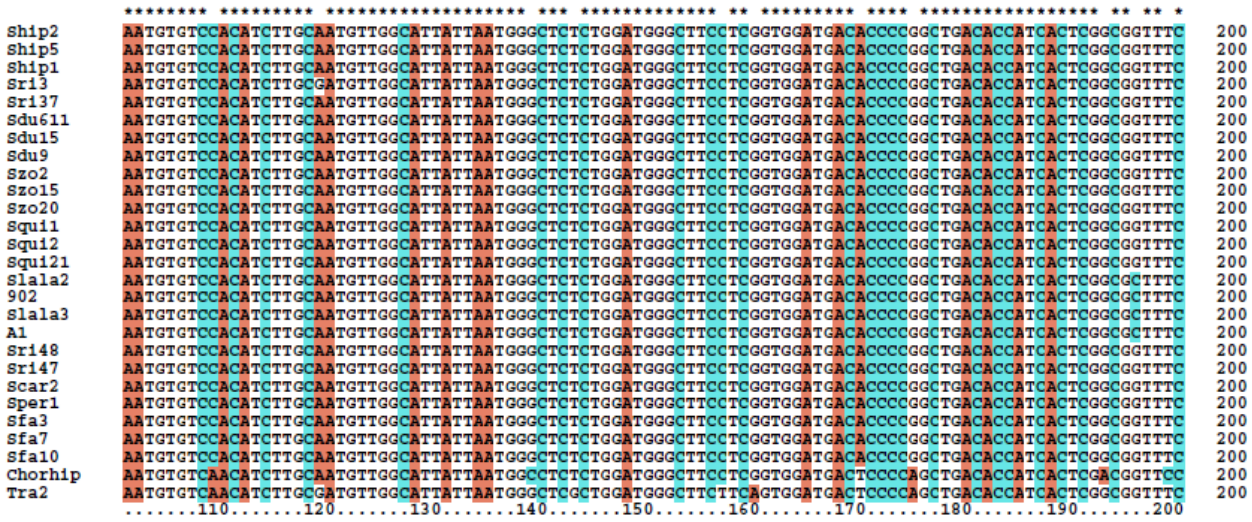


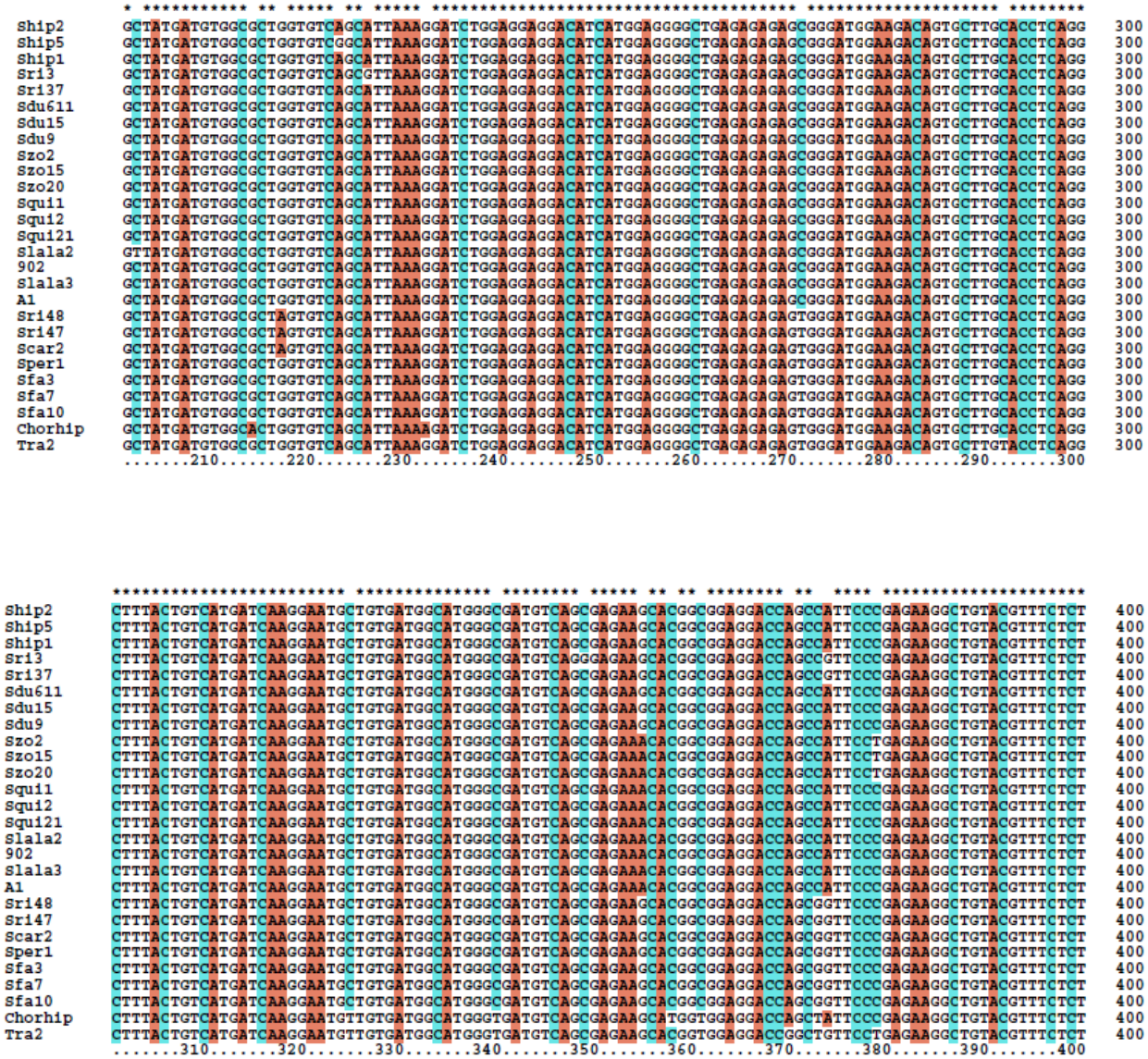


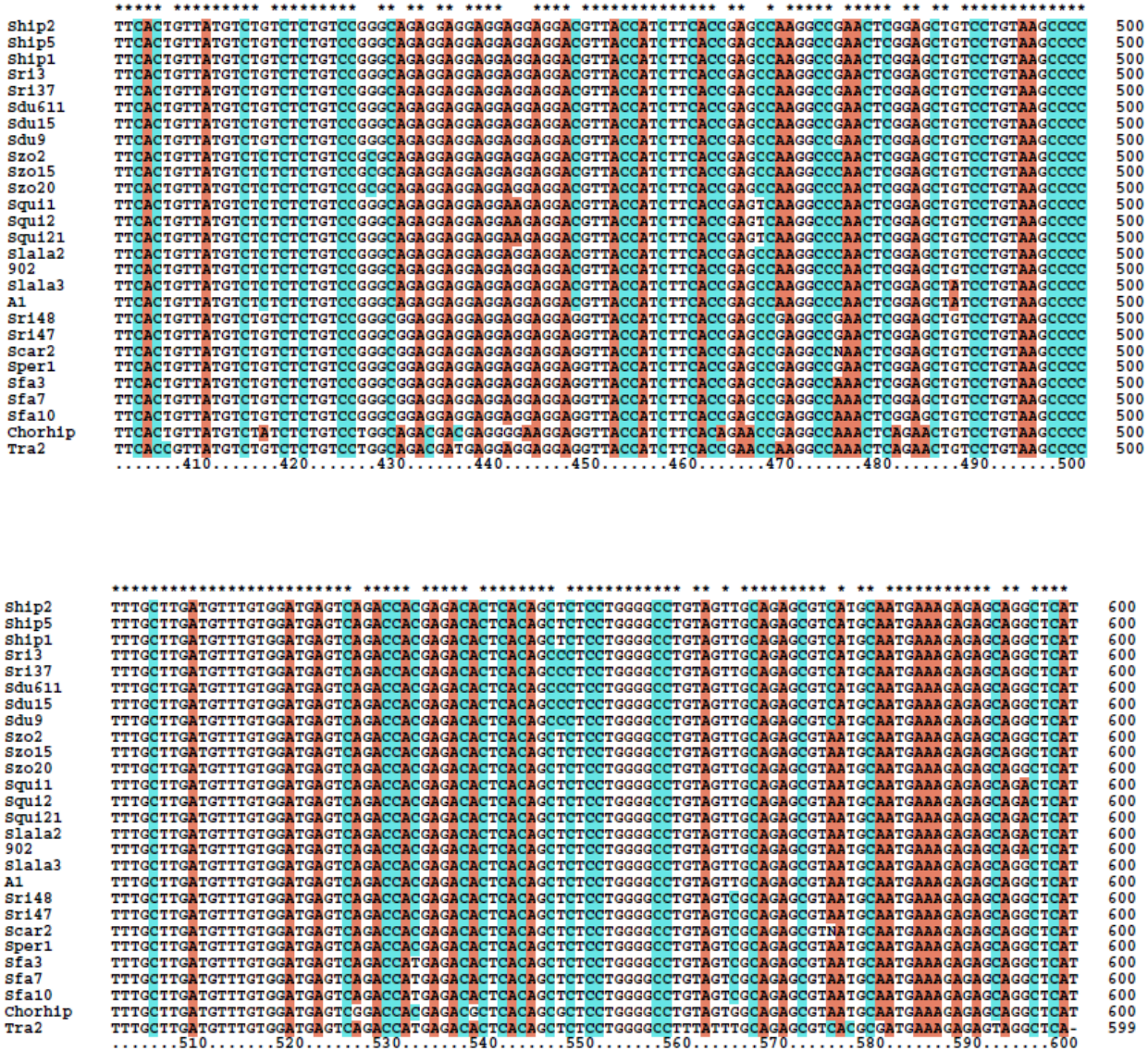


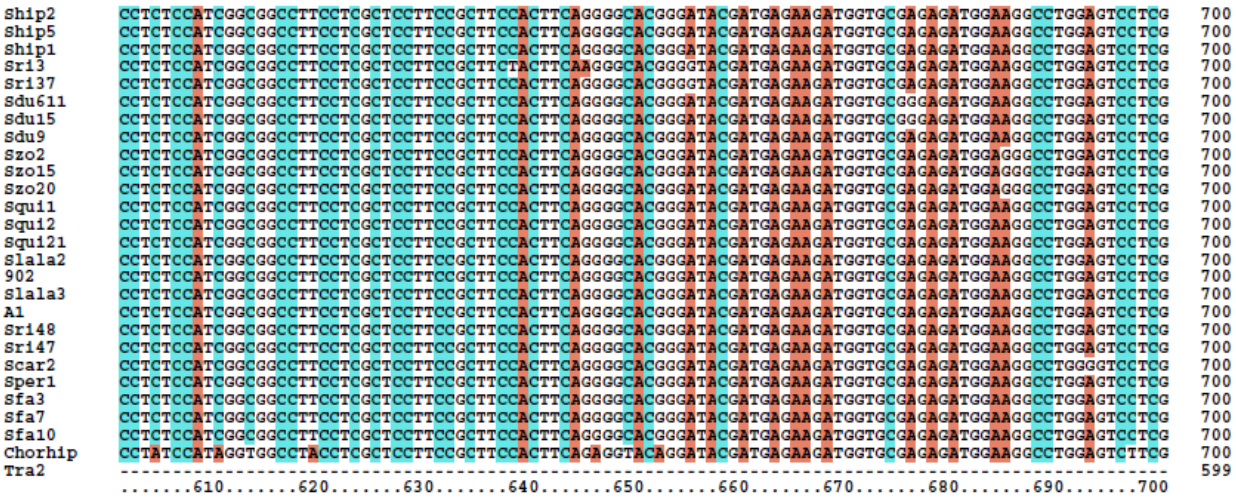
Appendix D: *RAG1* sequence alignments











Chapter 3: The Global Genetic Structure of Yellowtail (*Seriola lalandi*)

3.1 Introduction

The genetic structure of populations in marine environments can be complex. Marine species often show patterns of low genetic structuring, which is frequently the product of high gene flow between ocean basins (Ward *et al*., 1994; Chow *et al*., 2009). Such high gene flow is generally the result of high dispersal capabilities (as either adult or larvae), their large distribution ranges and large effective population sizes. However, ecological (*e.g.* homing behaviour or discrete spawning units) and physical (*e.g.* past changes in sea temperature levels or / and present-day oceanic barriers such as currents or deep oceanic waters) factors can limit gene flow resulting in genetic variation between populations. The latter is evident through the many studies of pelagic species that show high levels of genetic structure between ocean basins (*e.g.* Ruzzante *et al*., 2000; McPherson *et al*., 2004; Chiang *et al*., 2008).

The yellowtail kingfish (*Seriola lalandi*; Valenciennes, 1833) is a commercially valuable pelagic fish species with a non-equatorial distribution, that is found along the coast of most continents and islands within the Atlantic and Pacific oceans (Fig. 3.1; Nugroho *et al*., 2001). Due to their fast growth rate, meat quality and suitability for off-shore cage farming, they are commercially farmed in Japan, China, New Zealand and Australia (Fowler *et al*., 2003; Poortenaar *et al*., 2003; Ohara *et al*., 2005; Nakada, 2008; Miller *et al*., 2011).

Within *S. lalandi* three morphologically similar, but geographically separated subspecies (Poortenaar *et al*., 2003) have been recognised (Smith-Vaniz, 1984): *S. lalandi dorsalis* (California yellowtail; Gill, 1863), *S. lalandi aureovittata* (Asian yellowtail; Schlegel, 1844) and *S. lalandi lalandi* (Southern yellowtail; Cuvier and Valenciennes, 1833). These subspecies are mainly recognised because of their disjunct distribution (Fig. 3.1) but it is unlikely that fishes from disjunct localities mix, although this has not been tested. California yellowtail are found off the coast of North America, Asian yellowtail off the coast of Japan and China, with the Southern yellowtail distributed throughout the waters of the southern hemisphere.

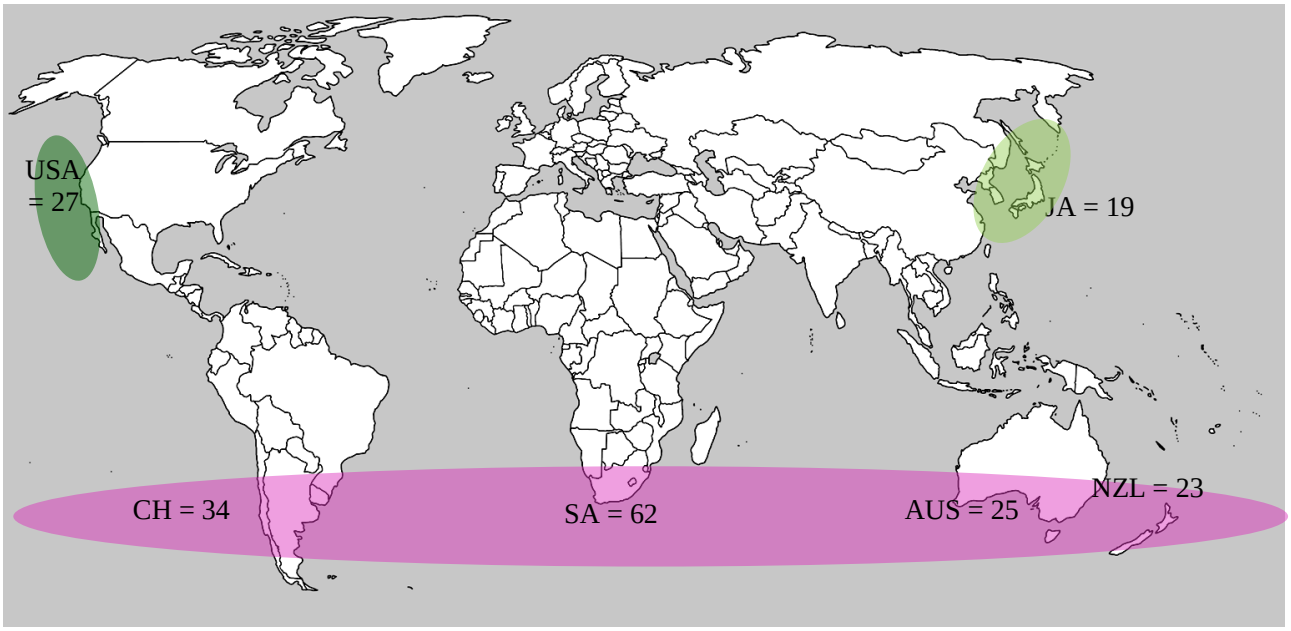


Fig. 3.1: Distribution ranges of *Seriola lalandi* subspecies: *S. l. dorsalis* (dark green), *S. l. aureovittata* (light green), *S. l. lalandi* (pink). Sampling locations and the number of fish sampled within these ranges are indicated.

Yellowtail can grow to approximately 2.5m in length, weigh on average 70kg (Gomon *et al*., 2008) and can be found in coastal waters with temperatures between 18 - 24°C, from the shore to the edge of the continental shelf (Wolvaardt, 2007). Interestingly, during an individual yellowtails' lifetime it can be both pelagic and demersal, which means they spend part of their lives roaming the oceans and the other part remaining close to the benthos. Juveniles are generally found schooling in warmer offshore waters with adults preferring to live a solitary life, or in small groups, inside the continental shelf. Juvenile yellowtail are often found sheltering within floating seaweed; suggesting the possible passive dispersal of juvenile *S. lalandi* (Diggles, 2002). In a tag-recapture study on yellowtail in New Zealand recapture rates varied among areas, fishermen and sizes of fish, with larger fish dispersing larger distances than smaller fish (Gillanders *et al*., 2001). The majority of fish recaptures occurred within 50km from where they were tagged; although some indication of further movement was found with the maximum distance travelled being 3 000km (Gillanders *et al*., 2001).

Currently, limited information is available on the biology and behaviour of *S. lalandi* and moreover, little is known about the genetic structure of this species. Only two studies have investigated phylogeographic patterns in *S. lalandi* to date. Nugroho *et al*. (2001) found significant differentiation between populations from Japan and those from Australia and New Zealand based on six microsatellite markers and mtDNA control region data. Furthermore, a fine-scale study along the Australian coast by Miller *et al*. (2011) found significant population differences between the west and east coast of Australia. These authors used seven microsatellite loci and mtDNA (ND4) sequence data to analyse a total of 272 individuals sampled from New Zealand, New South Wales, Victoria, South Australia and Western Australia. However, sampling

locations in these studies were limited and samples from over the entire distribution range of a species are needed to gain insights into the contemporary and historical factors shaping all extant *S. lalandi* populations.

In the current study global patterns of genetic variation within *S. lalandi* were examined with the use of mtDNA and nuclear DNA sequence data. The primary aim was to investigate the global genetic structure of *S. lalandi*. A secondary aim was to confirm the systematics of the three geographically distinct subspecies within *S. lalandi*. Due to the disjunct nature of the *S. lalandi* subspecies, one would expect three genetic groupings corresponding to the subspecies' distribution. A final aim was to infer the processes (such as barriers to gene flow and vicariant events) shaping the population structure within this species.

3.2 Material and methods

A total of 190 individuals (comprising individuals of all three subspecies) of *S. lalandi* were collected from locations around the globe (Table 3.1; USA = 27, JA = 19, SA = 62, NZL = 23, AUS = 25, CH = 34; Fig. 3.1). All samples were preserved in 95% (v/v) ethanol. Genomic DNA extraction, polymerase chain reaction (PCR) amplifications, PCR product purification, and DNA sequencing was carried out using the methods described in Chapter 2. Amplification and sequencing for *Cytb*, *COI* and *RAG1* was performed with the primer pairs described in Chapter 2. Sequences were edited and manually aligned with BioEdit v7 (Hall, 1999) and alignments were checked in ClustalX v1.81 (Thompson *et al.*, 1997).

Table 3.1: The *Seriola lalandi* specimens examined, with sampling locality and sample ID.

Country	Locality	Sample ID
Australia	New South Wales	SL3 [d]
(AUS)	New South Wales	SL4 [d]
	New South Wales	SL5 [d]
	New South Wales	SL6 [d]
	New South Wales	SL7 [d]
	New South Wales	SL8 [d]
	New South Wales	SL9 [d]
	New South Wales	SL10 [d]
	New South Wales	SL11 [d]
	New South Wales	SL12 [d]
	New South Wales	SL13 [d]
	New South Wales	SL14 [d]
	New South Wales	SL15 [d]
	New South Wales	SL16 [d]
	New South Wales	SL19 [d]
	Lord Howe Island	SL21 [c]
	Lord Howe Island	SL22 [c]
	Lord Howe Island	SL23 [c]
	Lord Howe Island	SL24 [c]
	Lord Howe Island	SL25 [c]
	Lord Howe Island	SL26 [c]
	Lord Howe Island	SL27 [c]
	Lord Howe Island	SL28 [c]
	Lord Howe Island	SL29 [c]
	Lord Howe Island	SL30 [c]
New Zealand	Tauranga	SL41 [f]
(NZL)	Tauranga	SL42 [f]
	Tauranga	SL43 [f]
	Tauranga	SL44 [f]
	Tauranga	SL45 [f]
	Tauranga	SL46 [f]
	Tauranga	SL47 [f]
	Tauranga	SL48 [f]
	Tauranga	SL49 [f]

	Tauranga	SL50 [f]
	Tauranga	SL51 [f]
	Tauranga	SL52 [f]
	Tauranga	SL53 [f]
	Tauranga	SL54 [f]
	Tauranga	SL55 [f]
	Tauranga	SL56 [f]
	Tauranga	SL57 [f]
	Tauranga	SL58 [f]
	Tauranga	SL59 [f]
	Tauranga	SL60 [f]
	Tauranga	SL65 [f]
	Tauranga	SL66 [f]
	Tauranga	SL67 [f]
Chile	Antofagasta coast	SL77 [g]
(CH)	Antofagasta coast	SL78 [g]
	Antofagasta coast	SL79 [g]
	Antofagasta coast	SL81 [g]
	Antofagasta coast	SL82 [g]
	Antofagasta coast	SL83 [g]
	Antofagasta coast	SL84 [g]
	Antofagasta coast	SL85 [g]
	Antofagasta coast	SL86 [g]
	Antofagasta coast	SL87 [g]
	Antofagasta coast	SL88 [g]
	Antofagasta coast	SL89 [g]
	Antofagasta coast	SL90 [g]
	Antofagasta coast	SL91 [g]
	Antofagasta coast	SL92 [g]
	Antofagasta coast	SL93 [g]
	Antofagasta coast	SL94 [g]
	Antofagasta coast	SL95 [g]
	Antofagasta coast	SL96 [g]
	Antofagasta coast	SL97 [g]
	Antofagasta coast	SL101 [g]
	Antofagasta coast	SL102 [g]

	Antofagasta coast	SL103 [g]
	Antofagasta coast	SL104 [g]
	Antofagasta coast	SL107 [g]
	Antofagasta coast	SL108 [g]
	Antofagasta coast	SL109 [g]
	Antofagasta coast	SL110 [g]
	Antofagasta coast	SL111 [g]
	Antofagasta coast	SL112 [g]
	Antofagasta coast	SL113 [g]
	Antofagasta coast	SL114 [g]
	Antofagasta coast	SL115 [g]
	Antofagasta coast	SL116 [g]
Japan	Fukuoka, Kyushu (Sea of Japan)	901 [b]
(JA)	Fukuoka, Kyushu (Sea of Japan)	902 [b]
	Fukuoka, Kyushu (Sea of Japan)	903 [b]
	Fukuoka, Kyushu (Sea of Japan)	904 [b]
	Fukuoka, Kyushu (Sea of Japan)	905 [b]
	Fukuoka, Kyushu (Sea of Japan)	906 [b]
	Fukuoka, Kyushu (Sea of Japan)	907 [b]
	Fukuoka, Kyushu (Sea of Japan)	908 [b]
	Fukuoka, Kyushu (Sea of Japan)	909 [b]
	Fukuoka, Kyushu (Sea of Japan)	910 [b]
	Fukuoka, Kyushu (Sea of Japan)	911 [b]
	Fukuoka, Kyushu (Sea of Japan)	912 [b]
	Fukuoka, Kyushu (Sea of Japan)	913 [b]
	Fukuoka, Kyushu (Sea of Japan)	914 [b]
	Fukuoka, Kyushu (Sea of Japan)	915 [b]
	Fukuoka, Kyushu (Sea of Japan)	916 [b]
	Fukuoka, Kyushu (Sea of Japan)	917 [b]
	Fukuoka, Kyushu (Sea of Japan)	918 [b]
	Fukuoka, Kyushu (Sea of Japan)	920 [b]
North America	California	700 [b]
(USA)	California	701 [b]
	California	702 [b]
	California	703 [b]
	California	704 [b]

	California	705 [b]
	California	706 [b]
	California	707 [b]
	California	708 [b]
	California	709 [b]
	California	710 [b]
	California	711 [b]
	California	712 [b]
	California	713 [b]
	California	714 [b]
	California	715 [b]
	California	716 [b]
	California	717 [b]
	California	718 [b]
	California	719 [b]
	California	720 [b]
	California	721 [b]
	California	722 [b]
	California	724 [b]
	California	725 [b]
	California	SL1 [e]
	California	SL2 [e]
South Africa	Suidwest rift	A1 [a]
(SA)	North Blinder	B1 [a]
	North Blinder	B2 [a]
	North Blinder	B3 [a]
	North Blinder	B4 [a]
	North Blinder	B5 [a]
	North Blinder	B6 [a]
	North Blinder	B7 [a]
	North Blinder	B8 [a]
	North Blinder	B9 [a]
	North Blinder	B10 [a]
	Elands Bay	I1 [a]
	Yzerfontein	C1 [a]
	Yzerfontein	C2 [a]

Yzerfontein	C3 [a]
Yzerfontein	C4 [a]
Yzerfontein	C5 [a]
Yzerfontein	C6 [a]
Yzerfontein	C7 [a]
Yzerfontein	C8 [a]
Yzerfontein	C9 [a]
Yzerfontein	C10 [a]
West of Robben Island	D1 [a]
West of Robben Island	D2 [a]
Olifantsbos	D3 [a]
Olifantsbos	D4 [a]
Cape Point / False Bay	E1 [a]
Cape Point / False Bay	E2 [a]
Cape Point / False Bay	E3 [a]
Cape Point / False Bay	E4 [a]
Cape Point / False Bay	E5 [a]
Cape Point / False Bay	E6 [a]
Cape Point / False Bay	E7 [a]
Cape Point / False Bay	E8 [a]
Cape Point / False Bay	E9 [a]
Cape Point / False Bay	E10 [a]
Whale Rock	D5 [a]
Whale Rock	D6 [a]
Struis Bay	F1 [a]
Struis Bay	F2 [a]
Struis Bay	F3 [a]
Struis Bay	F4 [a]
Struis Bay	F5 [a]
Struis Bay	St1 [a]
Struis Bay	St2 [a]
Struis Bay	St3 [a]
Struis Bay	St4 [a]
Struis Bay	St5 [a]
St. Helena Bay	H1 [a]
St. Helena Bay	H2 [a]

St. Helena Bay	H3 [a]
St. Helena Bay	H4 [a]
Port Elizabeth	Riy1 [a]
Port Elizabeth	Riy2 [a]
Port Elizabeth	Riy3 [a]
Port Elizabeth	Riy4 [a]
Port Elizabeth	Riy5 [a]
Port Elizabeth	Riy6 [a]
Port Elizabeth	Riy7 [a]
Port Elizabeth	Riy8 [a]
Port Elizabeth	G1 [a]
Port Elizabeth	G2 [a]

Samples were contributed by [a] Sven Kerwath, [b] Natalie Martinez-Takeshita, [c] Ian Kerr, [d] Kate Hudson, [e] HJ Walker, [f] Jane Symmonds, [g] Roberto Flores

3.2.1 Data analyses

Six different geographic populations were defined prior to analysis according to geographic origin (JA, USA, SA, NZL, AUS, CH). Sample localities within countries were pooled. Haplotype diversity (*h*), nucleotide diversity (π) and uncorrected genetic distances (*p*-distances) were calculated in ARLEQUIN v3.1 (Excoffier *et al*., 2005) for each of the six "populations" for each gene fragment. Median-joining haplotype networks for each of the three gene regions separately were constructed with NETWORK v4.610 (Bandelt *et al*., 1999).

3.2.1.1 Phylogenetic analysis

Phylogenetic relationships between the *S. lalandi* haplotypes identified by the haplotype network analyses were investigated using three phylogenetic reconstruction methods: Maximum parsimony (MP), Maximum likelihood (ML) and Bayesian inference (BI). The same parameters as in Chapter 2 were used for reconstructing the phylogenetic trees in MEGA v4.02 (Tamura *et*

al., 2007), PHYML v2.4.4 (Guindon and Gascuel, 2003) and MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003), respectively. Models implemented in ML and BI analysis were identified in jModelTest v0.1.1 (Posada, 2008) utilising the Akaike Information Criterion (AIC) method. These models were Hasegawa-Kishino-Yano with a proportion of invariable sites (HKY+I) for *Cytb*, general time-reversible model with a proportion of invariable sites (GTR+I) for *COI* and Hasegawa-Kishino-Yano with a proportion of invariable sites, plus gamma (HKY+I+G) for *RAG1*.

The *Cytb*, *COI* and *RAG1* datasets were subsequently also combined and haplotypes obtained from networks drawn in the program NETWORK. For phylogenetic analyses of the combined multi-fragment dataset, two strategies were followed. First, a single concatenated dataset containing all the genes fragments and haplotypes was used to construct ML and MP phylogenies. For ML analysis jModelTest identified the GTR+I model as the best model to fit the data. Secondly, a mixed-model Bayesian analysis was performed on the partitioned (by gene) dataset. All phylogenetic trees were rooted using the sister taxon, *S. quinqueradiata*, as an outgroup species.

3.2.1.2 Population structure

To assess population differentiation and possible structure, analysis of molecular variance (AMOVA; Weir and Cockerham, 1984; Excoffier *et al*., 1992) tests were performed with ARLEQUIN . Tests were performed based on two grouping hypotheses: 1) between the three main groupings identified in NETWORK (JA, USA and {AUS/CH/NZL/SA}); 2) between the three main groupings plus an additional weaker grouping identified by NETWORK (JA, USA,

{AUS/NZL/CH} and SA). Pairwise population F_{ST} and Φ_{ST} comparisons (10 000 permutations) were performed in ARLEQUIN between sampling localities. Φ_{ST} was calculated under the Tamura and Nei (1993) nucleotide substitution model parameters.

3.2.1.3 Demographic history

To further assess the population history of the *S. lalandi* populations, Tajima's *D* (Tajima, 1989) and Fu's *Fs* (Fu, 1997) neutrality tests were performed. The Fu's *Fs* and Tajima's *D* tests were implemented in ARLEQUIN for each gene fragment (*Cytb*, *COI*, *RAG1*). The "populations" tested were the three main groupings identified in NETWORK (JA, USA, southern hemisphere {AUS/CH/NZL/SA}); the weaker groupings found within the southern hemisphere ({AUS/NZL/CH} and SA) and all populations combined. The significance of all the tests was determined by 1 000 coalescent simulations.

Mismatch distributions (frequency distributions of pairwise differences between sequences) were constructed for the four groupings identified in NETWORK (JA, USA, {AUS/NZL/CH} and SA) and all populations combined. Mismatch distribution and the demographic parameters τ, θ_0 and θ_1 were estimated in ARLEQUIN and graphs were constructed using Microsoft Excel 2010. The sum of the squared differences (SSD) and raggedness index (r) between the observed and the estimated mismatch distribution test the overall validity of the estimated demographic model.

3.2.1.4 Time of divergence

Divergence times between the *S. lalandi* populations were estimated in BEAST v1.7.3 (Drummond *et al*., 2012). BEAST uses a Bayesian MCMC approach that estimate the time to

coalescence. The most recent common ancestor (TMRCA) was calculated for the four groupings identified in this study (JA, USA, {AUS/NZL/CH} and SA). The time of divergence between the northern and southern hemisphere populations was also investigated by pooling the populations from each hemisphere ({JA/USA} and {AUS/NZL/CH/SA}). The program BEAUti v1.7.3 (Drummond and Rambaut, 2007) was used to generate the input files for BEAST. Analyses were run under the strict molecular clock assumption using previously identified models of substitution for *Cytb* (HKY+I) and *COI* (GTR+I) and default priors. In the absence of clock calibration data (fossil) for *S. lalandi*, the conventional mutation rate of 2% per site per MYA for the mitochondrial genome was used for both gene fragments (*Cytb* and *COI*; Brown *et al*., 1979). A more conservative value of 1% per site per MYA for *Cytb* in teleost fish as suggested by Johns and Avise (1998) and Bermingham *et al*. (1997) was also used for the clock calibration. This conservative value was also used in the calculation of divergence times in other Carangidae species (Karaiskou *et al*., 2003; Cárdenas *et al*., 2005). Five independent runs with simulations of 10 million MCMC steps with sampling every 1 000 generations and a burn-in of 10% were computed in BEAST. After the first run the program TRACER v1.5 (Rambaut and Drummond, 2007) was used to examine effective samples sizes (ESS) to assess if this parameter was above 200 and if the parameter space had been efficiently searched. After the five runs TRACER was used to examine and combine the log files.

3.3 Results

Partial sequences were obtained for *Cytb*, *COI*, *Rhod* and *RAG1*. The discrepancy in the number of base pairs per gene fragment between this chapter en chapter 2 is the result of pruning of the 3' ends of the sequences.

3.3.1 Sequence variation

A total of 50 haplotypes were obtained from 190 *Cytb* sequences (800bp), of which 35 were from single individuals. Of the 800bp, 69 nucleotide sites were variable, of which 44 were parsimony informative and 25 were singletons. Base composition analysis revealed a bias against guanine (A: 0.263; C: 0.352; G: 0.150; T: 0.243). The transition: transversion ratio (3.8/1) showed some bias towards transitions with a rate of $R = 5.538$. For the 190 *COI* sequences, 43 haplotypes were retrieved. Thirty of these haplotypes were found only once. A sequence length of 460bp (45 variable sites, 33 parsimony informative sites) was obtained for the *COI* sequences. A bias against guanine (A: 0.226; C: 0.291; G: 0.199; T: 0.284) was observed. For the *COI* sequences, a transition bias was observed (transition: transversion ratio = 7.71/1; $R = 3.218$). A lower number of haplotypes (19) were obtained for the slower evolving nuclear gene *RAG1* (190 sequences). With a consensus length of 550bp, only 19 nucleotide sites were variable, while seven of these sites were parsimony informative. Base frequencies were A: 0.217; C: 0.252; G: 0.296; T: 0.235 and the transition: transversion ratio was 4.4/1 ($R = 14.124$).

Overall population haplotype diversity was high (*Cytb* = 0.923 ± 0.009; *COI* = 0.898 ± 0.012) with low nucleotide diversity (*Cytb* = 0.013 ± 0.007; *COI* = 0.009 ± 0.005). Moderate to high haplotype diversity and low nucleotide diversity for *Cytb* and *COI* sequences were found in all six sampling locations (Table 3.2). Overall haplotype- and nucleotide diversity was higher compared to those at each sampling location (Table 3.2). An overall high haplotype diversity (*Cytb* = 0.885 ± 0.013; *COI* = 0.847 ± 0.019) and low nucleotide diversity (*Cytb* = 0.004 ±0.002; *COI* = 0.003 ± 0.002) was found when only sampling sites in the southern hemisphere (AUS, NZL, CH, SA) were examined.

Table 3.2: Diversity measures for *Cytb* and *COI* in the *Seriola lalandi* populations.

Population	*N*	*nh*	S	*h*	*Π*
Cytb					
North-eastern Pacific (USA)	27	8	9	0.510 (0.116)	0.001 (0.001)
North-western Pacific (JA)	19	9	17	0.813 (0.081)	0.004 (0.002)
South-eastern Atlantic (SA)	62	16	17	0.7234 (0.055)	0.002 (0.001)
South-western Pacific (NZL)	23	7	8	0.577 (0.118)	0.002 (0.001)
South-western Pacific (AUS)	25	3	2	0.156 (0.096)	0.0002 0.0003)
South-eastern Pacific (CH)	34	9	12	0.463 (0.106)	0.001 (0.001)
COI					
North-eastern Pacific (USA)	27	8	6	0.459 (0.119)	0.002 (0.001)
North-western Pacific (JA)	19	6	6	0.602 (0.124)	0.002 (0.001)
South-eastern Atlantic (SA)	62	13	13	0.491 (0.077)	0.002 (0.001)
South-western Pacific (NZL)	23	7	9	0.517 (0.124)	0.003 (0.002)
South-western Pacific (AUS)	25	6	5	0.623 (0.100)	0.002 (0.002)
South-eastern Pacific (CH)	34	5	5	0.323 (0.102)	0.001 (0.001)

n, number of individuals; *nh*, number of haplotypes; S, number of polymorphic sites; *h*, gene diversity; and π, nucleotide diversity, with standard errors in brackets.

The average uncorrected *p*-distances between the six geographical regions for *Cytb* ranged from 0.1% between NZL, AUS and CH to 3.2% between SA and JA (Table 3.3). For *COI* it ranged

from 0.2% between NZL/AUS and CH to 2.2% between JA and NZL/AUS (Table 3.4). Low *p*-distances were found for *RAG1* (Table 3.5).

Table 3.3: The average uncorrected sequence divergence for *Cytb* sequences among the six sampling sites.

	North-eastern Pacific (USA)	North-eastern Pacific (JA)	South-eastern Atlantic (SA)	South-western Pacific (NZL)	South-western Pacific (AUS)	South-eastern Pacific (CH)
North-western Pacific (JA)	0.027	-				
South-eastern Atlantic (SA)	0.023	0.032	-			
South-western Pacific (NZL)	0.024	0.031	0.006	-		
South-western Pacific (AUS)	0.024	0.031	0.006	0.001	-	
South-eastern Pacific (CH)	0.024	0.031	0.006	0.001	0.001	-

Table 3.4: The average uncorrected sequence divergence for *COI* sequences among the six sampling sites.

	North-eastern Pacific (USA)	North-western Pacific (JA)	South-eastern Atlantic (SA)	South-western Pacific (NZL)	South-western Pacific (AUS)	South-eastern Pacific (CH)
North-western Pacific (JA)	0.017	-				
South-eastern Atlantic (SA)	0.017	0.019	-			
South-western Pacific (NZL)	0.019	0.022	0.005	-		
South-western Pacific (AUS)	0.019	0.022	0.005	0.002	-	
South-eastern Pacific (CH)	0.018	0.021	0.004	0.003	0.002	-

Table 3.5: The average uncorrected sequence divergence for *RAG1* sequences among the six sampling sites.

	North-eastern Pacific (USA)	North-western Pacific (JA)	South-eastern Atlantic (SA)	South-western Pacific (NZL)	South-western Pacific (AUS)	South-eastern Pacific (CH)
North-western Pacific (JA)	0.004	-				
South-eastern Atlantic (SA)	0.008	0.006	-			
South-western Pacific (NZL)	0.006	0.004	0.002	-		
South-western Pacific (AUS)	0.006	0.004	0.002	0.001	-	
South-eastern Pacific (CH)	0.006	0.004	0.002	0.000	0.000	-

3.3.2 Haplotype networks

Three distinct *S. lalandi* clades were identified in the median-joining networks for all three DNA fragments analysed (Fig. 3.2) corresponding to three different geographic regions (North-western Pacific - JA, North-eastern Pacific - USA, and the southern hemisphere). The southern hemisphere clade comprised of samples from the southern Pacific (AUS, NZL and CH) and the southern-eastern Atlantic (SA). Interestingly within this southern hemisphere clade, no haplotypes were shared between the southern Pacific and the South-eastern Atlantic sampling areas. However, within the southern Pacific clade haplotypes were shared over a vast geographical area (*i.e.* between Chile, New Zealand and Australia). Clades identified by the haplotype networks can be characterised in having a star-like shape; thus having several low frequency haplotypes that were one or a limited number of mutational steps removed from the most common haplotype(s).

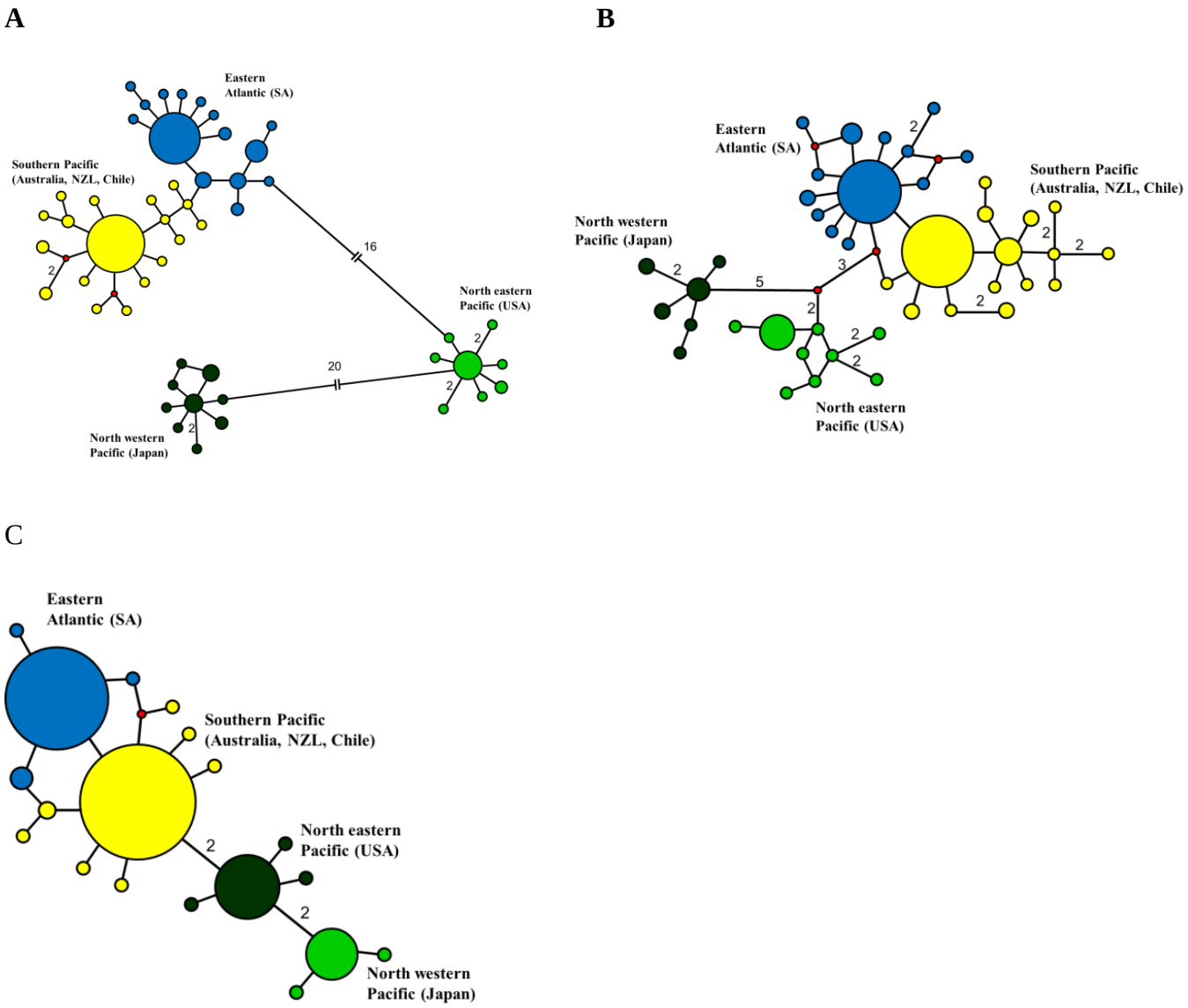


Fig. 3.2: The median joining networks indicating the genetic relationships of the *Seriola lalandi* samples in this study. (a) network of the 50 *Cytb* haplotypes; (b) network of the 43 *COI* haplotypes; (c) network of the 19 *RAG1* haplotypes. Genetic haplotypes are represented by circles with sizes proportional to the number of individuals represented by that haplotype. Intermediate missing haplotypes are indicated as red dots. Connecting branch lengths are proportional to one base change, except where the actual lengths are indicated on the branches.

3.3.3 Phylogenetic relationships

Haplotype sequences for *Cytb* (50; Appendix E), *COI* (42; Appendix F), and *RAG1* (19; Appendix G) were used to reconstruct the phylogenetic relationship between *S. lalandi* populations. Three inference methods (MP, ML, and BI) were used to construct the phylogenetic trees using the datasets for each of the three gene fragments separate as well as for a combined dataset. The ML phylogenetic trees are presented for all datasets with bootstrap values (ML/MP) and posterior probabilities (BI) given for supported nodes (Fig. 3.3 - 3.6). The relationship between the *S. lalandi* populations will be discussed in detail following the summary of the basic results; however the phylogenetic relationship between *S. lalandi* populations differed somewhat depending on the dataset examined and inference method used. MP analysis of the *Cytb* dataset generated 1 957 equally parsimonious trees of 149 steps (CI = 0.792; RI = 0.935), for the *COI* dataset it produced 4 274 equally parsimonious trees with a length of 72 (CI = 0.847; RI = 0.930) and for *RAG1* it resulted in 2 594 equally parsimonious trees (length = 32; CI = 0.813; RI = 0.813). The phylogenetic analyses of the combined dataset was constructed using 91 haplotypes with a sequence length of 1 810bp of which 192 were variable and 135 parsimoniously informative. MP analysis produced 2 797 equally parsimonious trees of 255 steps (CI = 0.780; RI = 0.950).

The presence of three main geographical groupings (North-western Pacific - JA, North-eastern Pacific - USA, and the southern hemisphere) was confirmed by the phylogenetic pattern obtained with the separate as well as combined datasets (Fig. 3.3 - 3.6). These three geographical clades are monophyletic and strongly supported (bootstrap $\geq$ 80 and posterior probabilities = 1.00; Fig. 3.3, 3.4, 3.6) for the *Cytb*, *COI* and combined dataset. Within the *RAG1* trees all three

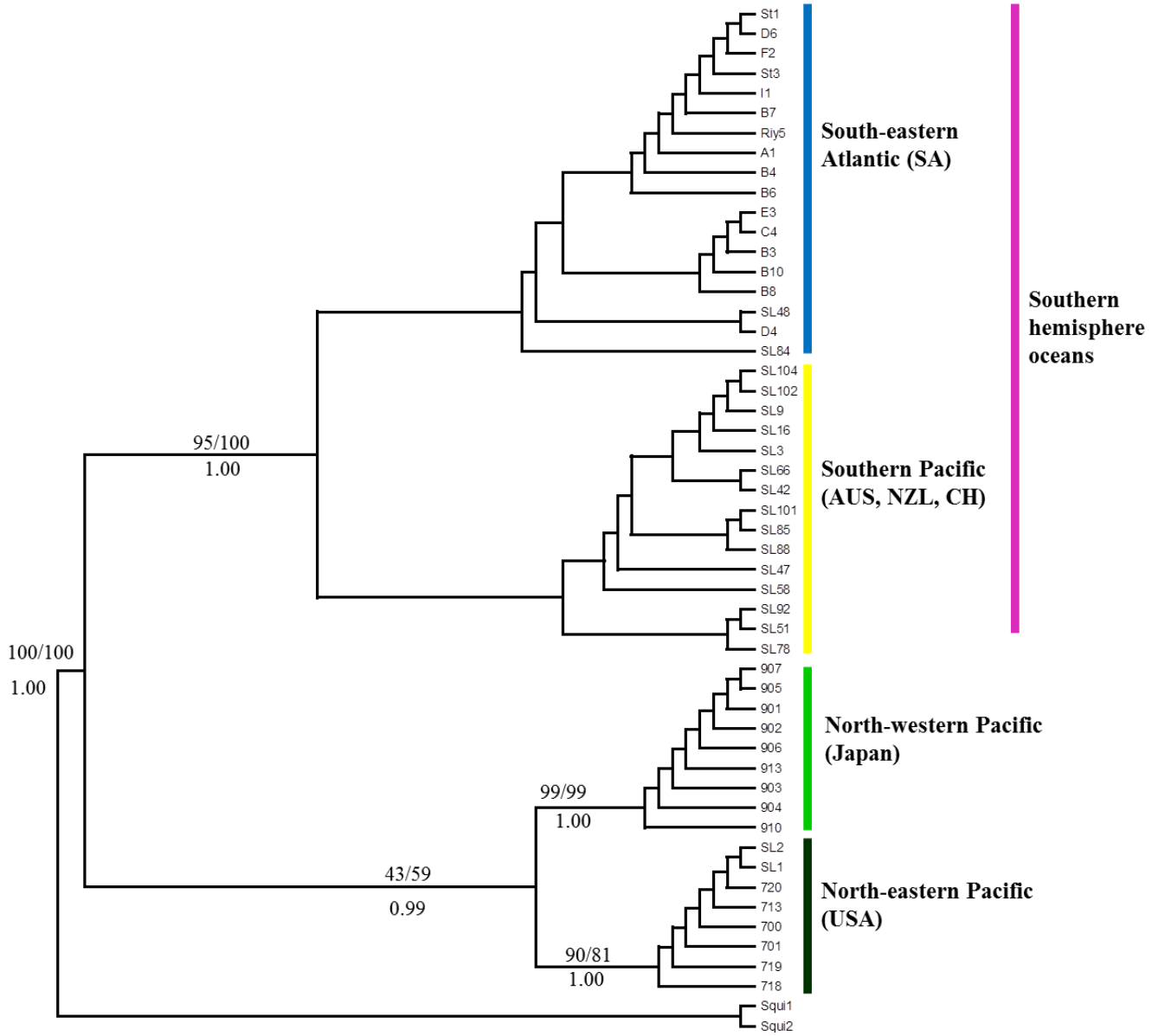


Fig. 3.3: The ML tree of the *Cytb* haplotypes. Numbers above branches are MP and ML bootstrap support. Only support values higher than 50 are indicated. Numbers below branches are Bayesian posterior probabilities.

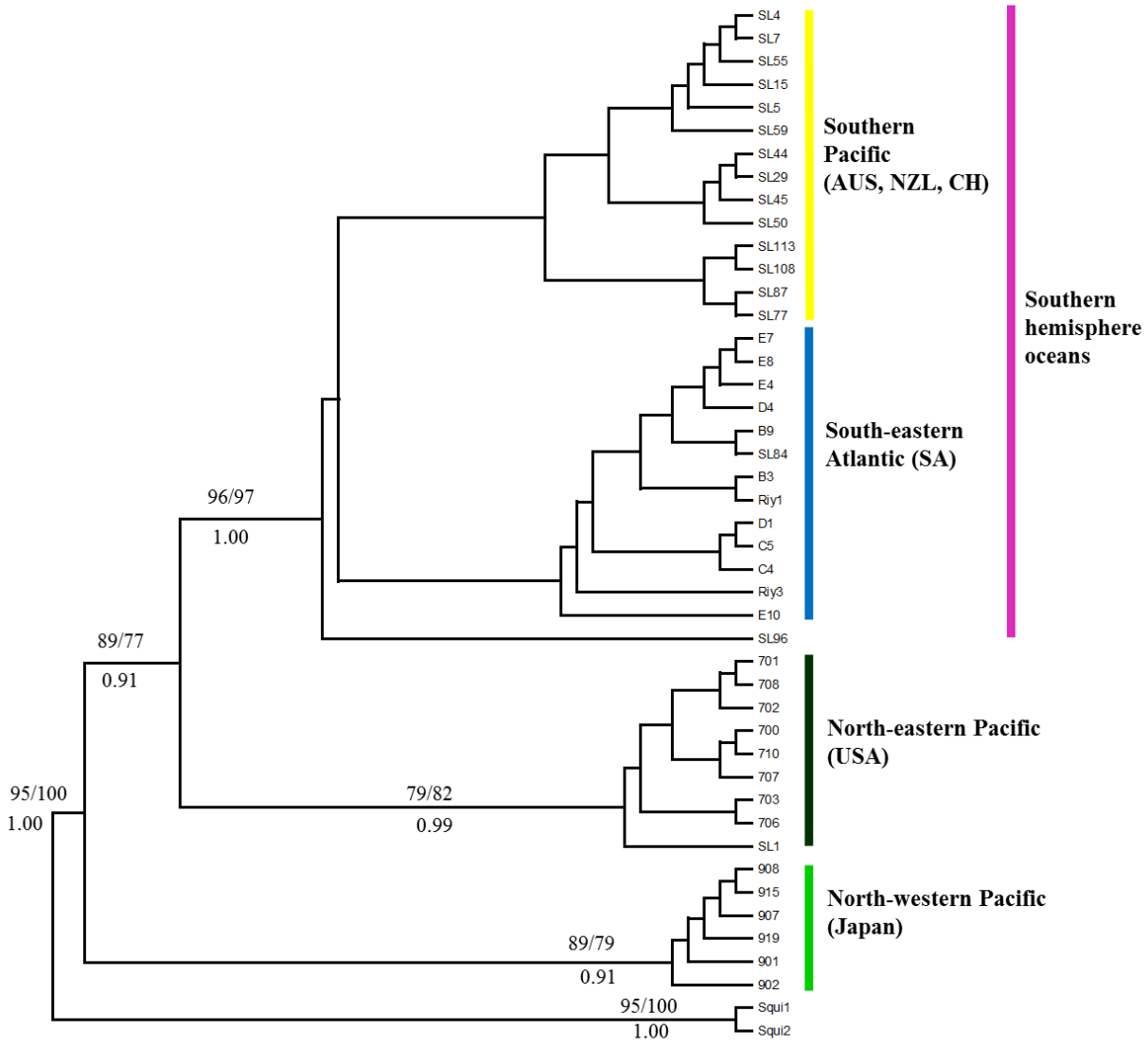


Fig. 3.4: The ML tree of the *COI* haplotypes. Numbers above branches are MP and ML bootstrap support. Only support values higher than 50 are indicated. Numbers below branches are Bayesian posterior probabilities.

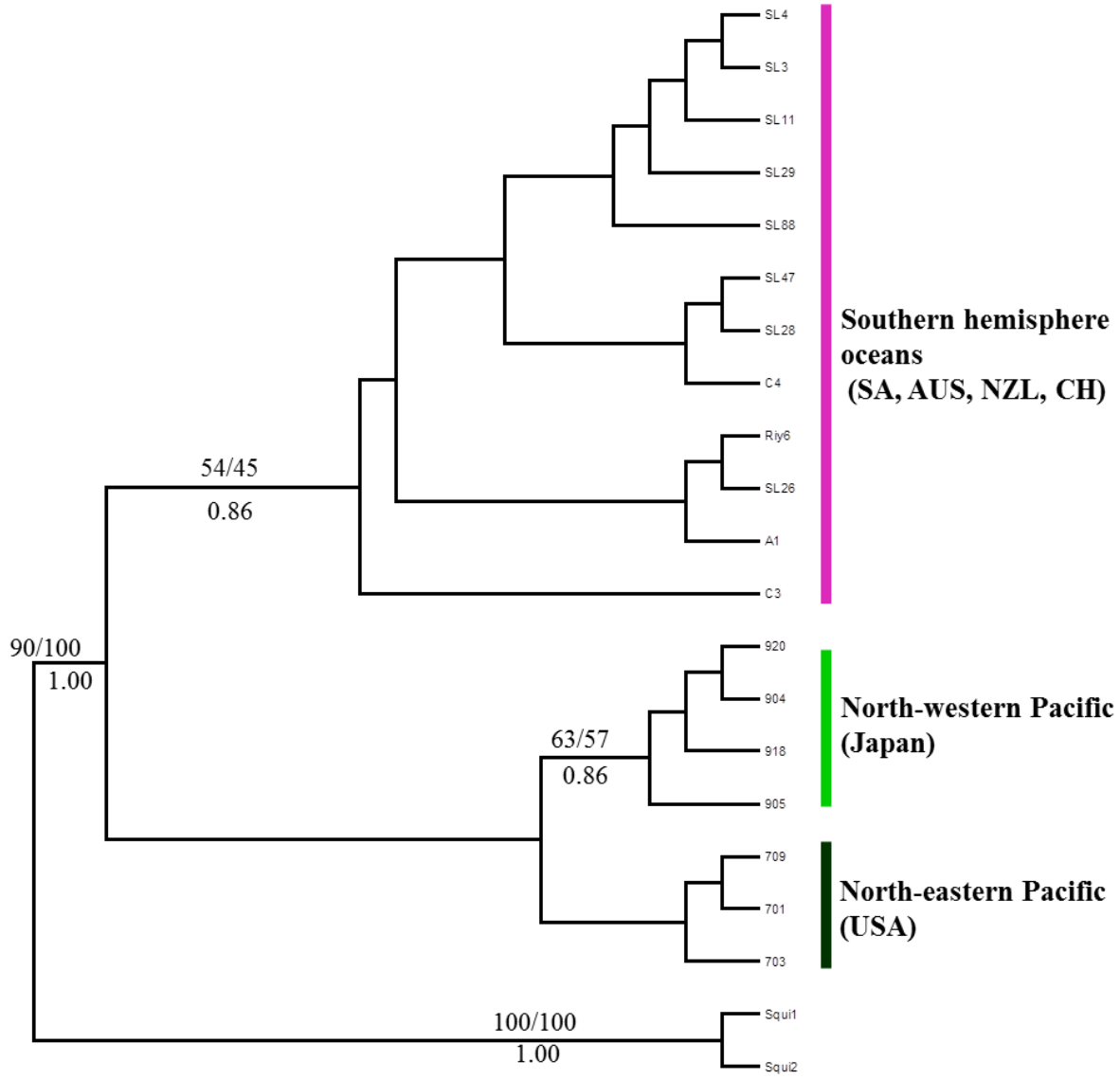


Fig. 3.5: The ML tree of the *RAG1* haplotypes. Numbers above branches are MP and ML bootstrap support. Only support values higher than 50 are indicated. Numbers below branches are Bayesian posterior probabilities.

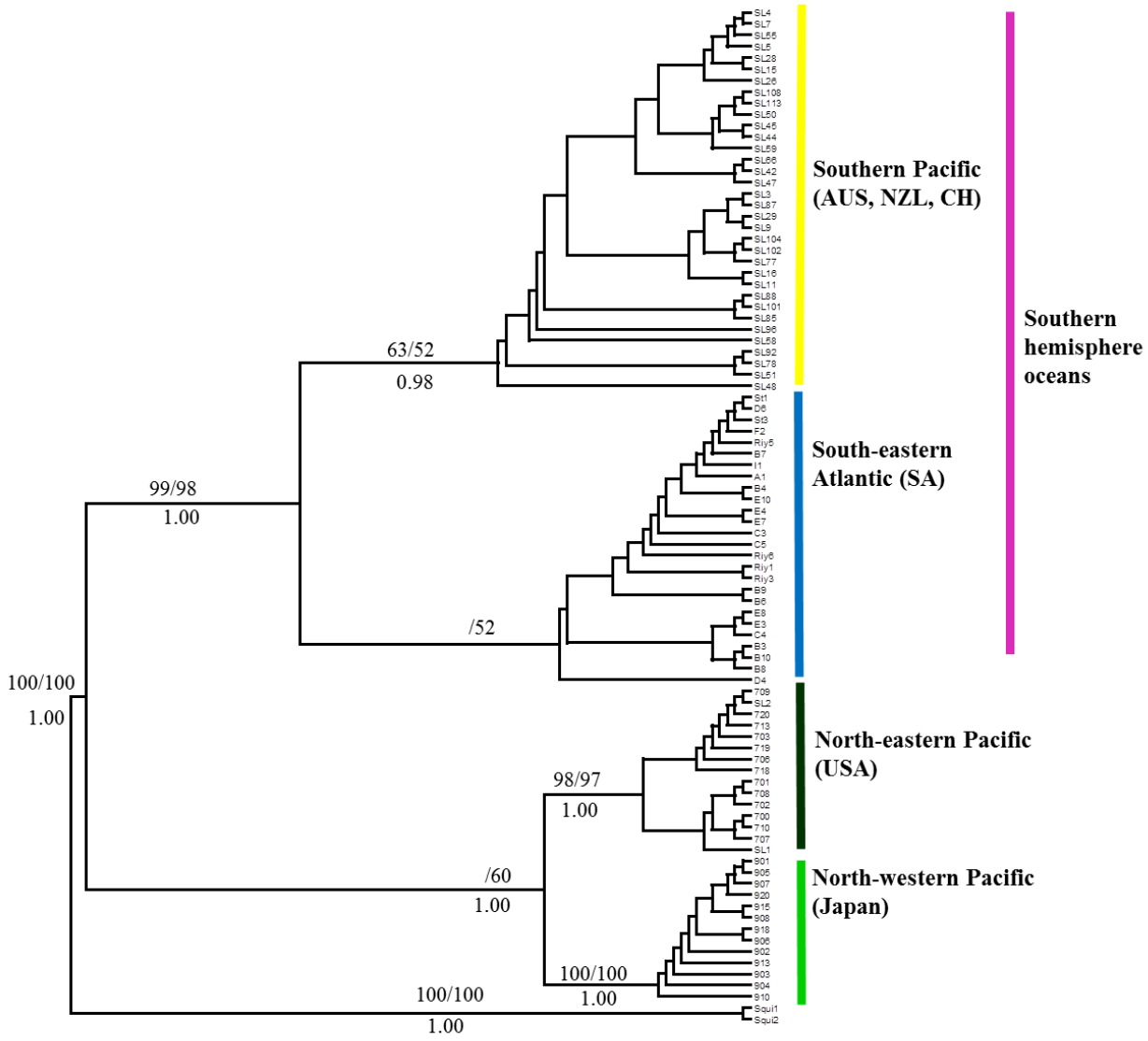


Fig. 3.6: The ML tree of the combined haplotype dataset. Numbers above branches are MP and ML bootstrap support. Only support values higher than 50 are indicated. Numbers below branches are Bayesian posterior probabilities.

geographic clades are resolved (albeit with weak or no support; Fig. 3.5). The relationships between the three geographical clades are not consistent in all dataset analyses. Even though the North-western Pacific (JA) and the North-eastern Pacific (USA) are sister taxa in the analysis of the *Cytb*, *RAG1* and combined dataset (Fig. 3.3, 3.5, 3.6), the *COI* phylogenetic hypothesis shows a sister relationship between the North-eastern Pacific (USA) and the southern hemisphere (Fig. 3.4). There is also some discrepancy within the same dataset between phylogenetic methods. The sister taxa relationship between the North-western Pacific (JA) and the North-eastern Pacific (USA) is supported by ML and BI analysis (bootstrap = 60 and posterior probabilities = 1.00; Fig. 3.6) in the combined dataset while in the MP phylogeny (not shown) the closer association of the North-eastern Pacific (USA) and the southern hemisphere is weakly supported with a bootstrap value of 55.

Two clades can be recognised by the *Cytb*, *COI* and combined phylogenies (Fig. 3.3, 3.4, 3.6) within the southern hemisphere lineage: the southern Pacific (AUS, NZL and CH) and the South-eastern Atlantic (SA). The southern Pacific grouping was only supported in the combined dataset for all three phylogenetic inferences (bootstrap value = 63/52 and posterior probabilities = 0.98; Fig. 3.6), while the validity of the South-eastern Atlantic grouping was only supported by ML bootstrap analysis.

3.3.4 Population structure

The AMOVA analysis of the three main clades (North-western Pacific, North-eastern Pacific, and the southern hemisphere) indicated high differentiation among clades and thus, among the geographic regions (*COI*: $F_{ST} = 0.895$, $p < 0.001$; $\Phi_{ST} = 0.896$, $p < 0.001$; *Cytb*: $F_{ST} = 0.940$, $p <$

Table 3.6: Pairwise F_{ST} and *p*-values (in brackets) below diagonal and Φ_{ST} and *p*-values (in brackets) above diagonal based on *Cytb* region.

	North-eastern Pacific (USA)	North - western Pacific (JA)	South-eastern Atlantic (SA)	South-western Pacific (NZL)	South-western Pacific (AUS)	South-eastern Pacific (CH)
North-eastern Pacific (USA)		0.920 ($p < 0.001$)	0.927 ($p < 0.001$)	0.952 ($p < 0.001$)	0.977 ($p < 0.001$)	0.953 ($p < 0.001$
North-western Pacific (JA)	0.918 ($p < 0.001$)	-	0.924 ($p < 0.001$)	0.915 ($p < 0.001$)	0.940 ($p < 0.001$)	0.927 ($p < 0.001$)
South-eastern Atlantic (SA)	0.931 ($p < 0.001$)	0.928 ($p < 0.001$)	-	0.675 ($p < 0.001$)	0.734 ($p < 0.001$)	0.690 ($p < 0.001$)
South-western Pacific (NZL)	0.951 ($p < 0.001$)	0.913 ($p < 0.001$)	0.694 ($p < 0.001$)	-	0.058 ($p = 0.003$)	0.034 ($p = 0.025$)
South-western Pacific (AUS)	0.976 ($p < 0.001$)	0.938 ($p < 0.001$)	0.750 ($p < 0.001$)	0.058 ($p < 0.001$)	-	0.021 ($p = 0.056$)
South-eastern Pacific (CH)	0.952 ($p < 0.001$)	0.924 ($p < 0.001$)	0.706 ($p < 0.001$)	0.034 ($p = 0.018$)	0.021 ($p = 0.081$)	-

0.001; $\Phi_{ST} = 0.939$, $p < 0.001$; *RAG1*: $F_{ST} = 0.876$, $p < 0.001$; $\Phi_{ST} = 0.923$, $p < 0.001$). A second AMOVA analysis was carried out on four geographical groups where the southern hemisphere sampling sites were divided into the Atlantic (SA) and South Pacific groups (AUS/NZL/CH). These results corroborate the population structuring found in networks and phylogenetic analysis; however an additional split in the southern hemisphere is also evident (*COI*: $F_{ST} = 0.845$, $p < 0.001$; $\Phi_{ST} = 0.812$, $p < 0.001$; *Cytb*: $F_{ST} = 0.909$, $p < 0.001$; $\Phi_{ST} = 0.911$, $p < 0.001$; *RAG1*: $F_{ST} = 0.896$, $p < 0.001 \Phi_{ST} = 0.897$, $p < 0.001$). Pairwise F_{ST} and Φ_{ST} values showed high levels of genetic differentiation between the northern and southern hemisphere groups (Table 3.6

Table 3.7: Pairwise F_{ST} and *p*-values (in brackets) below diagonal and Φ_{ST} and *p*-values (in brackets) above diagonal based on *COI* region.

	North-eastern Pacific (USA)	North-western Pacific (JA)	South-eastern Atlantic (SA)	South-western Pacific (NZL)	South-western Pacific (AUS)	South-eastern Pacific (CH)
North-eastern Pacific (USA)	-	0.860 ($p < 0.001$)	0.886 ($p < 0.001$)	0.862 ($p < 0.001$)	0.878 ($p < 0.001$)	0.903 ($p < 0.001$)
North-western Pacific (JA)	0.859 ($p < 0.001$)	-	0.911 ($p < 0.001$)	0.886 ($p < 0.001$)	0.902 ($p < 0.001$)	0.927 ($p < 0.001$)
South-eastern Atlantic (SA)	0.884 ($p < 0.001$)	0.909 ($p < 0.001$)	-	0.550 ($p < 0.001$)	0.588 ($p < 0.001$)	0.560 ($p < 0.001$)
South-western Pacific (NZL)	0.860 ($p < 0.001$)	0.883 ($p < 0.001$)	0.550 ($p < 0.001$)	-	0.008 ($p = 0.242$)	0.092 ($p < 0.001$)
South-western Pacific (AUS)	0.877 ($p < 0.001$)	0.900 ($p < 0.001$)	0.588 ($p < 0.001$)	0.007 ($p = 0.261$)	-	0.184 ($p < 0.001$)
South-eastern Pacific (CH)	0.902 ($p < 0.001$)	0.926 ($p < 0.001$	0.599 ($p < 0.001$)	0.092 ($p < 0.001$)	0.184 ($p < 0.001$)	-

Table 3.8: Pairwise F_{ST} and *p*-values (in brackets) below diagonal and Φ_{ST} and *p*-values (in brackets) above diagonal based on *RAG1* region.

	North-eastern Pacific (USA)	North-western Pacific (JA)	South-eastern Atlantic (SA)	South-western Pacific (NZL)	South-western Pacific (AUS)	South-eastern Pacific (CH)
North-eastern Pacific (USA)		0.767 ($p < 0.001$)	0.947 ($p < 0.001$)	0.916 ($p < 0.001$)	0.887 ($p < 0.001$)	0.948 ($p < 0.001$)
North-western Pacific (JA)	0.767 ($p < 0.001$)	-	0.932 ($p < 0.001$)	0.878 ($p < 0.001$)	0.830 ($p = 0.000$)	0.930 ($p < 0.001$)
South-eastern Atlantic (SA)	0.946 ($p < 0.001$)	0.931 ($p < 0.001$)	-	0.828 ($p < 0.001$)	0.786 ($p < 0.001$)	0.874 ($p < 0.001$)
South-western Pacific (NZL)	0.916 ($p < 0.001$)	0.877 ($p < 0.001$)	0.827 ($p < 0.001$)	-	0.009 ($p = 0.714$)	0.038 ($p = 0.143$)
South-western Pacific (AUS)	0.886 ($p < 0.001$)	0.830 ($p < 0.001$)	0.784 ($p < 0.001$)	0.007 ($p = 0.748$)	-	0.009 ($p = 0.029$)
South-eastern Pacific (CH)	0.948 ($p < 0.001$)	0.929 ($p < 0.001$)	0.872 ($p < 0.001$)	0.036 ($p = 0.117$)	0.008 ($p = 0.036$)	-

- 3.8). Within the southern hemisphere moderate genetic differentiation was found between the eastern Atlantic (SA) and the South Pacific (AUS/NZL/CH) groups. Little genetic structure was found in the southern Pacific, indicating high levels of gene flow within this region.

3.3.5 Demographic history and neutrality

The Tajima's *D* and Fu's *Fs* neutrality tests indicate whether a population's size is constant (neutral); thus at mutation-drift equilibrium. Deviations from neutrality could be the consequence of selection, population expansion, -bottleneck, or mutation rate heterogeneity (Aris-Brosou and Excoffier, 1996). In addition to testing for neutrality, these statistics are also widely used to test for changes in population size (Ramos-Onsins and Rozas 2002; Mousset *et al*., 2004). Negative Tajima's *D* values may either indicate population growth (after a bottleneck or after a small founder event) or a selective sweep. Positive Tajima's *D* values on the other hand can imply positive and/or balancing selection, or an admixture of isolated populations (Rand, 1996). When these positive values are significant it can be a signal of genetic subdivision, population contraction, or diversifying selection. Negative Fu's *Fs* values indicate an excess of recent mutations (thus an excess of rare alleles), with a large negative value taken as evidence for population growth (Fu, 1997). The Tajima's *D* and Fu's *Fs* neutrality tests, performed for each gene fragment, were all negative and mostly significant for each grouping examined as well as globally (Table 3.9 - 3.11) indicating that, under a neutral model, a demographic expansion event may have occurred. For the southern hemisphere and its two groupings ({AUS/NZL/CH} and SA) the *D*- and *Fs* values were all significant and negative, suggesting population growth. Further evidence for this is the large negative values of Fu's *Fs*. Contrasting results were obtained for JA and USA. For JA, Tajima's *D* values are all negative, but only *RAG1* Tajima's *D*

values showed significant results; while Fu's *Fs* values for *RAG1* and *Cytb* were significant. Non-significant Fu's *Fs* values were found for USA with the *RAG1* dataset while non-significant Tajima's *D* values were observed for the *Cytb* sequences. Non-significant neutrality test values usually means the population size is constant. However in this case limited sample size for these groupings probably influenced the results (JA = 19, USA = 27) as these neutrality tests are more accurate in detecting population expansion for larger sample sizes (Fu, 1997; Ramos-Onsins and Rozas, 2002). Tajima's *D* and Fu's *Fs* values for the combined population gave contrasting results: although all values were negative for all the gene fragments, the values were non-significant for Tajima's *D* and significant for Fu's *Fs*.

Table 3.9: The results of the neutrality tests for the *Cytb* region, analysed per geographical grouping and for the overall population.

Locality	Tajima's *D*	*p*	Fu's *Fs*	*p*
North-eastern Pacific (USA)	-0.785	0.233	-3.642	**0.008**
North-western Pacific (JA)	-1.340	0.091	-2.333	**0.031**
Southern hemisphere (SA, NZL, AUS, CH)	-2.008	**0.010**	-28.570	**0.000**
South-eastern Atlantic (SA)	-2.114	**0.007**	-11.819	**0.000**
South Pacific (NZL, AUS, CH)	-1.902	**0.017**	-16.927	**0.000**
Overall	-1.318	0.087	-24.820	**0.000**

Significant *p*-values indicated in bold

Table 3.10: The results of the neutrality tests for the *COI* region, analysed per geographical grouping and for the overall population.

Locality	Tajima's *D*	*p*	Fu's *Fs*	*p*
North-eastern Pacific (USA)	-2.182	**0.004**	-5.665	**0.000**
North-western Pacific (JA)	-1.046	0.160	-1.363	0.223
Southern hemisphere (SA, NZL, AUS, CH)	-1.630	**0.039**	-23.874	**0.000**
South-eastern Atlantic (SA)	-1.618	**0.043**	-9.035	**0.000**
South Pacific (NZL, AUS, CH)	-2.284	**0.003**	-21.061	**0.000**
Overall	-0.627	0.284	-10.708	**0.033**

Significant *p*-values indicated in bold

Table 3.11: The results of the neutrality tests for the *RAG1* region, analysed per geographical grouping and for the overall population.

Locality	Tajima's *D*	*p*	Fu's *Fs*	*p*
North-eastern Pacific (USA)	-1.889	**0.019**	-0.981	0.173
North-western Pacific (JA)	-1.719	**0.033**	-2.677	**0.000**
Southern hemisphere (SA, NZL, AUS, CH)	-1.592	**0.044**	-12.964	**0.000**
South-eastern Atlantic (SA)	-1.684	**0.036**	-2.975	**0.016**
South Pacific (NZL, AUS, CH)	-2.125	**0.006**	-16.737	**0.000**
Overall	-1.311	0.089	-12.328	**0.001**

Significant *p*-values indicated in bold

In most cases the mismatch distributions from individual geographical groupings were unimodal, (Fig. 3.7) indicating sudden expansion. This is confirmed by the small and non-significant SSD and raggedness index values in all populations, suggesting that the distributions observed did not differ significantly from the distributions expected under a sudden demographic expansion model (Roger and Harpending, 1992; Schneider and Excoffier, 1999; Table 3.12). The overall mismatch distribution including all populations was bimodal for *Cytb* and *COI* (Fig. 3.7), with one mode related to the number of differences within the clades, and the other corresponding to the differences between the clades.

These results agree with the network analysis (Fig. 3.2). The haplotype network of *S. lalandi* was characterised by a highly dominant central haplotype for each geographic grouping. Most other haplotypes were unique and separated from the central haplotypes by only one or two mutational steps, resulting in a strong "star-like" phylogeny pattern for each grouping.

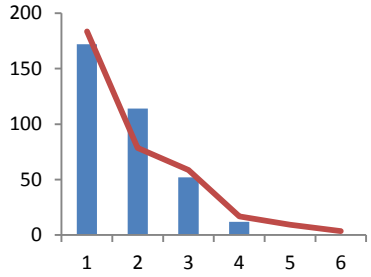
Table 3.12: Mismatch distribution estimates for the geographical groupings found in *Seriola lalandi* populations and for the overall population. The parameters of the sudden expansion model and goodness-of-fit test are shown as well as Harpending's index of raggedness and its *p*-value (Rogers and Harpending, 1992; Harpending, 1994).

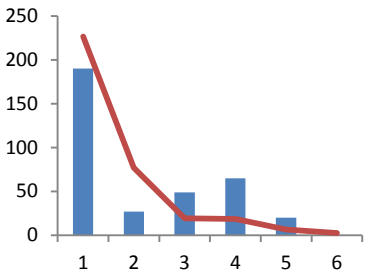
	North-eastern Pacific (USA)	North-western Pacific (JA)	South-eastern Atlantic (SA)	South Pacific (AUS, CH, NZL)	Overall
Cytb					
Parameters					
Θ_0	0.000	0.000	0.001	0.000	8.490
Θ_1	2.200	3.621	3.051	0.726	8.491
τ	0.943	9.826	2.842	3.951	3.120
Goodness of fit test					
SSD	0.00002	0.046	0.010	0.017	0.035
p	0.750	0.200	0.650	0.400	0.250
R	0.072	0.074	0.038	0.216	0.356
p	0.850	0.550	0.800	0.550	0.300
COI					
Parameters					
Θ_0	0.000	0.000	0.000	0.00	0.000
Θ_1	0.993	2.223	31.040	0.971	4.941
τ	4.203	1.530	0.666	4.042	9.778
Goodness of fit test					
SSD	0.047	0.004	0.006	0.006	0.028
p	0.300	0.750	**0.000**	0.600	0.600
R	0.241	0.048	0.117	0.119	0.033
p	0.600	0.900	0.650	0.850	0.750
RAG1					
Parameters					
Θ_0	0.094	0.000	0.000	0.000	0.275
Θ_1	0.107	0.446	0.179	0.223	3.035

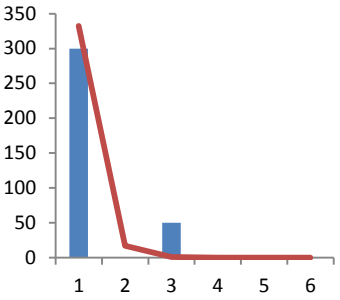
τ	3.00	0.848	3.036	3.025	2.326
Goodness of fit test					
SSD	0.023	0.005	0.0002	0.0007	0.0123
p	**0.000**	**0.000**	0.200	0.250	0.500
R	0.771	0.246	0.534	0.471	0.051
p	0.900	0.950	0.800	0.750	0.550

Θ_0 = pre-expansion, Θ_1 = post-expansion, τ = time in generations since expansion, SSD = sum of squared deviations, R = raggedness index, *p* = *p*-values.

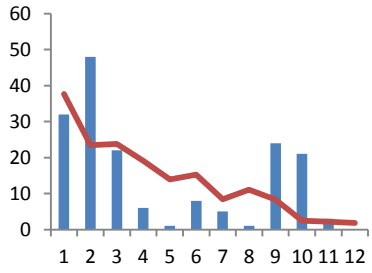
Cytb *COI* *RAG1*

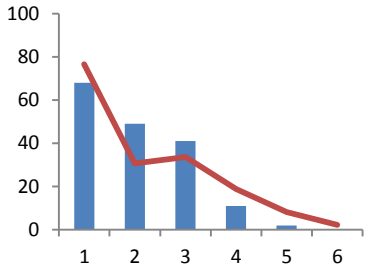


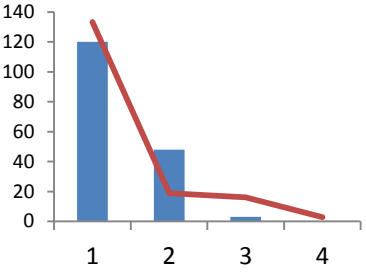




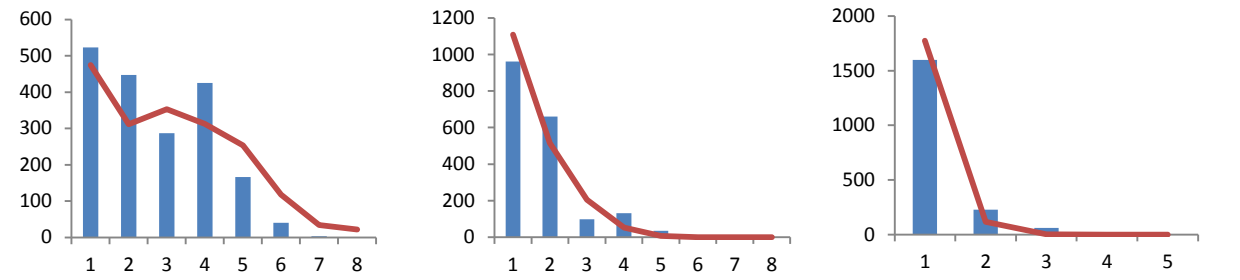
USA



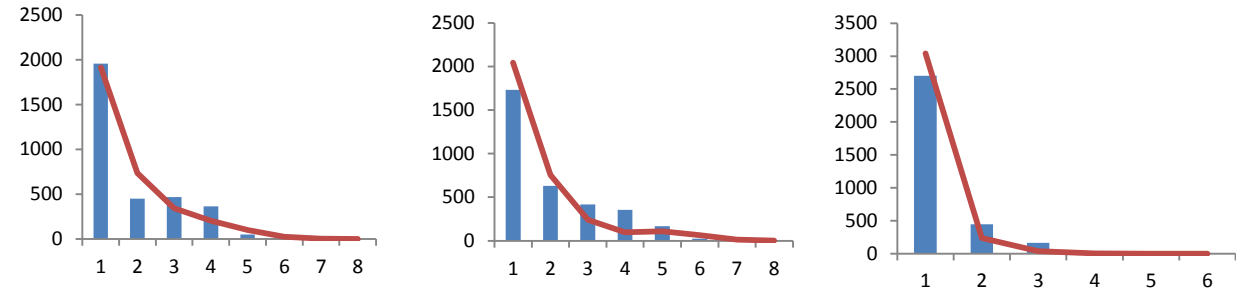




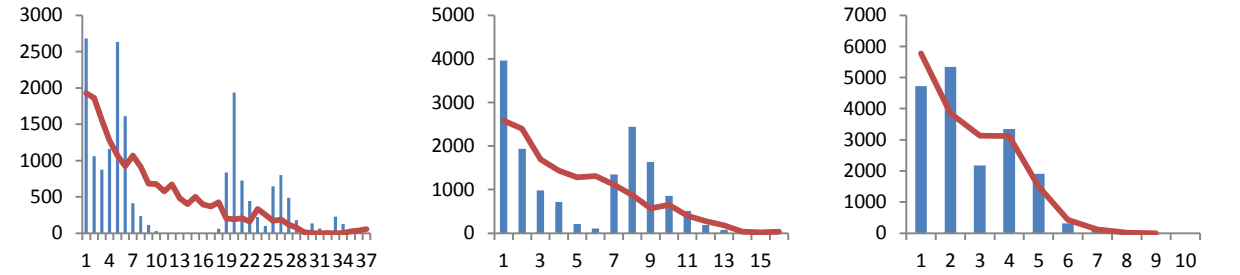
JA



SA



South Pacific



ALL

Fig. 3.7: Mismatch distributions obtained with the mitochondrial (*Cytb* and *COI*) and nuclear (*RAG1*) DNA datasets. The simulated (red line) and observed (blue bars) frequency of distribution was plotted against the number of pairwise differences.

3.3.6 Divergence estimates

The estimated ages of divergence (TMRCA) for the four geographical groupings are given in Table 3.13. Relative similar estimates for TMRCA for each grouping was recovered, with the North-eastern Pacific (USA) probably the youngest (USA = 116 700 - 234 100 years, JA = 197 700 - 449 700 years; SA = 217 100 - 432 700 years; South Pacific = 230 400 - 459 700 years). The South Pacific populations showed slightly older population expansions when compared to the Atlantic population (SA). When using a mutation rate of 2% per MYA, similar values for TMRCA was estimated for the two mtDNA fragments (*Cytb* and *COI*), suggesting that mutations accumulated within these regions at a similar rate. The northern hemisphere population originated approximately 681 500 - 1 565 400 years ago while different ages of origin was predicted by the two mtDNA fragments for the southern hemisphere groups (*Cytb* = 2 280 600 - 4 564 000 and *COI* = 326 000).

Table 3.13: Divergence times for *Seriola lalandi* populations. TMRCA values estimated using different mutation rates (%) and 95% confidence intervals (CI) in brackets for the two mtDNA fragments (*Cytb* and *COI*) are shown.

	Cytb		***COI***
	1%	**2%**	**2%**
North-eastern Pacific (USA)	234 100	116 700	225 100
	(79 200 - 412 600)	(37 829 - 205 100)	(82 226 - 381 500)
North-western Pacific (JA)	449 700	224 000	197 700
	(207 000 - 702 100)	(107 700 - 351 500)	(55 043 - 372 100)
South-eastern Atlantic (SA)	432 700	217 100	287 900
	(202 600 - 670 800)	(106 600 - 344 000)	(153 800 - 455 000)
South Pacific (NZL, AUS, CH)	459 700	230 400	309 300
	(239 200 - 709 600)	(122 100 - 355 600)	(168 900 - 458 200)
Northern hemisphere (USA, JA)	1 565 400	777 800	681 500
	(1 000 300 - 2 259 400)	(495 200 - 1 113 200)	(367 300 - 1 018 300)
Southern hemisphere (SA, NZL, AUS, CH)	4 564 000	2 280 600	326 000
	(3 554 600 - 5 570 800)	(1 793 000 - 2 790 000)	(197 900 - 475 800)

3.4 Discussion

In the current study the analyses of multi-locus datasets revealed for the first time the global population dynamics, diversification and history of *S. lalandi*. Three distinct geographical groupings (North-western Pacific - JA, North-eastern Pacific - USA, and the southern hemisphere) were identified over the entire distributional range of this species. Analyses of these lineages showed strong signatures of recent demographic expansions. These three clades correspond with the previously identified *S. lalandi* subspecies: North-eastern Pacific clade - *S. lalandi dorsalis* (California yellowtail; Gill, 1863), North-western Pacific clade - *S. lalandi aureovittata* (Asian yellowtail; Schlegel, 1844) and southern hemisphere clade - *S. lalandi lalandi* (Southern yellowtail; Cuvier and Valenciennes, 1833).

3.4.1 Population structure

Global population structure in marine vertebrates can range from relative little (*e.g.* Ward *et al*., 1994; Chow *et al*., 2009) to highly structured populations (*e.g.* Ruzzante *et al*., 2000; McPherson *et al*., 2004; Chiang *et al*., 2008). For example, in pelagic species with a global distribution, population structure was detected only between ocean basins in species in highly mobile adults (*e.g.* shortfin mako, *Isurus oxyrinchus*; Schrey and Heist, 2003; basking shark, *Cetorhinus maximus;* Hoelzel *et al*., 2000; whale shark, *Rhincodon typus*; Castro *et al*., 2007). In contrast for less mobile, demersal or benthic species with more restricted geographic ranges, higher levels of intraspecific genetic differentiation were found (*e.g.* shovelnose guitarfish, *Rhinobatus productus*; Sandoval-Castillo *et al*., 2004; thornback ray, *Raja clavata*; Chevolot *et al*., 2006; zebra shark, *Stegostoma fasciatum*; Dudgeon *et al*., 2008). These differences in genetic differentiation among species are most probably influenced by their life history traits (such as

mobility). On the other hand, different patterns in genetic variation are also found in populations of closely related species with similar life histories (*e.g.* comparing the European seabass, *Dicentrarchus labrax* and the spotted seabass, *D. punctatus*; Bonhomme *et al*., 2002). Therefore it is evident that a number of factors (historical and contemporary) are involved in shaping population genetic structure in marine species with circumglobal distributions.

The current study found three distinct geographical groupings (North-western Pacific - Japan, North-eastern Pacific - USA, and the southern hemisphere) of *S. lalandi* which are substantiated by the topology of the haplotype networks (Fig. 3.3), the phylogenetic trees (Fig. 3.4 - 3.6), F_{ST} values (Table 3.6 - 3.8), and the hierarchical AMOVA. Within the southern hemisphere two genetically divergent groups were found: the southern Pacific (AUS, NZL and CH) and the southern-eastern Atlantic (SA). The F_{ST} values (Table 3.6 - 3.8), and the hierarchical AMOVA show high levels of genetic structuring. However, these groupings were less distinct in the topology of the haplotype networks (only separated by a single haplotype; Fig. 3.3) and not strongly supported in the phylogenetic trees (Fig. 3.4 - 3.6; except *RAG1*).

High haplotype diversity was found at the mitochondrial level in *S. lalandi* populations and is similar to values reported for other fish populations (*e.g.* Viñas *et al*., 2004; Zardoya *et al*., 2004). Theoretically, genetic variability in populations is positively correlated with the effective population size (Fuji and Nishida, 1997). Therefore, high levels of haplotype diversity suggest large, stable, effective population sizes. In contrast with the high haplotype diversity, the nucleotide diversity values were low in all *S. lalandi* populations. Similar values have been described for populations with historically small population sizes (*e.g.* Chilean jack mackerel,

Trachurus murphyi; Cárdenas *et al*., 2009). This pattern of high haplotype diversity and low nucleotide diversity is usually observed when genetic diversity that was reduced (for example as would occur in events such as population bottlenecks), is regained after a relatively recent population expansion (Grant and Bowen, 1998). This pattern has been observed in several marine species that experienced recent population growth (orange roughy, *Helicolenus dactylopterus*; Aboim *et al*., 2005; flame angelfish, *Centropyge loriculus*; Schultz *et al*., 2006; Atlantic whitebeaked dolphin, *Lagenorhynchus albirostris*; Banguera-Hinestroza *et al*., 2010; Cape hake, *Merluccius paradoxus*; von der Heyden *et al*., 2010b). This hypothesis of recent expansion in *S. lalandi* populations was supported by a star-like mtDNA genealogy, the haplotype networks, Tajima's *D* and Fu's *Fs* statistics and the mismatch distribution analyses. The star-like median networks are commonly found in marine species with recent population growth and high gene flow (Grant and Bowen, 1998; Beheregaray and Sunnucks, 2001; Bargelloni *et al*., 2003; Zardoya *et al*., 2004; Chow *et al*., 2009; Ravago-Gotanco and Juinio-Meñez, 2010). Interpreting neutrality tests result should be made with caution (Karl *et al*., 2012) because departures from neutrality may be due to background selection (Charlesworth *et al*., 1993), selective sweeps (Maynard-Smith and Haigh, 1974) or demographic expansion (Fu, 1997). In marine species, authors have favoured demographic expansion as an explanation for mitochondrial variation patterns found by these tests (Fauvelot *et al*., 2003; Bay *et al*., 2004; Craig *et al*., 2007; Chen *et al*., 2008). In *S. lalandi* populations these significantly negative values probably also indicate population growth, especially when interpreted in conjunction with the other analyses such as the mismatch distributions. Here, unimodally distributed populations were found and the statistical analysis confirmed the demographic expansion model (Rogers, 1995; Excoffier, 2004).

3.4.2 Contemporary biogeography and barriers to gene flow

Between the northern and southern Pacific populations it seems that the warmer waters of the equator could act as an important isolating mechanism for this subtropical species. Similar scenarios have also been found in other marine species (sardines, *Sardinops*, Bowen and Grant, 1997; lemon shark, *Negaprion brevirostris*, Schultz *et al*., 2008; tope shark, *Galeorhinus galeus*, Chabot and Allen, 2009; spiny dogfish, *Squalus acanthias*, Verissimo *et al*., 2010). Grant and Leslie (2001) found that two Old-World *Merluccius* clades spanning the equator included pairs of sister taxa that were probably separated by tropical waters. The Chilean- and Californian jack mackerels (*Trachurus symmetricus* and *T. murphyi*) are also closely related taxa (*Cytb* sequence divergence, 0.90%) that are separated by the equator (Poulin *et al*., 2004). The high level of genetic divergence detected across the equatorial Pacific for *S. lalandi* and other marine organisms suggest the warm equatorial waters seem to function as effective barrier to gene flow for species adapted to more temperate waters.

Within the North Pacific, marine fish are known to exhibit several intraspecific geographical groupings (Sato *et al*., 2004; Dodson *et al*., 2007; Liu *et al*., 2007). These groupings are generally explained by isolation in refugias and subsequent divergence during the Pleistocene glacial periods, with the East Pacific Barrier maintaining that isolation. The East Pacific Barrier, a 4 000 to 7 000km stretch of uninterrupted deep seawater between the central Pacific and the Americas, is an important oceanic barrier for several marine fish species (Ekman, 1953; Schultz *et al*., 2008) and has also been described as the greatest marine biogeographic obstacle (Ekman, 1953; Briggs, 1961; Grigg and Hey, 1992; Rocha *et al*., 2005; Lessios and Robertson, 2006). This is because of the origin of this barrier, possibly since the beginning of the Cenozoic (up to

65 MYA; Grigg and Hey, 1992) with no islands within the region that could serve as stepping stones for migration (Ekman, 1953; Briggs, 1974; Vermeij, 1978, 1987a, b; Veron, 1995; Robertson and Allen, 1996). Two species of the lemon shark (*Negaprion*), for example, are geographically separated by the East Pacific Barrier (Schultz *et al*., 2008). Although *S. lalandi* has great dispersal capabilities, this species can usually be found in the waters between the coast and continental self. Thus, once divergence between the two northern Pacific groups was established, the isolation was probably maintained by the East Pacific Barrier.

Results from the southern hemisphere *S. lalandi* populations show some differentiation between the Atlantic (SA) and the Pacific populations (NZL, AUS, CH) with no further genetic structure found within the southern Pacific populations (NZL, AUS, CH). Several studies have similarly found population differentiation between the Atlantic and Indo-Pacific populations of globally distributed species (Alvarado-Bremer *et al*., 1995, 1998; Graves and McDowell, 2003; Alvarado-Bremer *et al*., 2005). For example, genetic differentiation was found between the Atlantic and Indo-Pacific populations of bigeye tuna (*Thunnus obesus*; Chow *et al*., 2000); rafting crab (*Plagusia depressa*; Schubart *et al*., 2001); green turtle (*Chelonia mydas;* Bowen and Karl, 2007), and hawksbill turtle (*Eretmochelys imbricate*; Bowen and Karl, 2007). The connectivity between the eastern- and western southern Pacific observed in the current study can be explained by long-distance dispersal between these regions via the southern Pacific islands (Chin *et al*., 1991; Waters and Roy, 2004). The population structure of the southern hemisphere grouping will be further investigated in the Chapter 4 where the fine-scale population structure within the southern hemisphere and South Africa specifically was explored using six microsatellite markers.

3.4.3 Origin and phylogeography

The North-eastern Pacific group and North-western Pacific group, correspond with the eastern North Pacific- and western North Pacific biogeographical provinces, respectively described by Briggs and Bowen (2012, 2013), while the southern hemisphere group is distributed over several of the warm-temperate and cold-temperate provinces in the southern hemisphere. These authors discuss several hypotheses on how species in these regions originated (see Briggs and Bowen, 2012, 2013), however according to the dating analysis in the current study, events within the Pleistocene played an important role in shaping *S. lalandi* population structure.

Climatic changes during the Pleistocene generated several episodes of range expansions and contractions that influenced temperate species (Hewitt, 2000; Wares, 2002; Hewitt, 2004) resulting in rapid demographic growth (as seen in the results). The TMRCA results suggest that the four S. *lalandi* populations originated in the late Pleistocene period (about 115 000 - 500 000 years ago). These TMRCA estimates should however be interpreted with caution, because standard mutation rates for the *Cytb* and *COI* mitochondrial regions were used. As different mutation rates for these regions could have occurred, using standard mutation rates could have resulted in an error in the estimations. However, the fact that similar time estimations were generated for both genes with the 2% mutation rate suggests this may not be the case. Regardless, TMRCA results should be viewed as very conservative time frames in which the results can be interpreted.

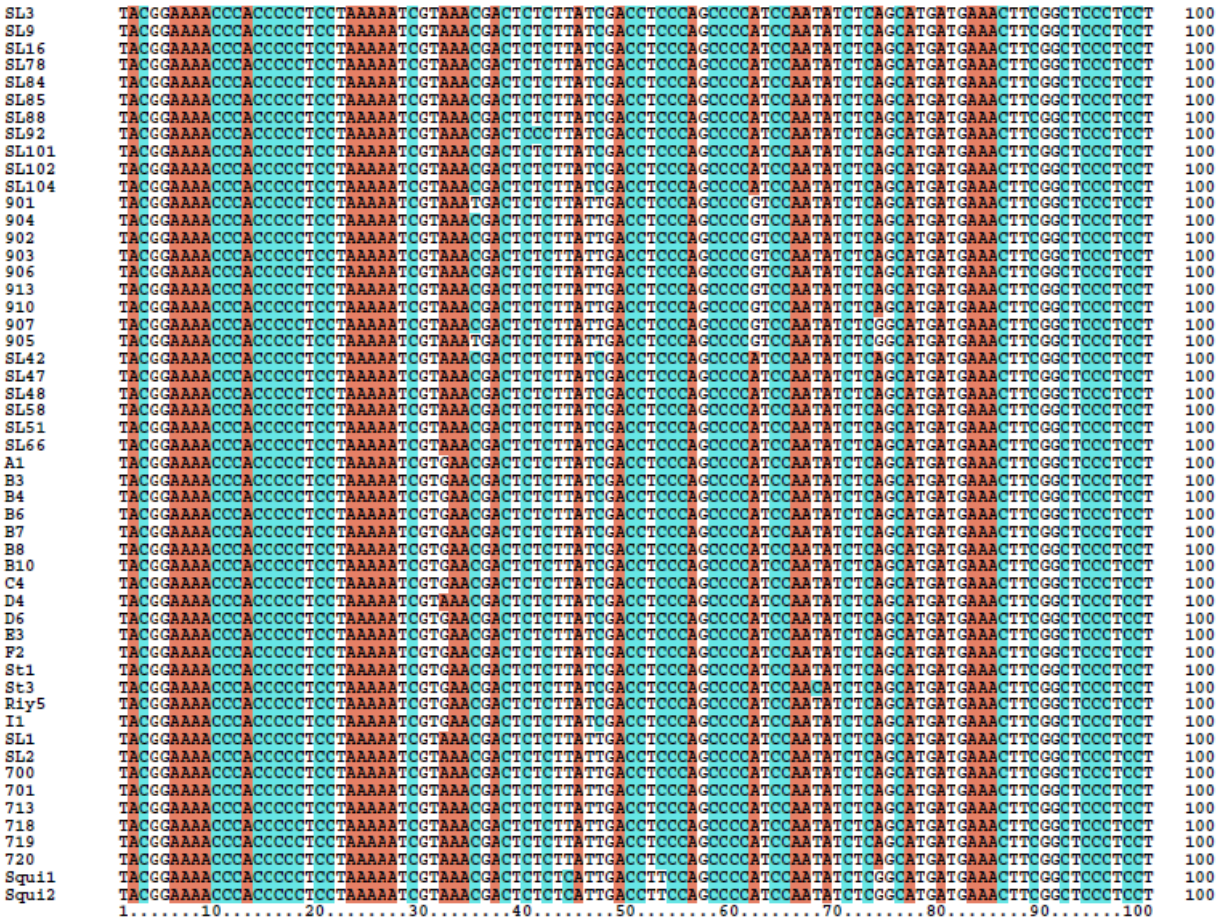
Seriola lalandi populations probably originated in the Pacific due to Pleistocene vicariant events. The results show a distinct break between the North- and South Pacific. The TMRCA results

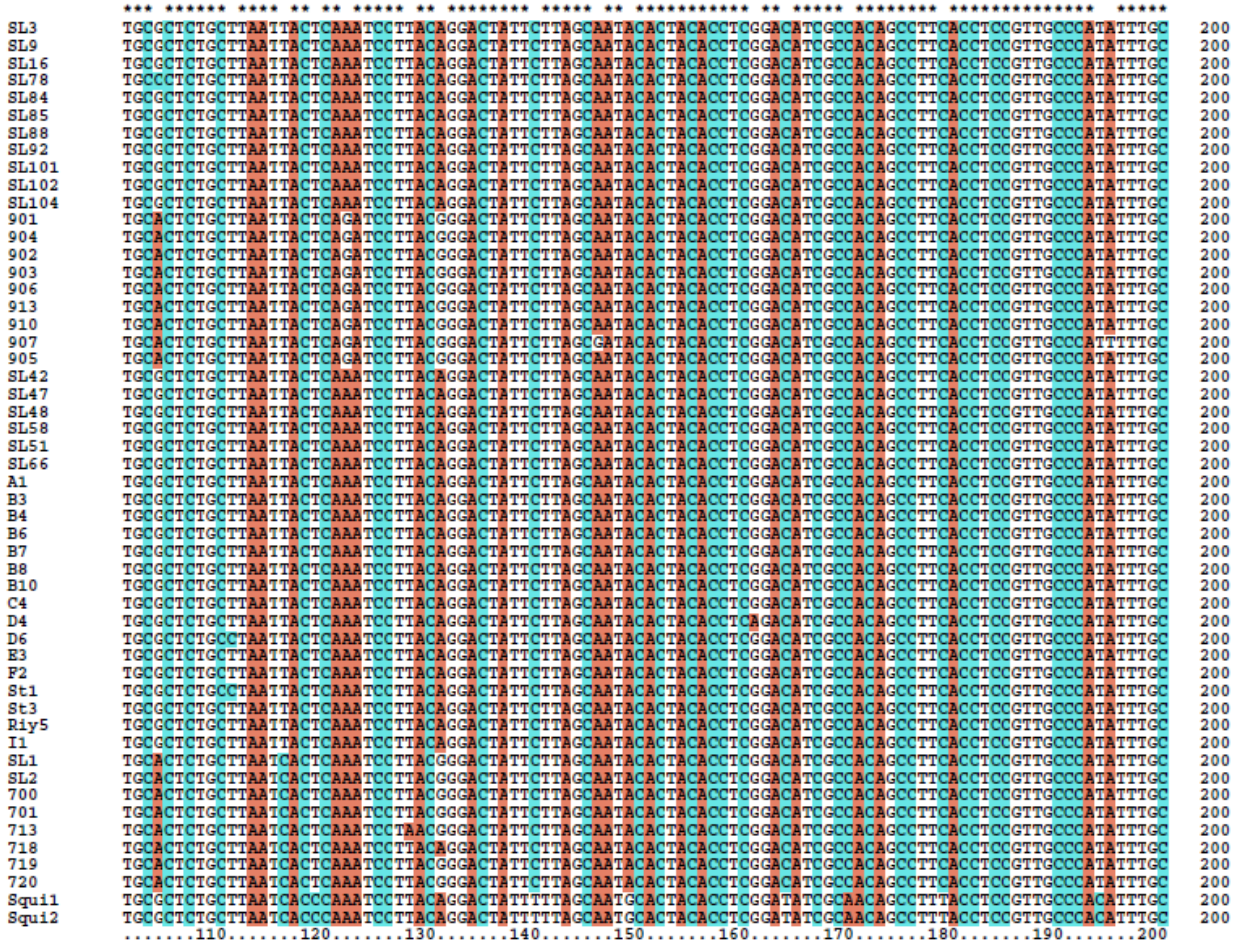
suggest that the common ancestor of the southern hemisphere groups originated around 2.28 - 4.5 MYA. A less pronounced latitudinal temperature gradient existed along the tropics until 2.6 MYA (Savin *et al*., 1975; Crame, 1993); this presented a less significant barrier to temperate organisms. Therefore, before the Pleistocene, the ancestral *S. lalandi* population could have been more widespread across equatorial regions. However, with repeated Pleistocene glacial- and interglacial periods and the establishment of a more pronounced latitudinal temperature gradient along the equator, the widespread ancestral *S. lalandi* population was then divided into two groups occupying the northern- and southern Pacific. Within the phylogenetic trees (*Cytb, RAG1* and combined) a prominent separation between northern and southern hemisphere clades is evident, suggesting the existence of these two groups. However, with in the *COI* phylogeny the North-eastern Pacific group (USA) is more closely related to the southern ocean group. This is probably the result of lineage sorting and not a true representative of the species (haplotype) tree. Within the North Pacific, the North-western Pacific group diverged first (JA = 197 700 - 449 700 years) from the northern ancestral *S. lalandi* population, with the North-eastern Pacific group being the most recently diverged (USA = 116 700 - 234 100 years). Intra-species divergence in the southern hemisphere seems to be somewhat older (SA = 217 100 - 432 700 years; South Pacific = 230 400 - 459 700 years). The late Pleistocene (the past one million years) was characterised by a number of large glacial-interglacial changes (Imbrie *et al*., 1992). Glaciated periods resulted in changes in sea levels, temperatures, salinity, ocean currents, upwelling patterns and prey distribution which in return affected the connectivity and isolation of populations (*e.g.* Costedoat *et al*., 2006; Harlin-Cognato *et al*., 2007). This led to a complicated and dynamic pattern of population colonisation and expansion within and between oceans and

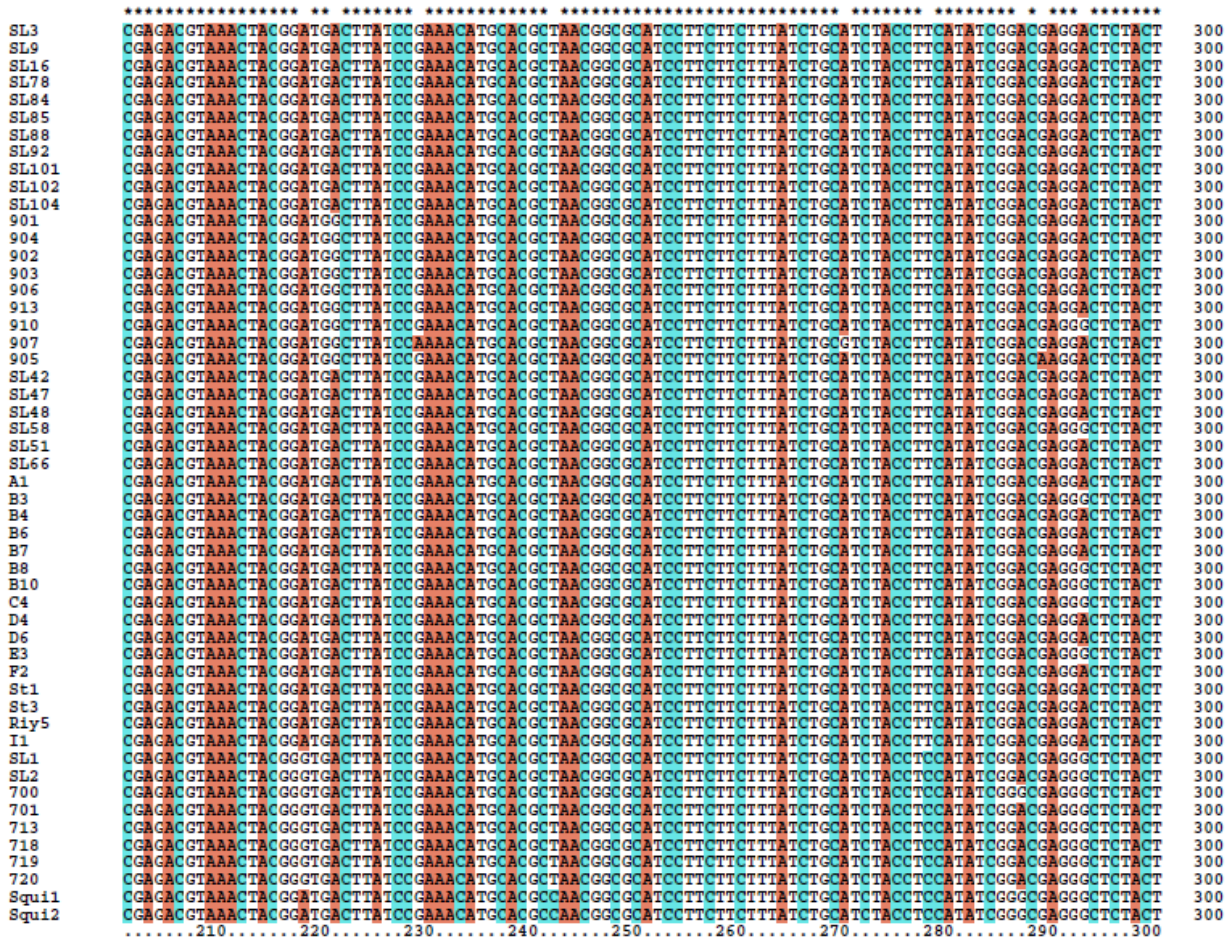
ocean basins. Therefore the divergence of the four *S. lalandi* groups could in part be explained by these oscillations in climate and ocean temperatures.

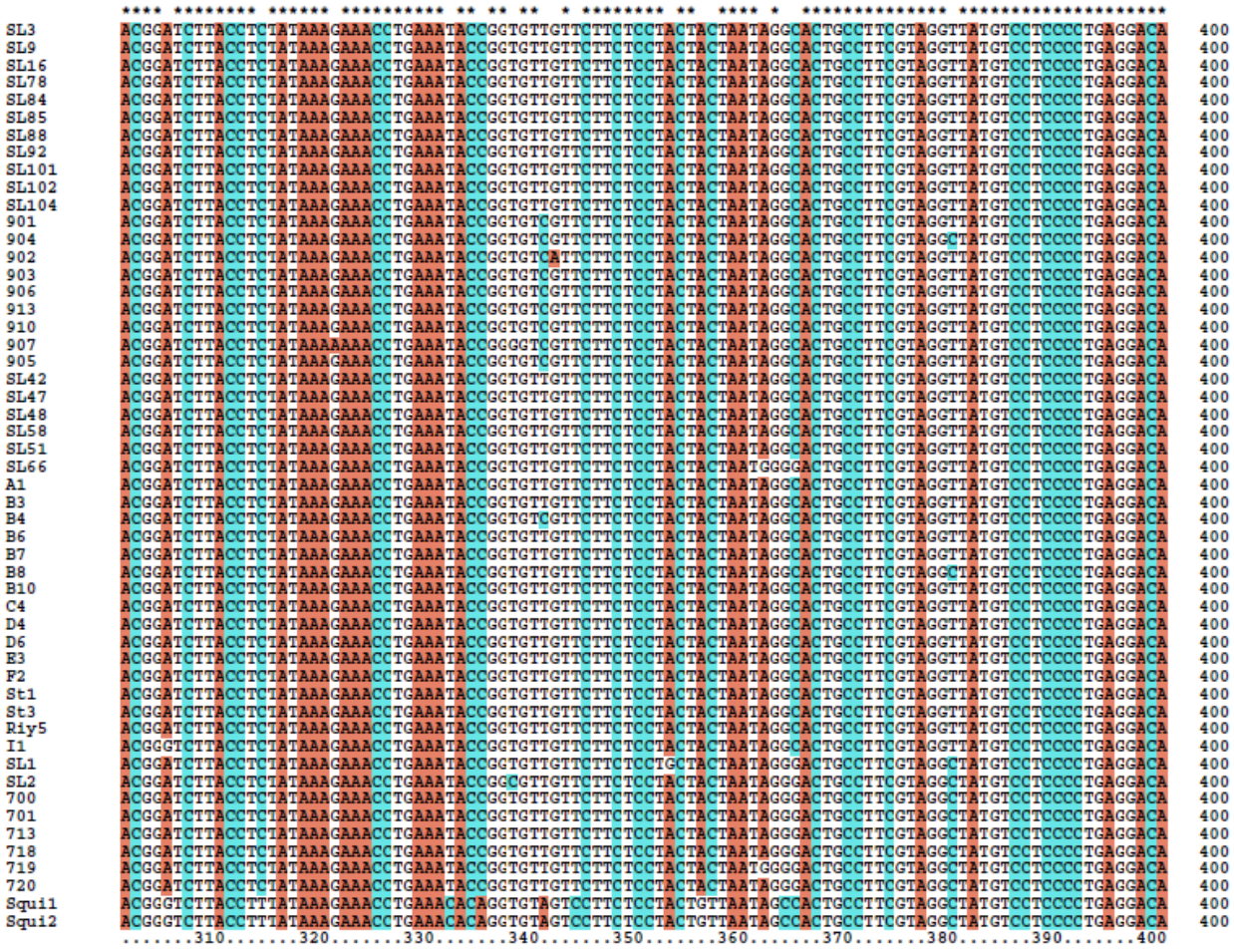
In conclusion, this study verified the existence of three genetically distinct groups in *S. lalandi* across its distribution range and postulated how Pleistocene glacial-interglacial changes played a major role in the formation of these three clades.

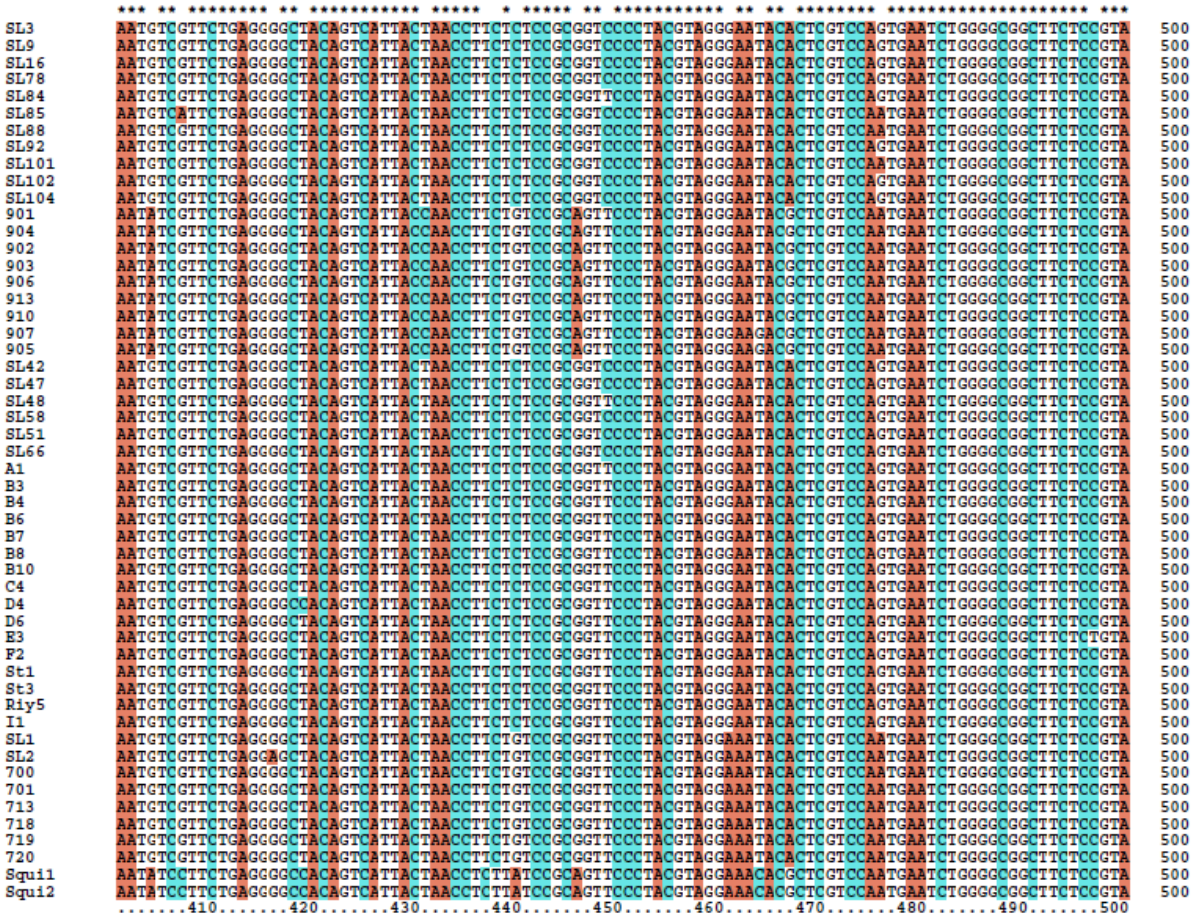
Appendix E: *Cytb* haplotype sequence alignments

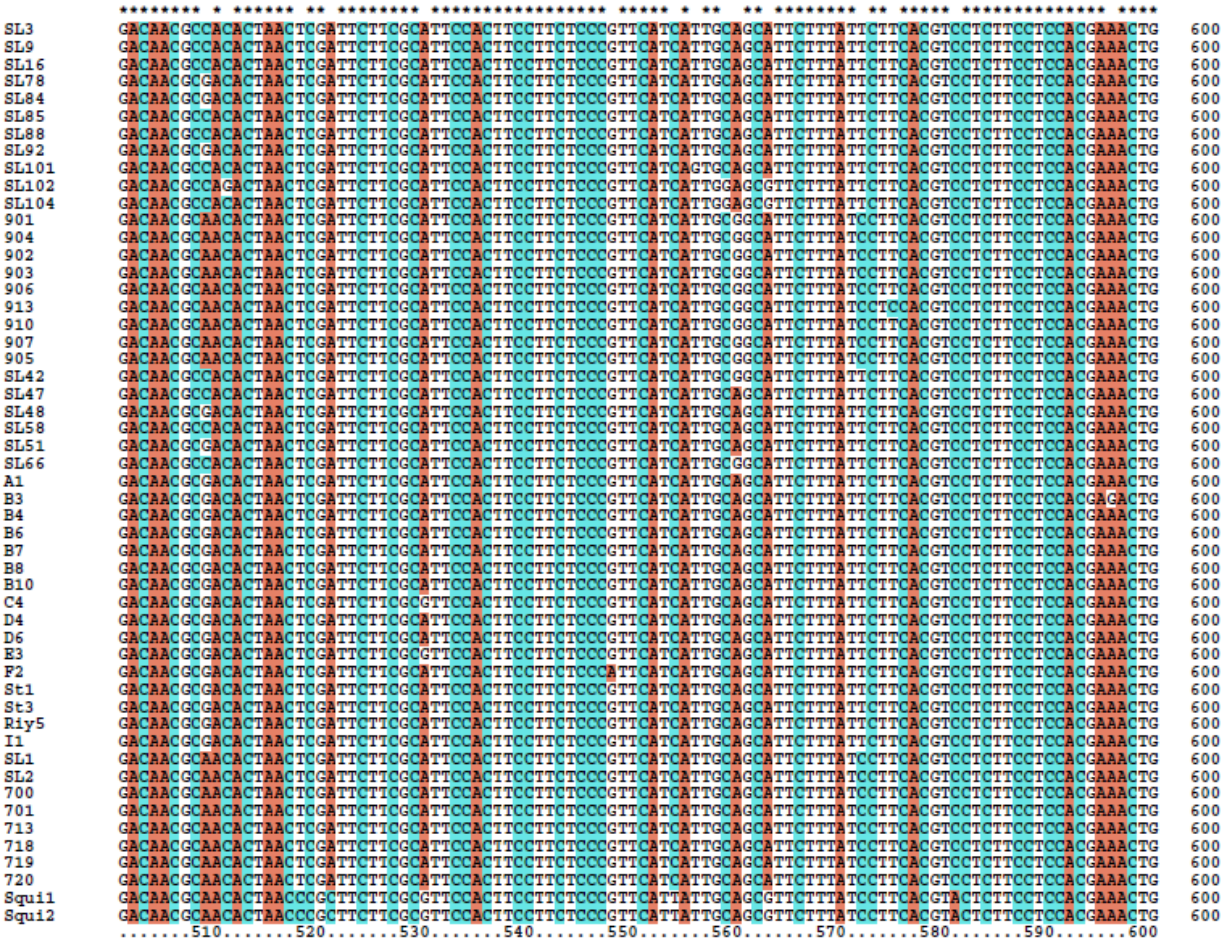


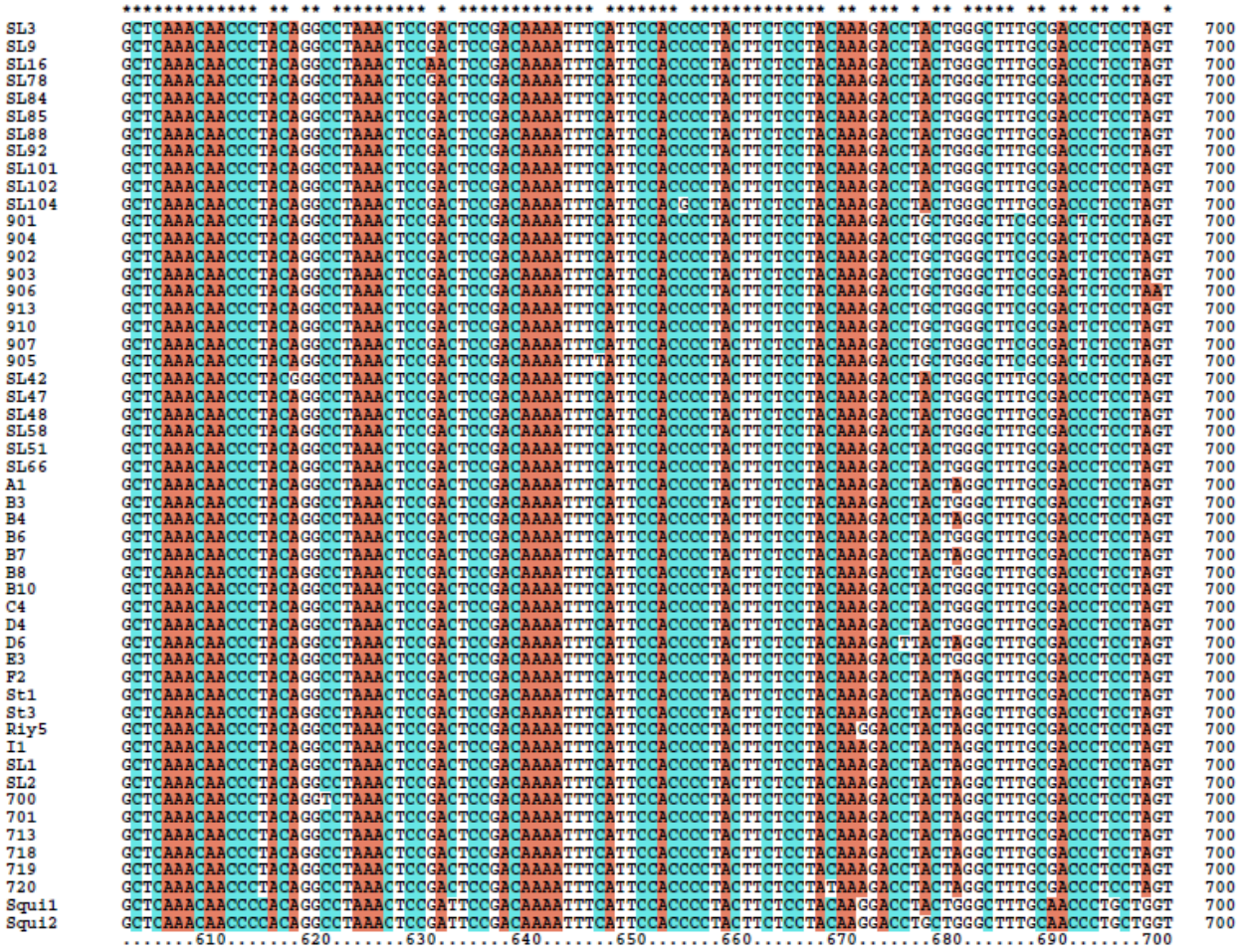


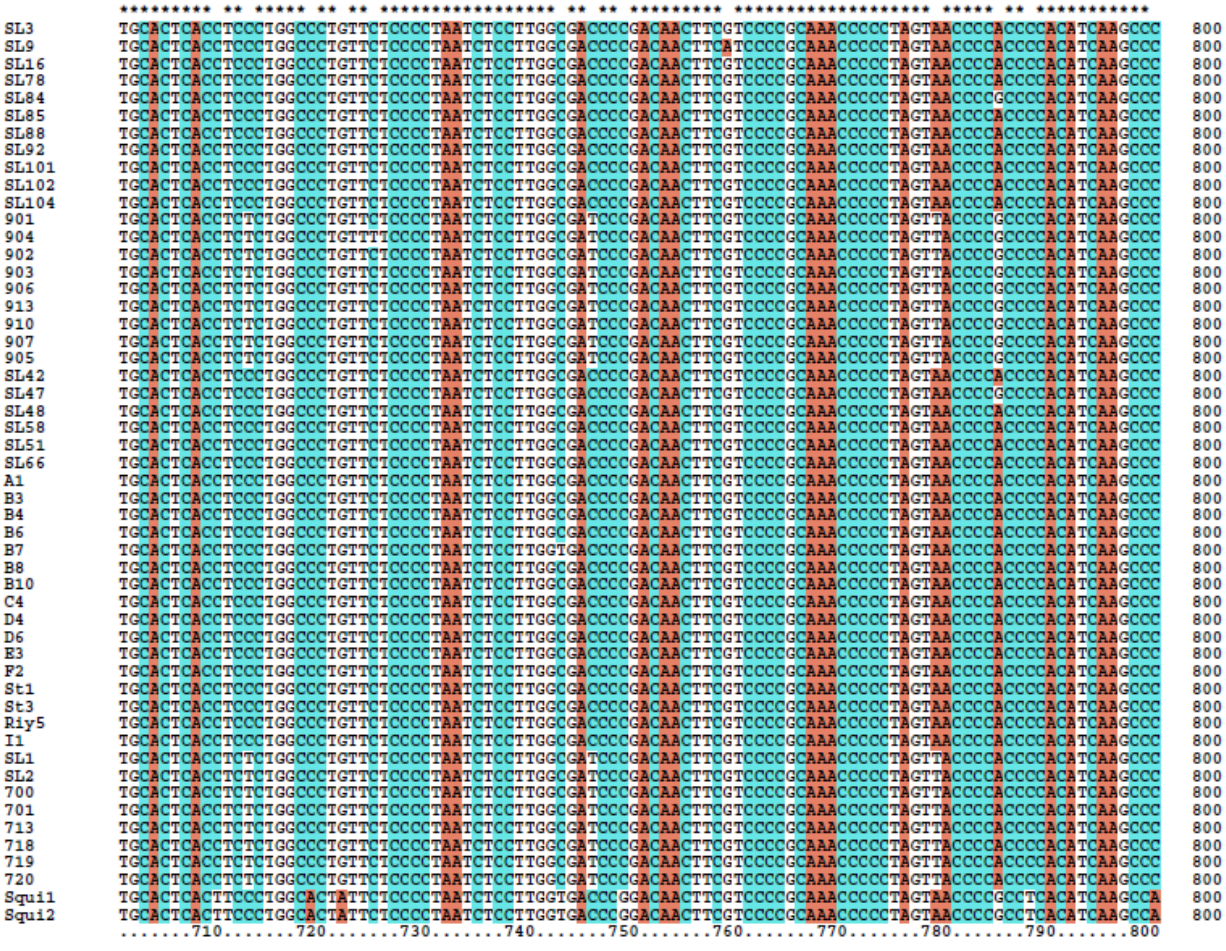




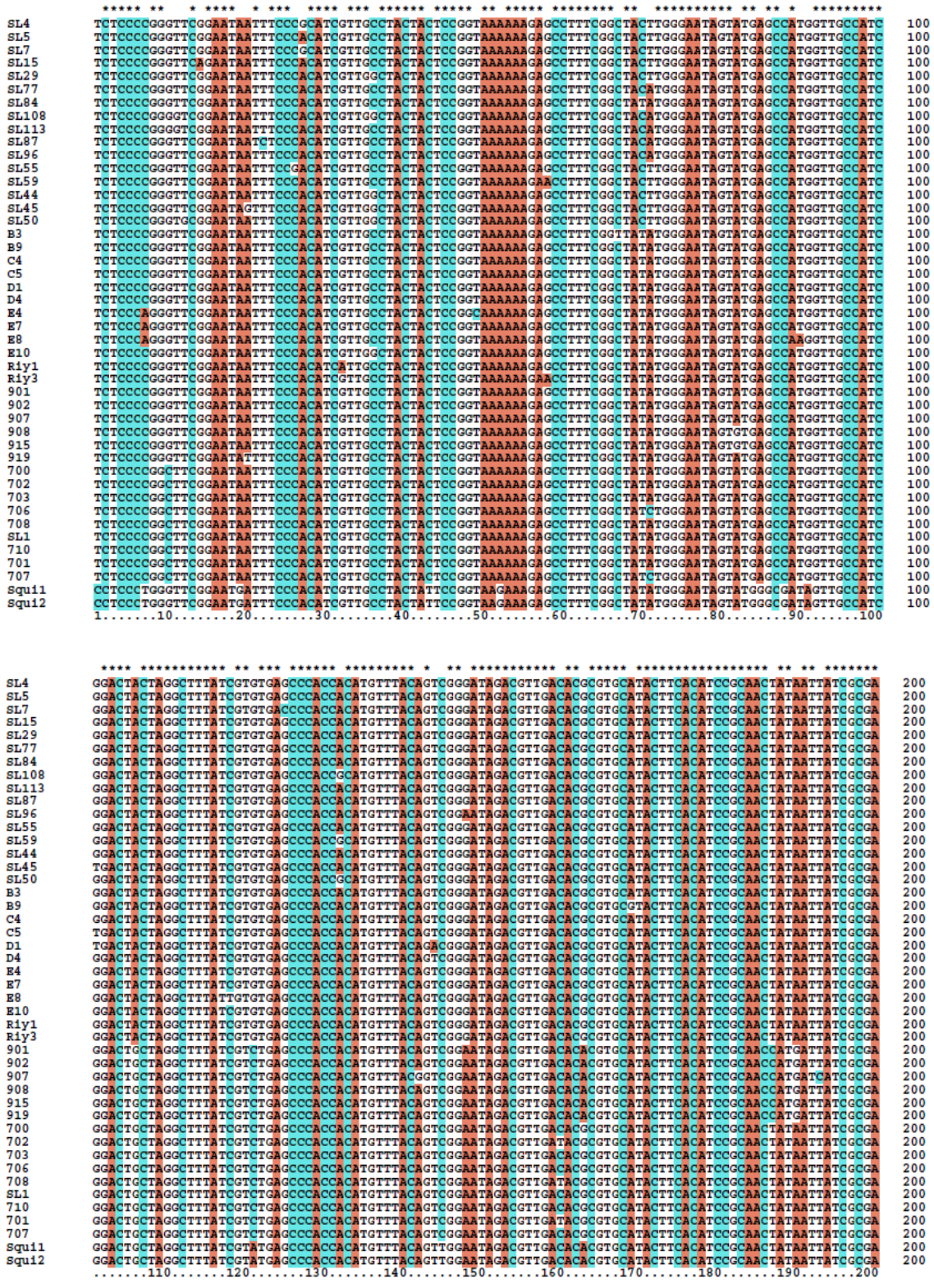


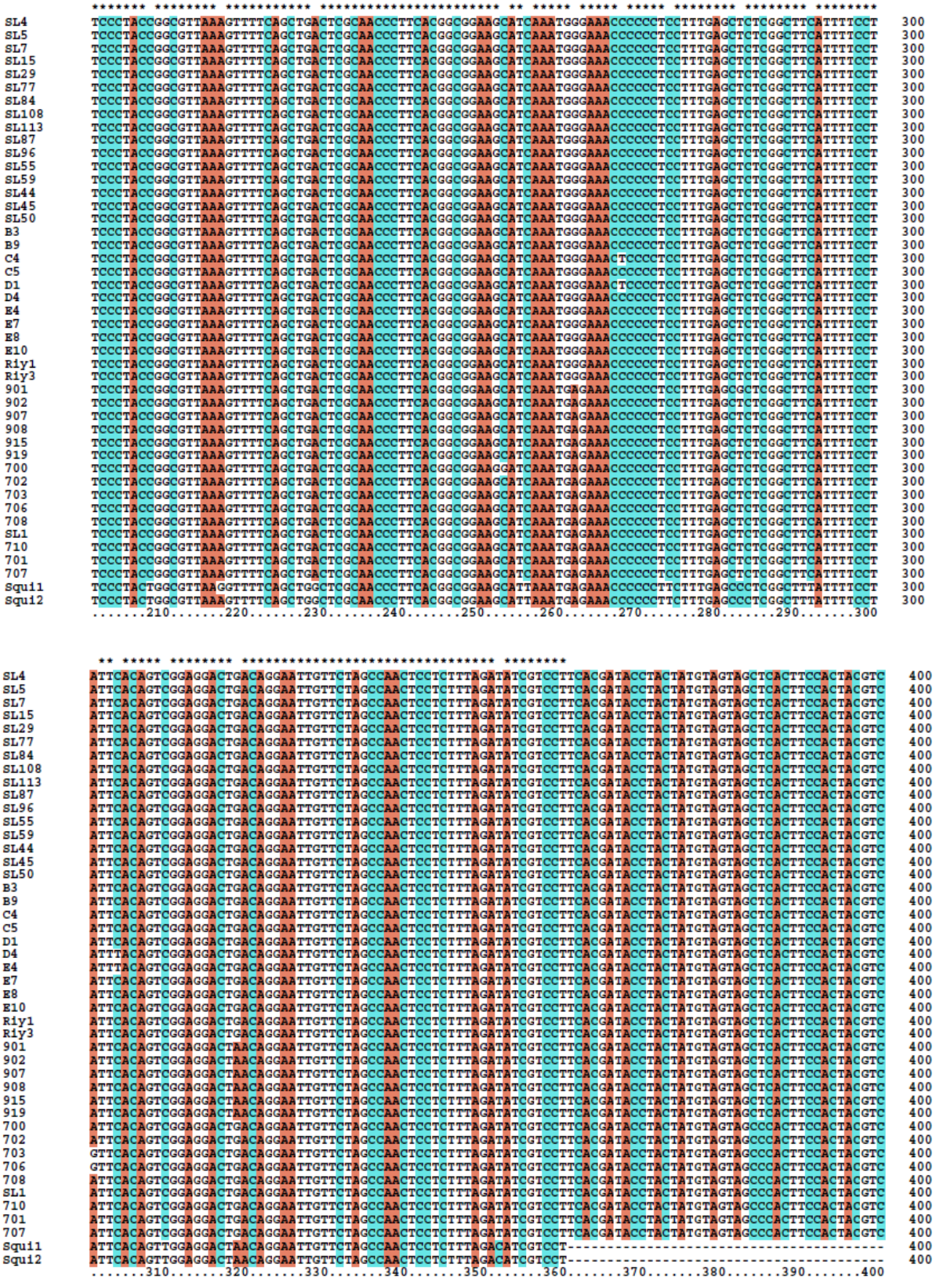


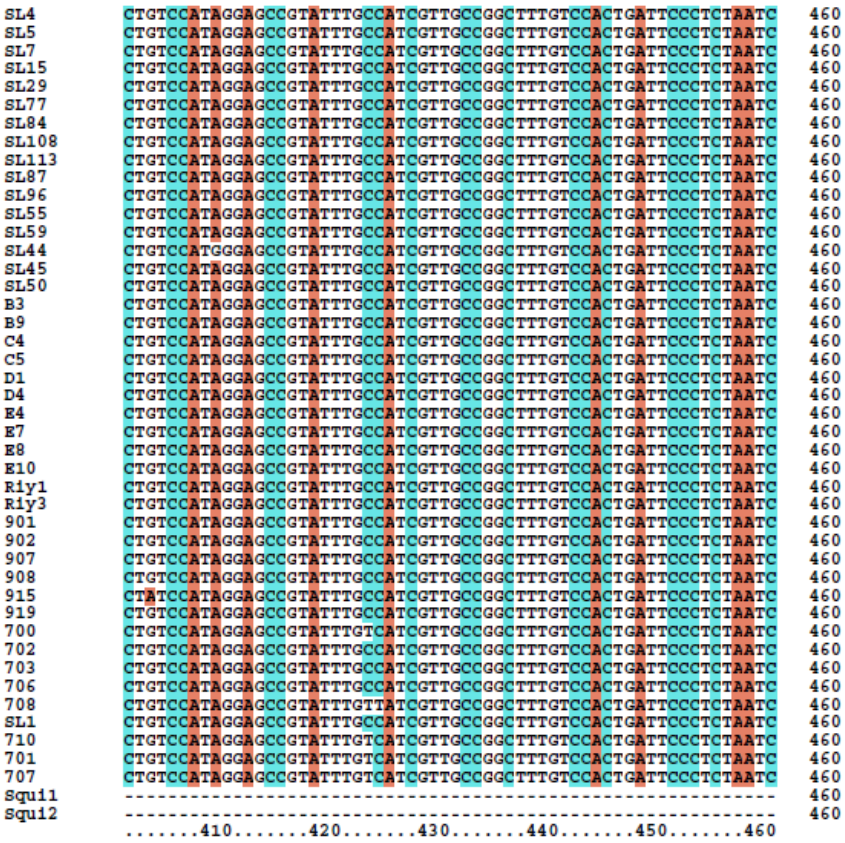




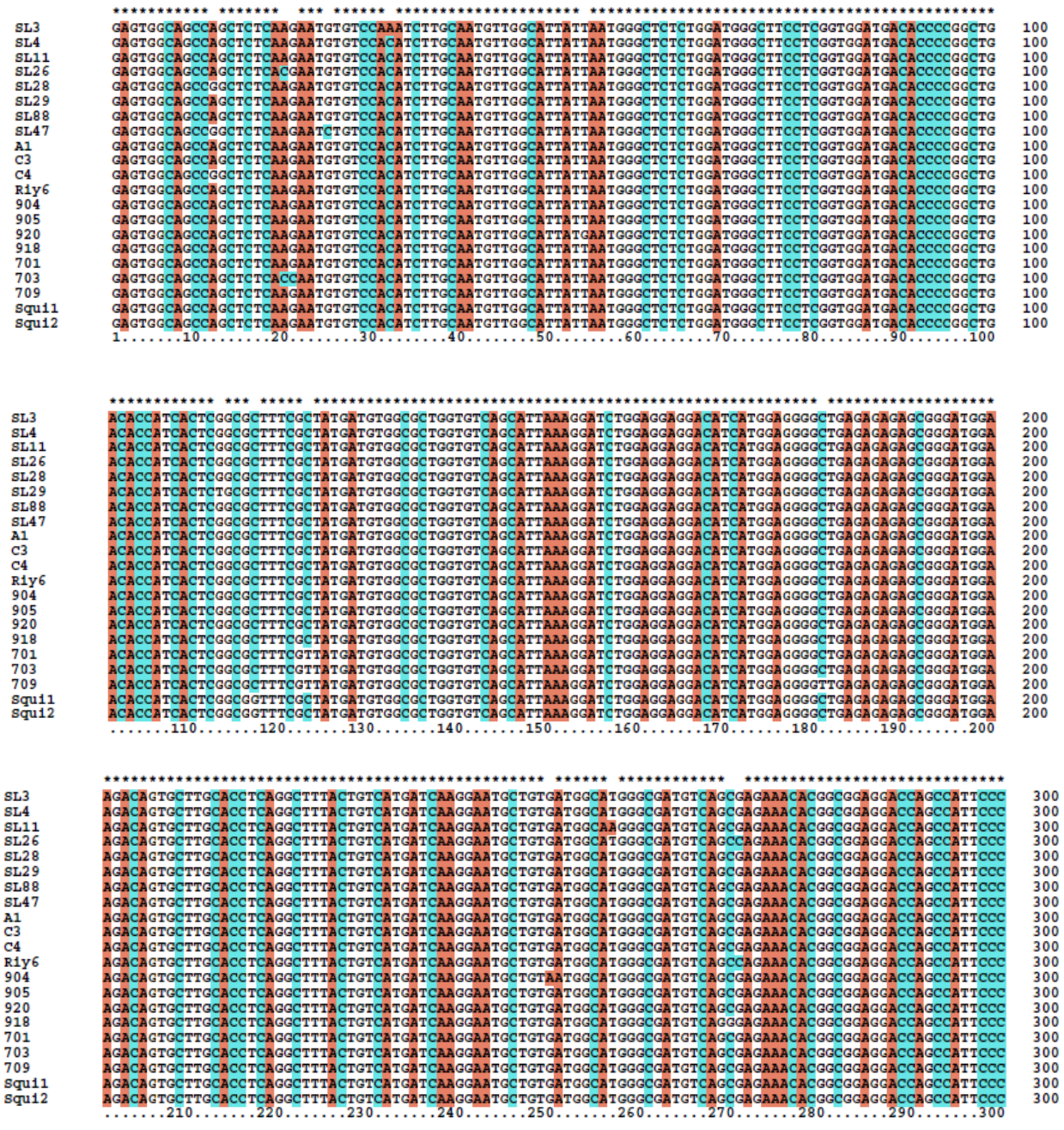
Appendix F: *COI* haplotype sequence alignments

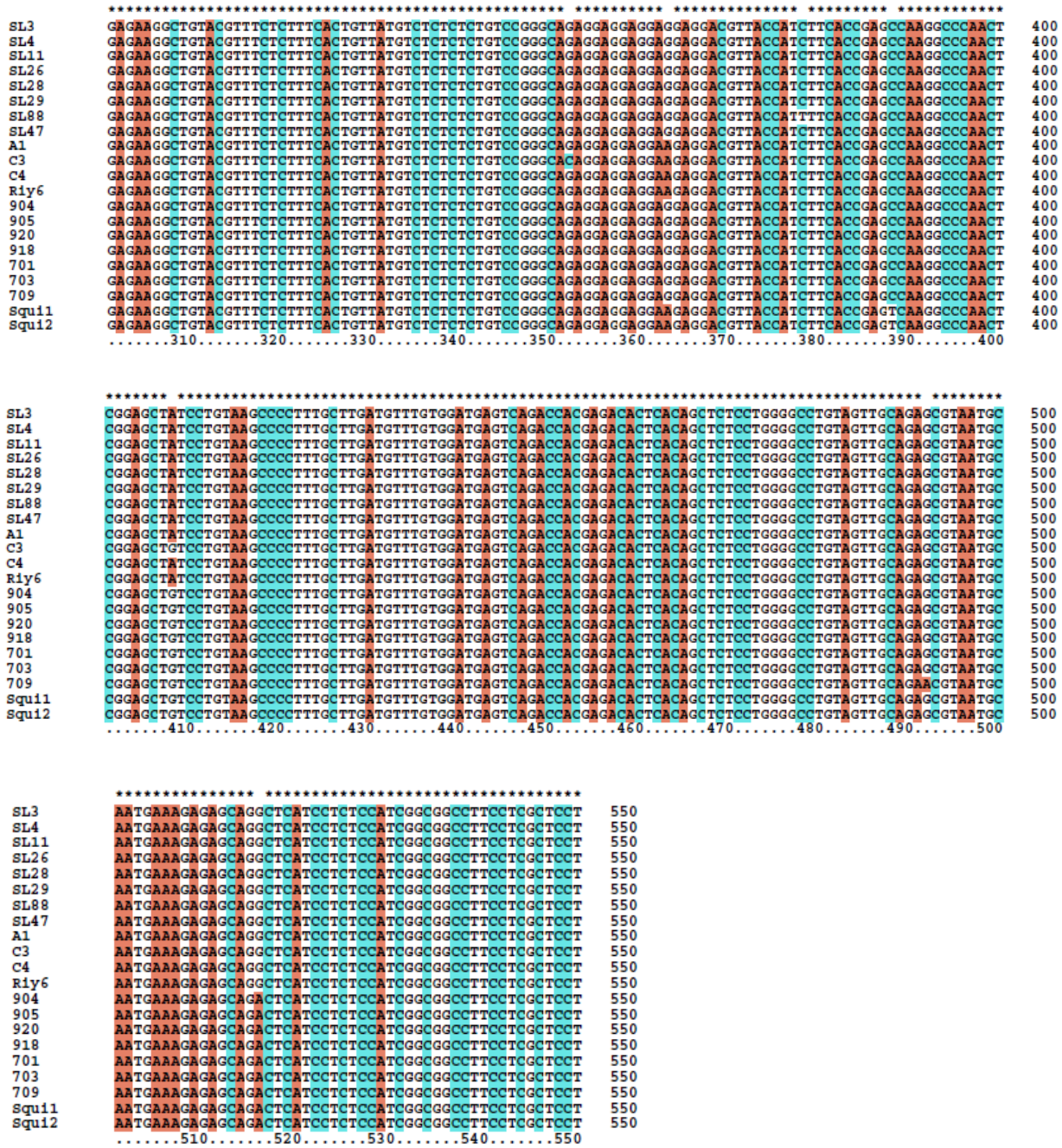






Appendix G: *RAG1* haplotype sequence alignments





Chapter 4: Population Structure of Yellowtail (*Seriola lalandi*) in the Southern Atlantic and Pacific Oceans, with Emphasis on the South African Coastline

4.1 Introduction

South African waters are home to a high diversity and abundance of marine species. This can be attributed to the biogeographic complexity of the region. Along the South African coastline two contrasting current systems can be found: the cold, northward flowing Benguela Current on the west coast and the warmer, southward moving Agulhas Current on the east coast (Fig. 4.1; Branch and Branch, 1995; Griffiths *et al*., 2010). The dynamic features of the south coast make it difficult if not impossible to locate the 'exact' point at which the two currents meet, although the contact zone is broadly believed to be between Cape Agulhas and the Cape Peninsula (Griffiths *et al*., 2010). The widening of the continental shelf (Agulhas Bank) beginning at Algoa Bay, causes the Agulhas Current to move offshore, and to retroflect (turns almost completely back on itself) at the southern tip of the continental shelf (Agulhas retroflection; Peterson and Stramma, 1991; Quartly and Srokosz, 1993; Stramma and Lutjeharms, 1997; Lutjeharms, 2006). In addition, as the Agulhas Current moves away from the coastline, circular currents (eddies) are formed along the south coast (Branch and Branch, 1995) whereas other eddies (called Agulhas rings) continue westwards and mix with the cooler Benguela waters (Lutjeharms, 2006).

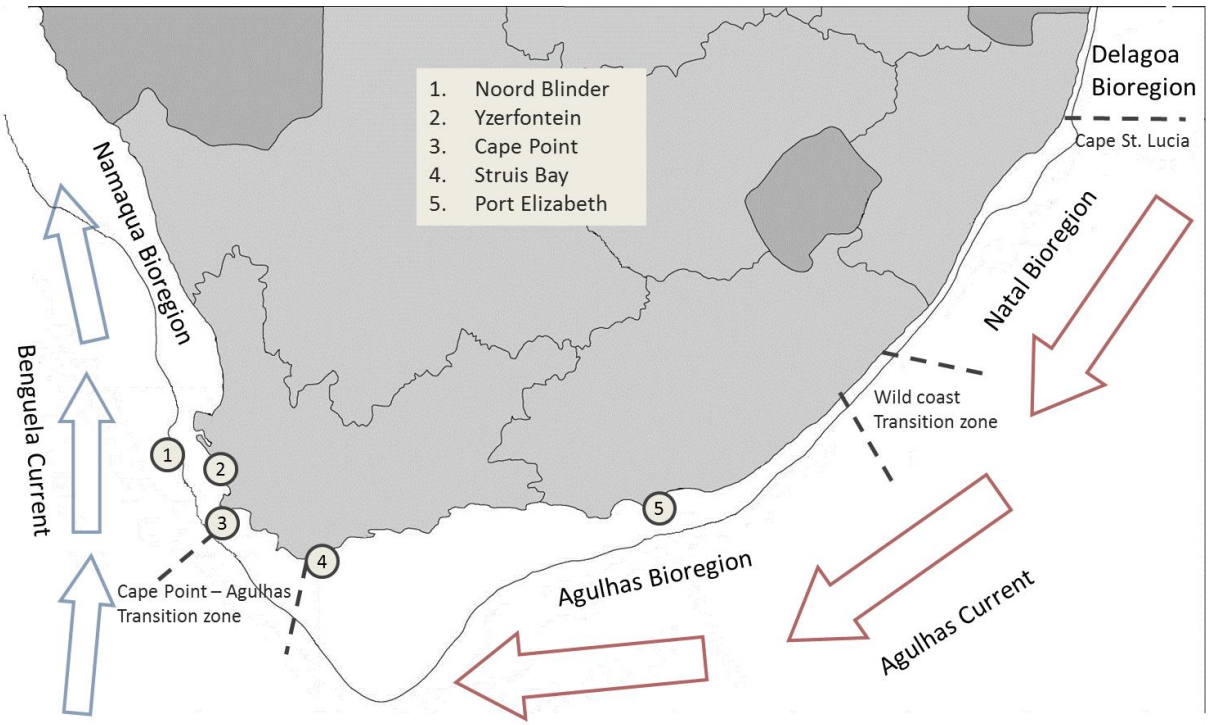


Fig. 4.1: Map of South Africa showing the major bioregions, potential genetic barriers and transition zones, major current systems, and position of the continental shelf break. Sampling localities are also indicated.

As a result of the dynamic oceanography, four major biogeographic provinces can be identified along the South African coastline: the tropical Delagoa Bioregion (extends southwards from Mozambique into KwaZulu-Natal); the subtropical Natal Bioregion (ranges from KwaZulu-Natal to east of Algoa Bay), the warm-temperate Agulhas Bioregion of the south coast (from Algoa Bay to Cape Point) and the cold-temperate Namaqua Bioregion of the west coast (from Cape Point to the Namibian border; Griffiths *et al*., 2010; Fig. 4.1). These provinces differ significantly in their abiotic and biotic make-up. Strikingly different faunal and floral composition has been observed between the east and west coasts of South Africa. However, due to the dynamic factors that influence the composition of the rest of the biogeographic bioregions, no defined boundaries has been identified between these regions; only transition regions.

Several barriers and transition zones have been identified along the coast of South Africa. The Cape Point region limits gene flow in marine species (for example brown mussel, *Perna perna*; Grant *et al*., 1992; bluntnose klipfish, *Clinus cottoides*; von der Heyden *et al*., 2008) from the west- and south coast. However within some marine species such as abalone (*Haliotis midae*; Evans *et al*., 2004; Bester-van der Merwe *et al*., 2011) and the bluntnose klipfish (von der Heyden *et al*., 2008), the Cape Agulhas region was identified as a barrier between the west- and south coast. The area between Cape Point and Cape Agulhas has been identified as a transition zone between the west- and south coast (Teske *et al*., 2013).

Several additional, less prominent, population genetic discontinuities can be found along the south and east coasts. One lies near Algoa Bay (Norton, 2005; Teske *et al*., 2006; von der Heyden *et al*., 2008) and one east of Port Alfred, in the region between Kenton on Sea and Haga Haga (Zardi *et al*., 2007). A last discontinuity can be found between Haga Haga and the Mkomazi Estuary (Teske *et al*., 2006).

While for various South African marine species these breaks have resulted in reduced gene flow; there are also several studies that found no population structuring along the South African coast. It is important to note that most of the species showing population structuring are intertidal or estuarine species while those species with high gene flow along the South African coast are mostly fish species (Grant and Leslie, 2005; Klopper, 2005; Oosthuizen, 2007; von der Heyden *et al*., 2007) or invertebrate species with a longer pelagic larval cycle (Oosthuizen *et al*., 2004; Tolley *et al*., 2005; Matthee *et al*., 2007). However von der Heyden *et al*. (2007), found significant population differentiation between South African and Namibian samples of the Cape

hake species, *Merluccius paradoxus* when examining the population structure of mature fish older than three and four years. Thus these barriers and paleo-oceanography events affect sympatrically distributed marine species differently; most probably depending on their life history and / or larval stages (Oosthuizen *et al*., 2004; Norton, 2005; Tolley *et al*., 2005; von der Heyden *et al*., 2007).

Within South Africa no information on population genetic structuring and -diversity is available / known for yellowtail, *Seriola lalandi*. Only two previous studies on this species have been published: a broad-scale genetic study showed significant differentiation between populations from Japan and those from Australia and New Zealand, with no differentiation between the Australian and the New Zealand populations (Nugroho *et al*., 2001). A more fine-scale study of *S. lalandi* along the coast of Australia, however found significant differentiation between the east- and west coast of Australia (Miller *et al*., 2011).

The aim of this chapter was to determine if genetically distinct populations of *S. lalandi* exist along the coast of South Africa. Two questions / hypotheses were considered: 1) *Seriola lalandi* is a panmictic population (*i.e.* genetically homogeneous); 2) *Seriola lalandi* comprise of multiple, genetically differentiated populations. Due to the high dispersal capabilities of *S. lalandi*, panmixia would be expected; as found in other linefish species. Despite this expectation, population structure has been found in several pelagic fish species (Martínez *et al*., 2005; Díaz-Jaimes *et al*., 2010; López *et al*., 2010). Furthermore, several studies have found restrictions to gene flow associated with the biogeographic regions along the coastline of South Africa for species that rely on larval dispersal (Evans *et al*., 2004; Bester-van der Merwe *et al*., 2011).

Although *S. lalandi* is a pelagic fish with potentially high dispersal capabilities, the same forces influencing these aforementioned species might also influence the South African yellowtail; thereby influencing population structure. The hypotheses were tested by investigating individuals from five localities along the South African coast with six microsatellite loci.

We further investigated the findings in Chapter 3 on the phylogeography of *S. lalandi* in the southern hemisphere. Based on sequence data (*COI*, *Cytb*, *RAG1*), individuals from the South Pacific (Australia, New Zealand, and Chile) were distinct from, but closely related to the South African individuals, with two genetically distinct groups observed. The same six microsatellite loci were used to further test the differentiation between the Atlantic Ocean (South Africa) and South Pacific samples.

4.2 Materials and methods

To investigate the population structure along the South African coastline a total of 201 samples was obtained from five regions (Port Elizabeth = 37, Struis Bay = 55, Cape Point = 50, Yzerfontein = 29, North Blinder = 30) ranging from south- to west coast (Fig. 4.1). Sample collection was conducted in the spawning season of *S. lalandi* from October to March in 2009, 2010, and 2011. For the three South Pacific regions a total of 118 samples (New Zealand - NZL = 35, Australia - AUS = 37, Chile - CH = 46) was obtained. All samples were preserved in 95% (v/v) ethanol and genomic DNA extractions were carried out using the methods described in chapter 2.

Six microsatellite markers previously published for *S. dumerili*: *Sdu10* (Renshaw *et al*., 2006), *Sdu29, Sdu32, Sdu46* (Renshaw *et al*., 2007), *SduCA107* and *SduCA4j* (Porta *et al*., 2009) were successfully amplified by polymerase chain reaction (PCR) and genotyped in all individuals (Table 4.1). Polymerase chain reactions were carried out in 15µl volumes comprising 0.1µM primer, 1X Go*Taq* buffer, 2.5mM $MgCl_2$, 0.5mM dNTPs, 0.5units Go*Taq* (Promega) and 15 - 20µg genomic DNA. Two PCR protocols were used in the amplification of loci. For *Sdu10, SduCA107* and *SduCA4j* the following PCR protocol was used: an initial denaturing step at 94°C for 3min, 35 cycles of denaturation at 94°C for 30s, annealing temperature (Renshaw *et al*., 2006; Porta *et al*., 2009) for 30s, extension at 72°C for 45s and a final extension step at 72°C for 5min. For *Sdu29, Sdu32* and *Sdu46* the thermal cycling conditions were: 2min at 94°C, followed by 34 cycles of 94°C for 30s, annealing (Renshaw *et al*., 2007) for 45s and extension at 68°C for 1min, with a final extension temperature of 68°C for 10min.

Table 4.1: Description of the six microsatellite loci used in the current study.

Locus	Primer name	Primer sequence (5'-3')	Repeat motif	Size (bp)	T_a (°C)
Sdu10	Sdu10-F	CCAAGTCCTCCTGCTACTACCAT			
	Sdu10-R	CCTTGTGGATGACCTGTTTG	$(CAA)_n$	295–346	56
Sdu29	Sdu29-F	CCTTGCCATACCGATGCCAG			
	Sdu29-R	GACTGCTCTGCCTGCTTGTTG	$(GA)_n$	311–377	60
Sdu32	Sdu32-F	CCTGTGAGAGCATTTGGTAT			
	Sdu32-R	GTGCTTGTCTCTTCTGTCAT	$(CA)_n$	99–177	53
Sdu46	Sdu46-F	GCAGTGTGAGCCATACATTAC			
	Sdu46-R	CTACAGGACAAAAGCCATT	$(GA)_n$	217–259	53
SduCA4j	SduCA4j-F	GTTGTTACTGGTGTGTAAGG			
	SduCA4j-R	AGTGGAAGTGGCTAGAGAGG	$(GT)_n$	149–175	61
SduCA107	SduCA107-F	TTCATCTGTTGAGTACATCC			
	SduCA107-R	CTGTTATCAGAACCTTCACC	$(GT)_n$	110–150	61

T_a = annealing temperature

Fragment analysis was done by capillary electrophoresis on the ABI 3100® automated sequencer (Applied Biosystems). Alleles were scored using MICROSATELIGHT v1 (Palero *et al*., 2011).

4.2.1 Data analyses

4.2.1.1 Population diversity

Genetic diversity estimates such as allele frequencies, observed- (*Ho*) and unbiased expected heterozygosity (*He*) were obtained with GENETIX v4.03 (Belkhir *et al*., 2000). FSTAT v2.9.3 (Goudet, 2001) was used to determine the number of alleles per locus and allelic richness based on a minimum sample size of 29. The inbreeding coefficient, F_{IS} was calculated by FSTAT and deviations from Hardy-Weinberg equilibrium (HWE) were tested with ARLEQUIN v3.01 (Excoffier *et al*., 2005). MICRO-CHECKER v2.2.3 (Shipley, 2003) was used to test for the presence of null alleles, genotyping errors or other reasons for deviations from HWE, such as heterozygote deficiency.

4.2.1.2 Population structure

Pairwise tests of F_{ST} and genetic distances were calculated with GENEPOP v4.0 (Rousset, 2007) by using 1 000 permutations. Pairwise tests of R_{ST} were calculated with ARLEQUIN (1 000 permutations). To visualise the ordination of populations along the factorial axes in a three-dimensional space, allele frequency data were subjected to Factorial correspondence analysis (FCA) available in the program GENETIX. Factorial correspondence analysis examines whether geographical distribution could be related to the relative distribution of individual genotypes on this three-dimensional scale. Component of correspondence analysis was calculated for 1) the entire data set to test for differences between the South African and South Pacific localities and

2) a partial data set containing only the South African localities. To test for population differentiation, hierarchial analysis of molecular variance (AMOVA) was carried out in ARLEQUIN. Different hypotheses were tested for genetic variation: 1) overall, 2) between South Africa and South Pacific, 3) between the samples of the west- and east coast of the South Pacific, 4) between the samples of the west- and south coast of South Africa, and 5) between three groups assuming barriers at Cape Point and Port Elizabeth for the South African samples.

Bayesian clustering analysis of populations was performed with STRUCTURE v2.1 (Pritchard *et al*., 2000). This method uses a Bayesian approach to generate the number of groupings that minimise the deviation from HWE and linkage disequilibrium by using multi-locus genotypes (such as microsatellites or SNPs) and a predefined number of clusters (*K*; Pritchard *et al*., 2000; Francois *et al*., 2006; Chen *et al*., 2007). Individuals are assigned to clusters based on their probability scores. For the analysis performed on the entire dataset, *K* was set between 1 and 9. For the South African sample only analysis *K* was tested from 1 to 6. For each *K*, 10 runs were performed consisting of 10 million MCMC iterations with a burn-in of 100 000. *K* was calculated assuming admixture and correlated allele frequencies. The statistic delta *K* (ΔK) was used to estimate the true number of clusters (Evanno *et al.*, 2005).For the selected *K* value, the individual membership coefficient was assessed to infer the probability of a certain individual belonging to a specific cluster. CLUMPP v1.1 (Jakobsson and Rosenberg, 2007) was used to average the runs and produce the admixture (Q) matrix. The cluster visualisation program DISTRUCT v1.1 (Rosenberg, 2004) was implemented to visualise the membership coefficients of the individuals within each population.

The program BARRIER v2.2 (Manni *et al*., 2004) was used to further explore the most likely area of restricted gene flow between South African populations. BARRIER identifies genetic barriers by implementing Monmonier's (1973) maximum difference algorithm and spatial coordinates. The coordinates for each individual was connected by Delauney triangulation to produce an associated distance for each connection. The genetic distances of Nei (1987) for each individual locus were analysed. Separate analysis per locus was done to ensure that strong differentiation at one locus did not cause bias to a certain barrier. In order to generate more validation for the barriers, the F_{ST}- and R_{ST} matrices were also included in the analysis. Robustness was assessed according to the number of different matrix sets supporting a particular barrier.

To determine long-term gene flow (migration rate) between the populations the program MIGRATE v3.3.2 (Beerli and Felsenstein, 2001) was used. MIGRATE is a coalescent-based program that implements the likelihood approach to calculate both population size θ ($4Ne\mu$; Ne = effective population size; μ = mutation rate) and migration rate M (m/μ; m = immigration rate; μ = mutation rate). Migration rates between populations within South Africa (5 sampling sites) and the South Pacific (3 sampling sites) were calculated separately. Migration rate between South Africa and the South Pacific was estimated by grouping the population data within these two areas. Runs consisted of 10 short and three long chains that were run for 500 and 5 000 sampled genealogies, respectively, following an adaptive heating scheme of four temperatures (1.0, 1.5, 3.0, 6.0). Convergence of chains was reached when a plateau was reached in likelihood values of chains.

To evaluate more recent migration rates between populations the program BAYESASS v3 (Wilson and Rannala, 2003) was used. This program implements a Bayesian approach with MCMC to calculate migration rates. The program was run with 8 million iterations, a burn-in period set at 2 million, and a sampling frequency of 1 000. Initial runs were performed to optimise the model parameters (allele frequency, migration rate and inbreeding coefficient). The model parameters were optimised using the acceptance rate for proposed change for each parameter. An acceptance rate between 20% and 40% was deemed to be optimal (Rannala, 2007). Ten runs, each starting at a different seed, were then performed with optimal model parameters for each dataset. The migration rates were calculated as mean values across those 10 runs.

4.3 Results

4.3.1 Genetic diversity

The population genetic variation for *S. lalandi* samples was characterised for five locations along the South African coastline. To further investigate population genetic variation in the southern hemisphere, three additional locations from the South Pacific were included (Australia, New Zealand and Chile). The average number of alleles per locus ranged from 2 (*Sdu10*) to 21 (*SduCA107*; Table 4.2). Allelic richness ranged from 2 (*Sdu10*) to 16.997 (*SduCA107*; Table 4.3), with similar average values for each putative population. Allele frequency plots for each microsatellite locus are shown in Appendix H. The amount of genetic variability, in terms of observed heterozygosity, was similar among sampling sites per microsatellite locus (Table 4.2). Among loci values of average observed heterozygosity per locus ranged from *Ho* = 0.4167at locus *Sdu46* to *Ho* = 1.000 at locus *SduCA4j*. Overall, the average observed heterozygosity per

locus and population was high (*Ho* = 0.722). Three loci conformed to Hardy-Weinberg expectations over all populations (*Sdu10*, *Sdu46* and *SduCA4j*). According to MICRO-CHECKER (Table 4.3) only two loci showed evidence of null alleles (*Sdu29* and *SduCA4j*).

Table 4.2: Summary of genetic variation for six analysed microsatellite loci at eight *Seriola lalandi* sampling sites.

Samples		Loci						
		Sdu10	*SduCA107*	*Sdu29*	*Sdu32*	*Sdu46*	*SduCA4j*	Mean
North	*He*	0.4753	0.8978	0.8377	0.8491	0.4561	0.8068	0.7205
Blinder	*Ho*	0.5556	0.8148	0.6552	0.8462	0.4444	0.6071	0.6539
(B)	*Na*	2	17	10	14	5	7	
N = 30	*Ar*	2.000	15.398	9.035	12.550	4.918	6.921	
	F_{IS}	-0.150	0.111	0.235	0.023	0.044	0.264	0.111
Yzerfontein	*He*	0.4898	0.8937	0.7997	0.8025	0.4297	0.8469	0.7104
(C)	*Ho*	0.4286	0.7778	0.5357	0.6500	0.4167	0.8571	0.6110
N = 29	*Na*	2	15	8	10	4	12	
	Ar	2.000	13.727	7.553	10.000	3.950	11.153	
	F_{IS}	0.143	0.148	0.346	0.215	0.052	0.006	0.160
Cape Point	*He*	0.4231	0.8869	0.8546	0.8629	0.5826	0.7689	0.7298
(E)	*Ho*	0.4615	0.8367	0.6800	0.8182	0.5278	0.9787	0.7172
N = 50	*Na*	3	20	15	17	6	6	
	Ar	2.513	13.718	11.162	13.433	5.437	5.655	
	F_{IS}	-0.078	0.067	0.214	0.063	0.108	-0.263	0.029
Struis Bay	*He*	0.4783	0.8946	0.8566	0.8350	0.6234	0.8178	0.7510
(F)	*Ho*	0.6667	0.7551	0.8400	0.8200	0.6000	0.9600	0.7736
N = 55	*Na*	2	18	16	16	6	9	
	Ar	2.000	13.169	11.472	11.649	5.479	7.842	
	F_{IS}	-0.385	0.166	0.029	0.028	0.050	-0.164	-0.020
Port Elizabeth	*He*	0.5019	0.8885	0.8355	0.8484	0.5696	0.8333	0.7462
(G)	*Ho*	0.4167	0.8056	0.9714	0.7778	0.6400	0.8485	0.7433
N = 37	*Na*	3	20	13	16	5	9	
	Ar	2.806	15.267	11.134	12.932	4.800	8.848	

	F_{IS}	0.184	0.107	-0.149	0.097	-0.103	-0.003	0.019
Australia	*He*	0.4824	0.9183	0.8535	0.8984	0.6812	0.8177	0.7753
(AUS)	*Ho*	0.5000	0.8710	0.8286	0.8286	0.7692	0.9697	0.7945
N = 35	*Na*	6	21	14	17	4	9	
	Ar	5.065	16.997	11.157	14.417	3.998	7.666	
	F_{IS}	-0.021	0.068	0.044	0.092	-0.110	-0.171	-0.009
New Zealand	*He*	0.5220	0.8711	0.8188	0.8704	0.6365	0.7915	0.7517
(NZL)	*Ho*	0.5556	0.7500	0.7714	0.6667	0.6786	1.0000	0.7370
N = 36	*Na*	6	17	11	15	6	7	
	Ar	4.584	14.118	9.212	12.589	5.143	6.498	
	F_{IS}	-0.050	0.153	0.072	0.247	-0.048	-0.249	0.034
Chile	*He*	0.4931	0.8113	0.8123	0.9062	0.7410	0.8261	0.7650
(CH)	*Ho*	0.4865	0.6923	0.7647	0.8500	0.6970	0.9714	0.7436
N = 40	*Na*	5	15	10	16	8	9	
	Ar	4.241	12.070	9.075	13.575	6.705	8.030	
	F_{IS}	0.027	0.159	0.073	0.075	0.075	-0.162	0.042
Overall	*He*	0.539	0.907	0.877	0.908	0.733	0.882	0.808
	Ho	0.509	0.788	0.756	0.782	0.597	0.899	0.722
	Na	11	32	21	25	9	14	
	Ar	3.380	14.844	11.711	14.020	6.233	9.938	
	F	-0.055	0.122	0.101	0.097	0.015	-0.112	0.038
	PHWE	0.860	0.000	0.000	0.000	0.383	0.999	

Ho = observed heterozygosity; *He* = expected heterozygosity; *Na* = number of alleles; *Ar* = allelic richness, F_{IS} = inbreeding coefficient , *F* = F_{IS} of Weir and Cockerham (1984); PHWE = probability of Hardy-Weinberg equilibrium.

Table 4.3: Estimates for null allele frequencies based on MICRO-CHECKER v2.2.3 (Shipley, 2003).

Locus	Null Present	van Oosterhout	Chakraborty	Brookfield 1	Brookfield 2
Sdu10	No	-0.0893	-0.0778	-0.0544	0.2136
SduCA107	No	0.05	0.0485	0.0437	0.2054
Sdu29	Yes	0.1098	0.1223	0.0993	0.1655
Sdu32	No	-0.0005	0.0017	0.0016	0.2201
Sdu46	No	0.0433	0.0129	0.008	0.2515
SduCA4j	Yes	0.1185	0.1412	0.1105	0.2298

4.3.2 Population differentiation

Pairwise genetic distances and F_{ST} values between the five South African sampling sites were relatively low (Table 4.4, 4.5), with the lowest between Cape Point, Struis Bay and Port Elizabeth. The F_{ST} values were not significant between these three sites (Table 4.5). However, significant pairwise F_{ST} values were obtained between the remaining two South African sampling sites (North Blinder and Yzerfontein). Overall large genetic distances and significant F_{ST} values were found between sampling sites from South Africa and the three sampling sites in

Table 4.4: Genetic distances between the eight *Seriola lalandi* sampling localities for all loci combined.

	North Blinder	Yzerfontein	Cape Point	Struis Bay	Port Elizabeth	AUS	NZL
North Blinder	-						
Yzerfontein	0.069	-					
Cape Point	0.026	0.105	-				
Struis Bay	0.016	0.077	0.018	-			
Port Elizabeth	0.027	0.046	0.013	0.016	-		
AUS	0.631	0.575	0.636	0.524	0.529	-	
NZL	0.648	0.581	0.630	0.555	0.526	0.002	-
CH	0.584	0.535	0.579	0.512	0.486	0.009	0.006

Table 4.5: Pairwise F_{ST} (1 000 permutations; above diagonal) and R_{ST} (1 000 permutations; below diagonal) values of eight *Seriola lalandi* populations.

	North Blinder	Yzerfontein	Cape Point	Struis Bay	Port Elizabeth	AUS	NZL	CH
North Blinder	-	0.023*	0.015*	0.010*	0.018*	0103*	0.114*	0.102*
Yzerfontein	0.013	-	0.038*	0.029*	0.026*	0.099*	0.107*	0.099*
Cape Point	-0.011	0.003	-	0.007	0.009	0.093*	0.103*	0.093*
Struis Bay	0.039	0.018	0.025	-	0.005	0.078*	0.091*	0.081*
Port Elizabeth	-0.009	0.017	-0.006	0.018	-	0.080*	0.084*	0.078*
AUS	0.092	0.121	0.102	0.109	0.058	-	0.003	0.006
NZL	0.158	0.167	0.156	0.150	0.113	0.005	-	0.003
CH	0.140	0.156	0.138	0.130	0.091	-0.004	-0.008	-

*Significant F_{ST} *p*-values (P <0.05)

the South Pacific (Table 4.4, 4.5). Within the South Pacific F_{ST} values were low and not significant (Table 4.5). The lowest genetic distance observed in the current study was found between AUS and NZL (Table 4.4) suggesting high gene flow between these regions.

AMOVA (Table 4.6) revealed overall significant genetic structuring between South Africa and the South Pacific samples (F_{ST} = 0.090 p = 0.000). Most of the total genetic variance was found between individuals within population (92.66%) while very little variance was distributed among populations within groups (1.13%). An AMOVA performed between the west coast sampling sites (B, C) and the rest of the South African samples (E, F and G) revealed weak, but significant population differentiation (F_{ST} = 0.021, p = 0.000). Variance was mostly distributed between individuals within populations (97.92%) with very little variance distributed among populations within groups (1.13%). All other structuring between areas across different biogeographical breaks tested for the South African samples were not significant.

Table 4.6: AMOVA results for *Seriola lalandi*. Analyses were performed under various hypotheses of regional genetic structure.

Hypothesised structure	**Among regions**			**Among populations within regions**			**Within populations**		
	d.f.	%var	F_{CT}	d.f.	%var	F_{SC}	d.f.	%var	F_{ST}
All populations									
South Africa – South Pacific	1	0.215	0.082*	6	0.030	0.123*	616	2.38	0.090*
South Africa									
West (B+C) – South (E+F+G)	1	1.00	0.010	3	1.08	0.011*	397	97.92	0.021*
South Pacific									
West (AUS/NZL) – East (CH)	1	0.14	0.001	1	0.31	0.003	219	99.55	0.004

d.f. = degrees of freedom, %var = percentage of variance, *significant F-statistics p-values

The FCA, which allows a three-dimensional view of the genetic relationship among populations, showed a strong separation between individuals from South Africa and those from the South Pacific (Fig. 4.2) with very little overlap between individuals from South Africa and those from the South Pacific population, with most variation seen on the first axis. Although the FCA performed only on the South African genotypes showed overlap of west- and south coast individuals, the distribution along the first axis indicated a clear separation of the west coast individuals from the rest (Fig. 4.3).

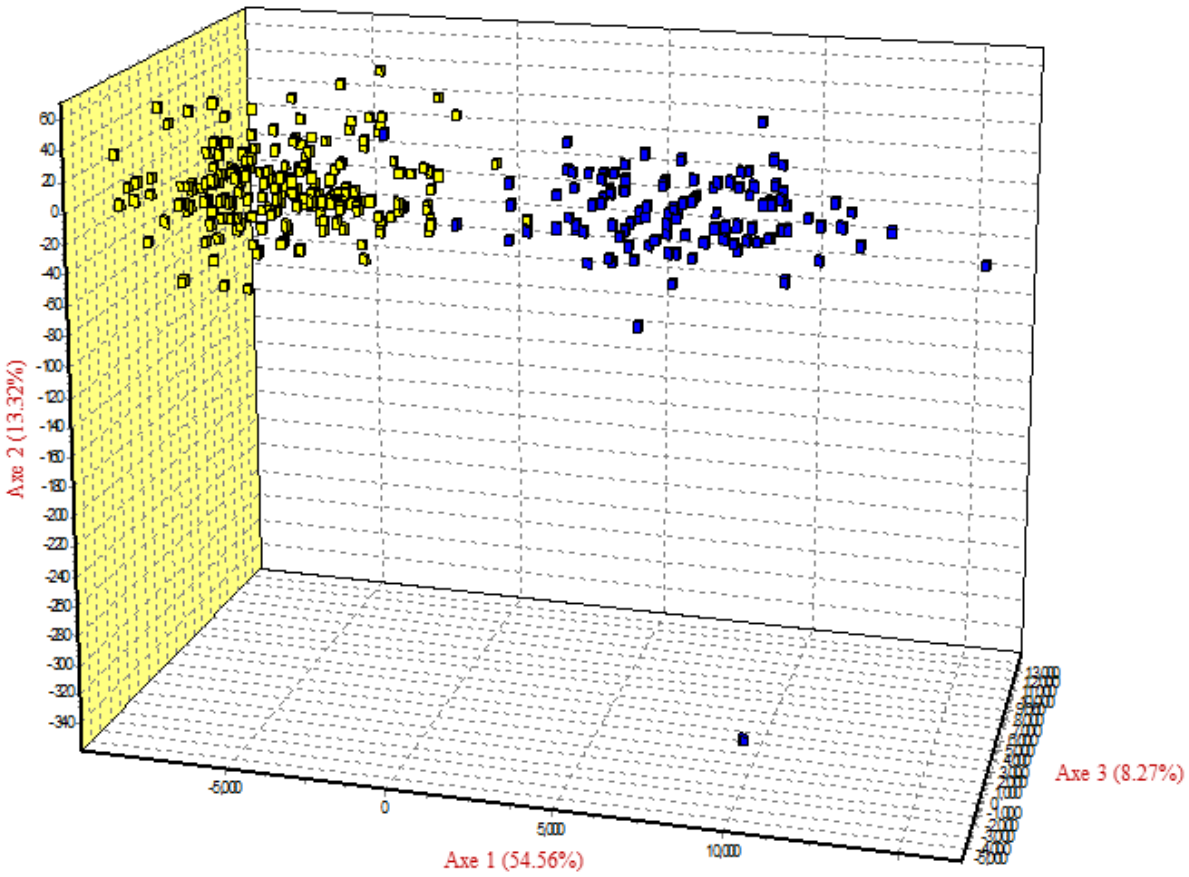


Fig. 4.2: Factorial correspondence analysis of all *Seriola lalandi* samples: yellow squares are South African samples and blue squares are South Pacific samples.

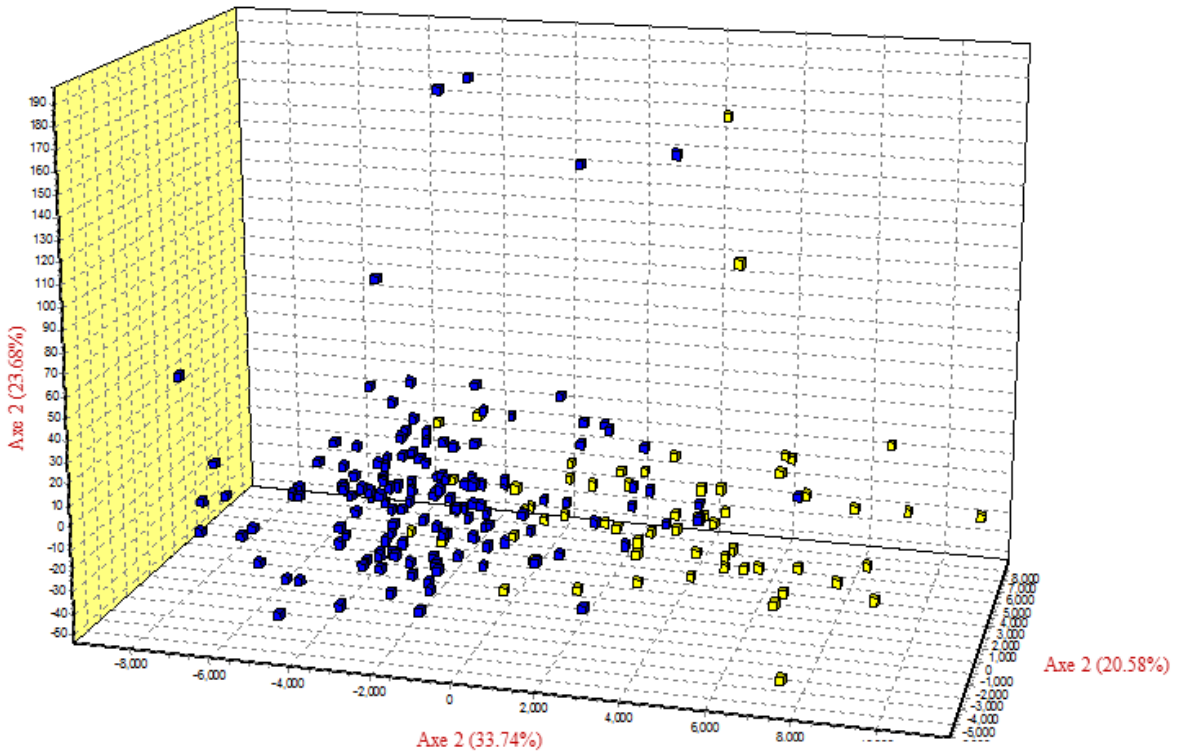
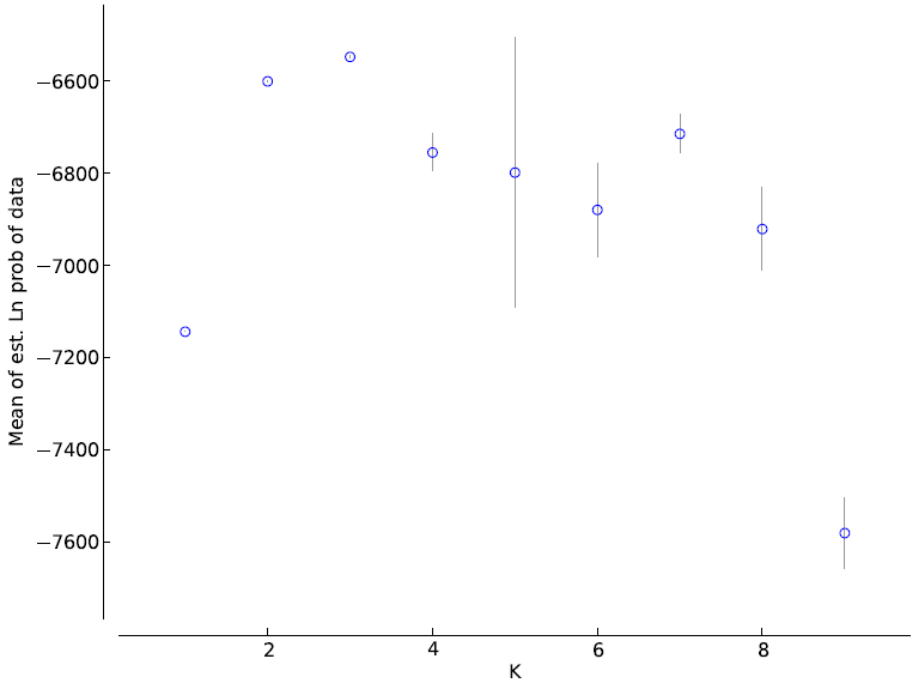


Fig. 4.3: Factorial correspondence analysis for the west coast (yellow squares) versus the south coast (blue squares) South African *Seriola lalandi* samples.

STRUCTURE was used to estimate the number of genetically distinct populations sampled, assuming no prior information on the number of sampling locations. When individuals from South Africa and the South Pacific were analysed in conjunction, only two clusters were detected (Fig. 4.4). The individual bar plots of $K = 2$ (Fig. 4.5), shows a clear separation between the South African- and the Pacific samples. The South African samples appear to be further separated into two clusters with ΔK peaking at $K = 2$ (Fig. 4.6, 4.7) although cluster assignment indicated admixture of the two clusters along the entire coastline (Fig. 4.7).



b)

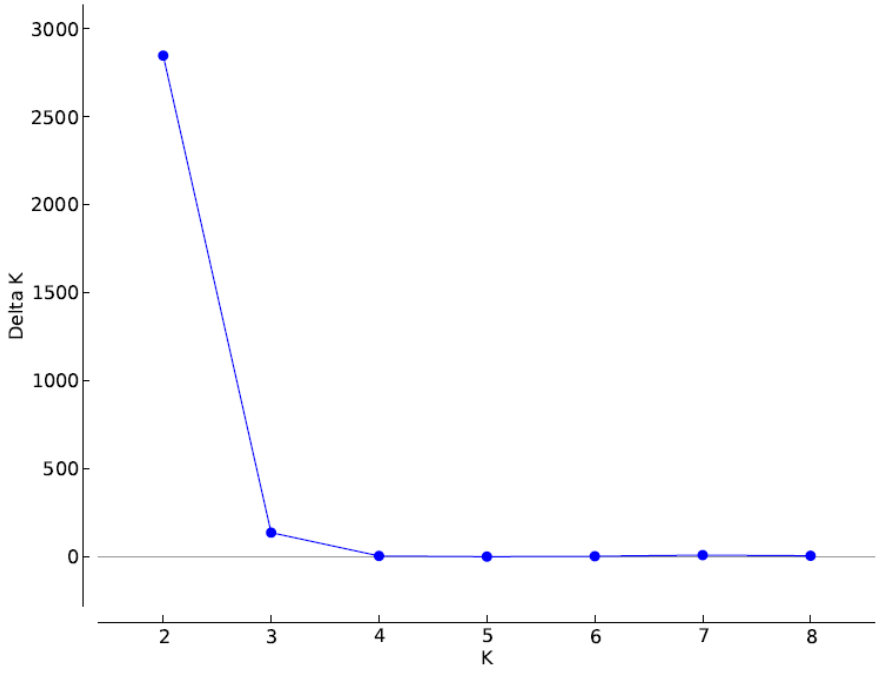


Fig. 4.4: (a) The posterior probability of the data, L(*K*) for each *K* and (b) Δ*K* as a function of *K* following Evanno *et al.* (2005) for all southern hemisphere sampling localities.

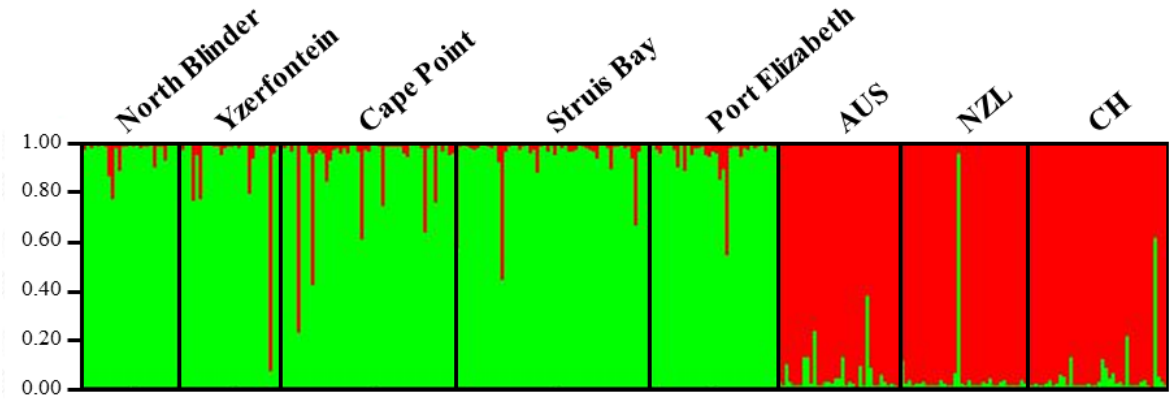
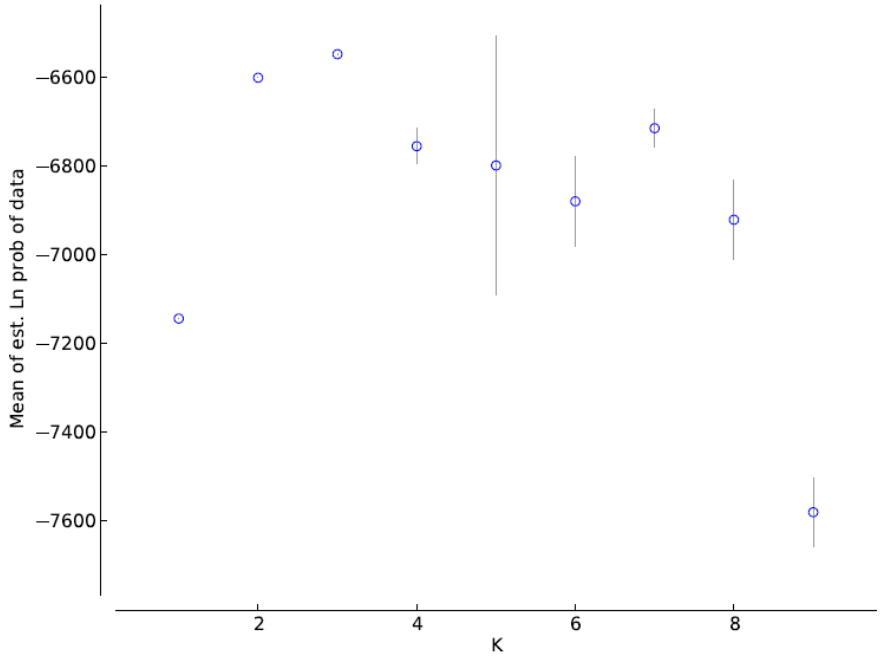


Fig. 4.5: STRUCTURE bar plots for *K* = 2 for all southern hemisphere sampling localities.

a)



b)

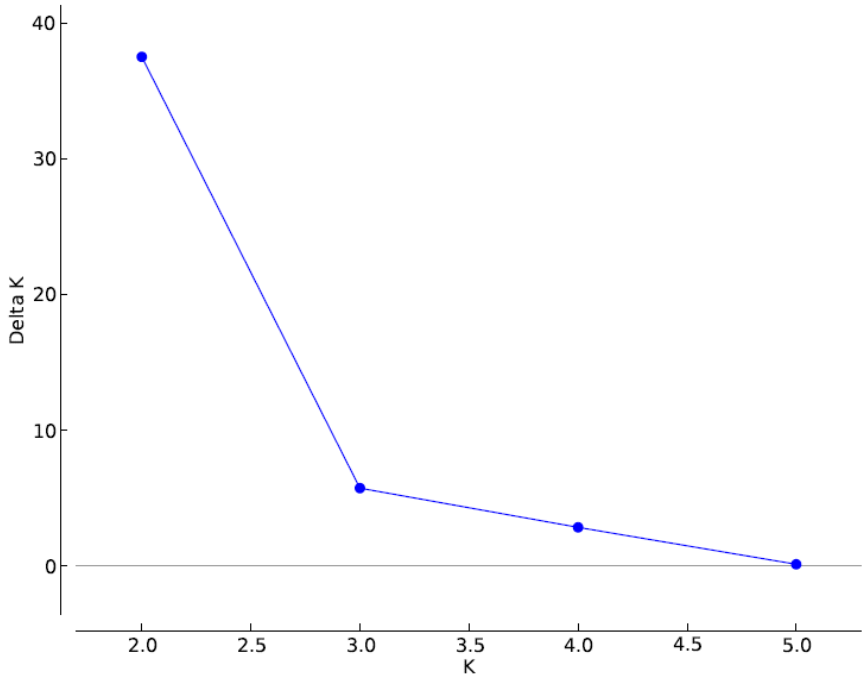


Fig. 4.6: (a) The posterior probability of the data, L(*K*) for each *K* and (b) Δ*K* as a function of *K* following Evanno *et al.* (2005) for only the South African sampling localities.

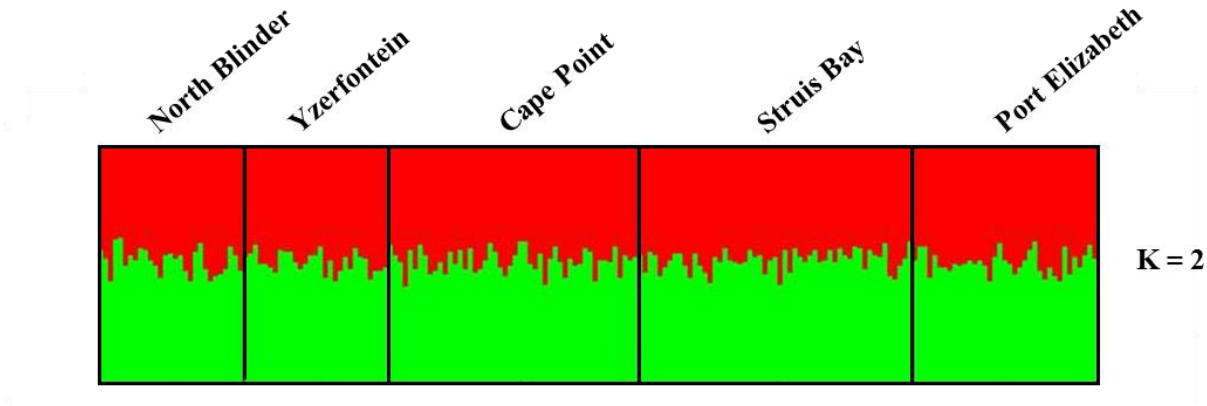


Fig. 4.7: STRUCTURE bar plots for *K* = 2 for the five South African sampling localities.

Three barriers decreasing in importance were identified by BARRIER (Fig. 4.8). The barrier that was best supported by the data was identified at Cape Point which further supports the division

between the south- and west coast samples. Two secondary barriers were found between Struis Bay and Port Elizabeth (thus between south- and south-east coast) and between Yzerfontein and North Blinder on the west coast.

The program MIGRATE generated similar migration rates between South Africa and the South Pacific as to those within South Africa localities (Fig. 4.9). The likelihood plots generated by MIGRATE are shown in Appendix I. However, for BAYESASS these rates were much lower between South Africa and the South Pacific compared to those within South African localities. This indicates that the reduced gene flow was a recent event. Migration results indicate that a similar degree of gene flow occurs between the geographical areas within South Africa. Within the South Pacific, migration rates are relative high and therefore support high gene flow and lack of structure in this area.

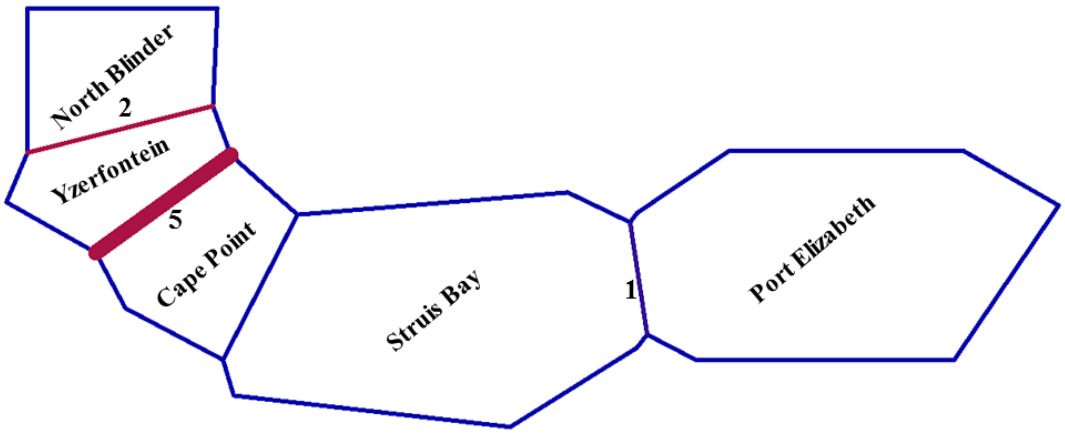


Fig. 4.8: The barriers (purples lines) to gene flow between the five sampling localities. The barrier width and number alongside the barrier line signify the number of matrices supporting the data.

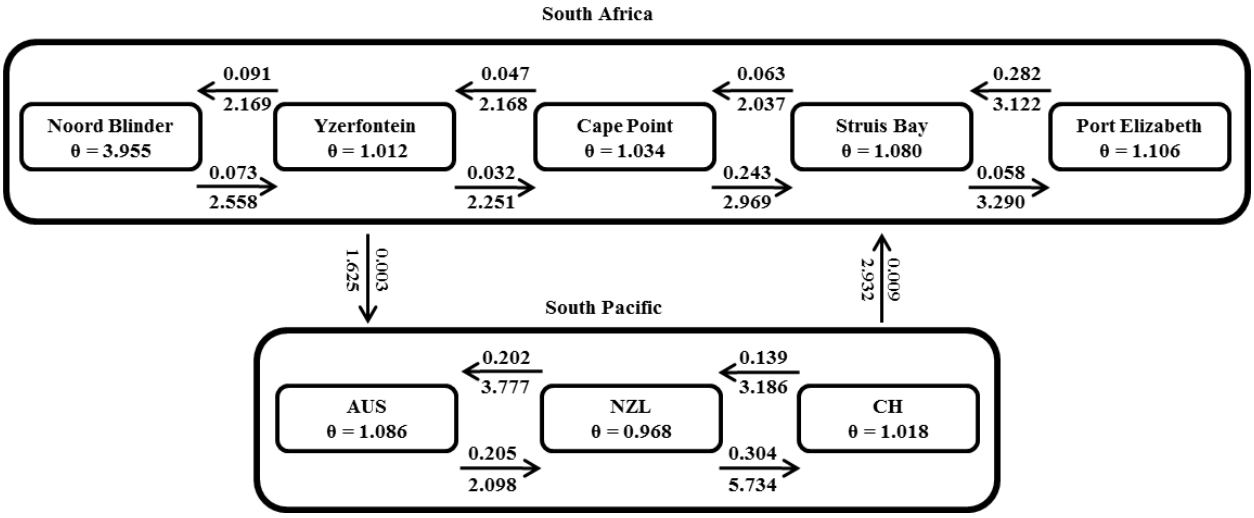


Fig. 4.9: Estimations of theta (θ) and migration rates between South Africa and the South Pacific, and between sampling localities within these two regions. Migration rates above the arrows were calculated using the program MIGRATE and the rates below the arrows were calculated by the program BAYESASS.

4.4 Discussion

This study is the first to investigate the genetic structure of the commercially important species, *S. lalandi* along the coast of South Africa and amongst populations along its distributional range in the southern hemisphere. The results suggest the following: 1) *Seriola lalandi* populations of South Africa are genetically distinct from those of the South Pacific ocean, 2) high gene flow occurs between populations within the South Pacific, 3) two groups of *S. lalandi* are found along the South African coast based on frequentist statistics.

4.4.1 Population differentiation in the southern hemisphere

The results clearly showed strong differentiation between the South African populations and the South Pacific (AMOVA, FCA, STRUCTURE). Several studies have found that genetic population differentiation between the Atlantic (South Africa) and Indo-Pacific may be

explained by recent vicariant events during the Pleistocene glacial- and interglacial periods (Alvarado-Bremer *et al*., 1995, 1998; Graves and McDowell, 2003; Alvarado-Bremer *et al*., 2005). For example, genetic differentiation was found between the Atlantic and Indo-Pacific populations of the bigeye tuna (*Thunnus obesus*; Chow *et al*., 2000; Durand *et al*., 2005); swordfish (*Xiphias gladius*; Alvarado-Bremer *et al*., 2005); yellowfin tuna (*Thunnus albacares*; Ely *et al*., 2005) and albacore (*Thunnus alalunga*; Viñas *et al*., 2004). The separation of the South African and the South Pacific populations was also seen for the sequence data results (Chapter 3) and dating analysis (200 000 - 300 000 years ago); suggesting that the Pleistocene climatic events played an important role in the divergence of these two clades.

4.4.2 Lack of population structure in the southern Pacific

The genetic homogeneity in the southern Pacific can be attributed to the high dispersal potential of *S. lalandi*. Several marine studies show this lack of genetic variation and high dispersal in the southern Pacific (*e.g.* the Topshell genera *Diloma*, *Melagraphia*, and *Austrocochlea;* Donald *et al*., 2005; the Chilean jack mackerel, *Trachurus murphy;*, Cárdenas *et al*., 2009), whereas some species show population differentiation between Australia / New Zealand populations and South America populations (Bryde's whales, *Balaenoptera brydei*; Kanda *et al*., 2007; Austral hake, *Merluccius australis*; Machado-Schiaffino *et al*., 2009). Passive dispersal by rafting on floating kelps has been put forth to explain the lack of genetic structure. Within the southern Pacific the high abundance of floating kelp rafts might contribute to passive dispersal (Smith, 2002; Nikula *et al*., 2012). Juveniles of several *Seriola* species are associated with drifting seaweeds, including *S. dumerili* (Ochiai and Tanaka, 1986) and *S. quinqueradiata* (Sakakura and Tsukarnoto, 1996); however this association has not been observed for *S. lalandi* (Ochiai and Tanaka, 1986). Thus

active migration of adults might be a more probable reason for the lack of genetic variation in the southern Pacific. Although *S. lalandi* prefer the deeper waters along the coast, this species does not venture further than the continental shelf. However within the South Pacific, several islands (such as the Polynesian islands) are found that may facilitate island-hopping between New Zealand and Chile (Reid, 2002). This species has been recorded at most of the Polynesian islands that are in the temperate water zone, and it has even been observed along the coast of the isolated Easter Island. The species might utilise such islands as resting stops during dispersal; thus explaining the low genetic differentiation. Another plausible explanation for the lack of genetic structure is a recent expansion event after the last glacial maxima (26 500 - 20 00 years ago; Coyer *et al*., 2001; Fraser *et al*., 2009). Thus *S. lalandi* populations could have recently become isolated within one region in the South Pacific during the last glacial maxima and then recolonised other areas of the South Pacific. A similar lack of genetic structure across the southern Pacific Ocean was also observed in another pelagic carangid species; the Chilean jack mackerel (*Trachurus murphyi*; Cárdenas *et al*., 2009), which was attributed to a recent colonisation of eastern populations (South America) to the west (New Zealand).

4.4.3 Origin of the genetic structure along the South African coastline

The results of this study suggest that there are potentially two genetically differentiated populations of *S. lalandi* in South Africa. Although the differentiation is shallow, the hypothesis of a single panmictic population across the South African coast can be rejected (AMOVA, FCA, F_{ST}). This can be explained by more than one potential scenario. The first scenario for the existence of two populations is that one population / group could have evolved elsewhere and subsequently spread to South Africa. A similar situation exists within most tuna and billfish

species, where secondary contact can be found along the South African coast between Indo-Pacific and Atlantic populations (Alvarado-Bremer *et al*., 1998; Graves and McDowell, 2003). However the fact that mtDNA analyses (Chapter 3) do not detect these two groups suggests that these groups represent a recent divergence that probably had its origin along the South African coast. This leads to a second potential scenario on how these two groups could have diverged. *Seriola lalandi* is a relatively recent, evolutionary speaking, occupant of the South African coast (± 220 000 - 290 000 years, chapter 3). Within this time period several climatic and oceanographic changes could have played a major role in the origin of these two *S. lalandi* populations, such as sea level changes (Yokoyama *et al*. 2000; Miller *et al*., 2005), latitudinal shifts in water temperature (Cutler *et al*., 2003; Bard and Rickaby, 2009), and changes in coastal topography and currents (Flores *et al*., 1999). One such an event occurred 41 000 and 25 000 years ago, with the increase of sea surface temperature prior to the last glacial maximum (Sachs *et al*., 2001). As previously mentioned, *S. lalandi* and even the genus *Seriola* are very sensitive to changes in sea temperature, and this may have caused a decline in populations and shift in suitable habitat. Furthermore, the reduction in upwellings in the Benguela Current as the result of introgression of this warmer water dramatically decreased productivity in the region. This in return could have influenced *S. lalandi* populations indirectly by causing significant population reductions of its main food sources, such as sardines (Boyd *et al*., 1997). In addition to this, during the glacial maximum sea levels were approximately 120m lower than today; thus raising the Agulhas Bank and further reducing the availability of suitable habitat. Although this represents a single example of how glacial and interglacial periods influenced the South African coastline, this pattern would have repeated itself to some degree during the last 200 000 years since this species inhabited this region. A combination of possible lower temperatures during

glacial periods and lower sea levels could have resulted in unsuitable habitats thereby giving rise to isolated refugia between the west- and south coast of South Africa. This is supported by several studies that have found genetic structure between species from the west- and south coast of South Africa (Evans *et al*., 2004; Norton, 2005; Teske *et al*., 2006, 2007; von der Heyden *et al*., 2008; Bester-van der Merwe *et al*., 2011).

4.4.4 Maintenance of genetic structure along South African coastline

Irrespective of the origin of these two groups, it is imperative to understand how the genetic integrity of these two groups is maintained. The AMOVA, FCA and F_{ST} results suggest that populations from the west coast (Yzerfontein and North Blinder) differ significantly from the Cape Point, Struis Bay and Port Elizabeth samples (south coast), suggesting the genetic break may be in the Cape Point region (BARRIER). However Bayesian clustering analysis (STRUCTURE) and the migration results (MIGRATE and BAYESASS) also suggest a high degree of gene flow between the regions.

The Cape Point region was previously identified as an important genetic break in a number of studies, for example the brown mussel (*Perna perna*; Grant *et al*., 1992) and bluntnose klipfish (*Clinus cottoides*; von der Heyden *et al*., 2008). The Cape Agulhas region has also been identified as a potential break for several marine species; for example abalone, *Haliotis midae* (Evans *et al*., 2004; Bester-van der Merwe *et al*., 2011), bluntnose klipfish (von der Heyden *et al*., 2008), and mudprawn, *Palaemon peringueyi* (Teske *et al*., 2009). In the current study, however, no population differentiation was found between the samples of Cape Point and Struis Bay; sampling localities on either side of the Agulhas break. The region between Knysna and

Port Elizabeth / Port Alfred along the southeast coast is another important boundary for gene flow as seen in similar studies of *Clinus cottoides* (von der Heyden *et al*., 2008), the crustacean, *Iphinoe truncata* (Teske *et al*., 2006) and round herring, *Gilchristella aestuaria* (Norton, 2005). This region also did not play a role in population differentiation in *S. lalandi* in the current study as samples from Struis Bay did not differ genetically from those of Port Elizabeth. The only genetic break therefore identified for *S. lalandi* in this study is the region around Cape Point. It is important to note that most of the above mentioned species are, however, either found in rock pools or in estuaries; therefore species with limited dispersal capabilities. Linefish species (kingklip, *Genypterus capensis*; Grant and Leslie, 2001; spotted grunter, *Pomadasys commersonnii*; Klopper, 2005; dusky kob, *Argyrosomus japonicus*; Klopper, 2005; Cape stumpnose, *Rhabdosargus holubi*; Oosthuizen, 2007; Cape hake, *Merluccius capensis*; von der Heyden *et al*., 2007) or species with a longer larval cycle (*Octopus vulgaris*; Oosthuizen *et al*., 2004; spiny lobster, *Palinurus gilchristi*; Tolley *et al*., 2005; west coast rock lobster, *Jasus lalandi*; Matthee *et al*., 2007), on the other hand, usually show no genetic discontinuity along the South African coast. Even though a species such as *Jasus lalandi* (Matthee *et al*., 2007) showed no overall phylogeographic structure, significant structure was found between Hout Bay individuals and some other sampling sites (Dassen Island, Olifantsbos, Elands Bay, Lüderitz and Hangklip); providing further evidence of the influence of the Cape Point region on gene flow.

Oceanic barriers are not the only force driving the separation of these two groups in *Seriola lalandi*, because high gene flow was found throughout its geographical range (MIGRATE, BAYESASS and STRUCTURE). This is supported by tagging studies that found movement from the west- to the east coast (ORI 2012). For example a juvenile (775mm fin length) travelled

a distance of 1 746km in just 30 days from Dassen Island on the west coast where it was first caught to Stiebel Rocks just south of Hibberdene on the KwaZulu-Natal south coast (ORI 2012). Therefore the life history of yellowtail should be considered to understand how separation of these two potential groupings is sustained. Several life history traits may explain these groupings, for instance these groups may have different spawning areas or larvae can only survive in certain oceanic conditions (*e.g.* water temperature, salinity, and pH). In marine species natal philopatry, the return of individuals to spawning areas where they originated from, is quite common. Several studies have found philopatry in marine teleosts (red drum, *Sciaenops ocellatus*; Gold *et al*., 1999; Atlantic cod, Robichaud and Rose, 2001; cutthroat trout, *Oncorhynchus clarkia*; Wenburg and Bentzen, 2001; shark species, Hueter *et al*., 2005). For example, in *M. paradoxus* significant genetic differentiation was found between Namibia and South Africa for adult individuals older than three years but not in juveniles (von der Heyden *et al*., 2007). Furthermore, several fish species in South African waters have evolved highly selective reproductive patterns due to the complex dynamics of the coastline. Four important areas, consisting of spawning sites, mechanisms for transport of larvae and nursery grounds, have been identified along the South African coast for a variety of pelagic, demersal and inshore-dwelling fish species: (1) the northern boundary of the Natal Bight; (2) off Port Elizabeth, where the Agulhas Current diverges from the coast; (3) off the tip of the Agulhas Bank, where the current diverges from the continental shelf and retroflects; (4) off Cape Columbine on the west coast, where the jet current appears to bifurcate (Hutching *et al.*, 2002). For instance, snoek (*Thyrsites atun*), which in some respects has a life history quite similar to *S. lalandi* has two spawning areas, one at the edge of the Agulhas Bank and one off Cape Columbine (Hutching *et*

al., 2002). *Seriola lalandi*, may utilise the same spawning sites, which can explain the pattern found in this study, however research is needed to test this.

While this is the first study to investigate the genetic structure of *S. lalandi* populations along the South African coast, the results obtained were only based on six microsatellite markers. These markers were obtained by cross-species amplification of 30 markers designed for *S. dumerili* (Renshaw *et al*., 2006, 2007; Porta *et al*., 2009). Therefore these markers were not specifically designed for *S. lalandi* which might have contributed to the low resolving power. Of the 30 microsatellites tested only these six microsatellite markers could be used for genotyping. Four of the markers used in the current study were also used in a study by Miller *et al*. (2011) to resolve the population structure in Australia and New Zealand. The levels of genetic diversities characterised in the current study are comparable to those observed by Miller *et al*. (2011).

In conclusion, *S. lalandi* in the southern hemisphere comprise of two distinct oceanic populations in the South Pacific and South Africa, with low levels of gene flow. Furthermore, two genetically distinct groupings are found within South African, with Cape Point being a barrier to gene flow.

Appendix H: Allele frequency bar charts for each microsatellite locus

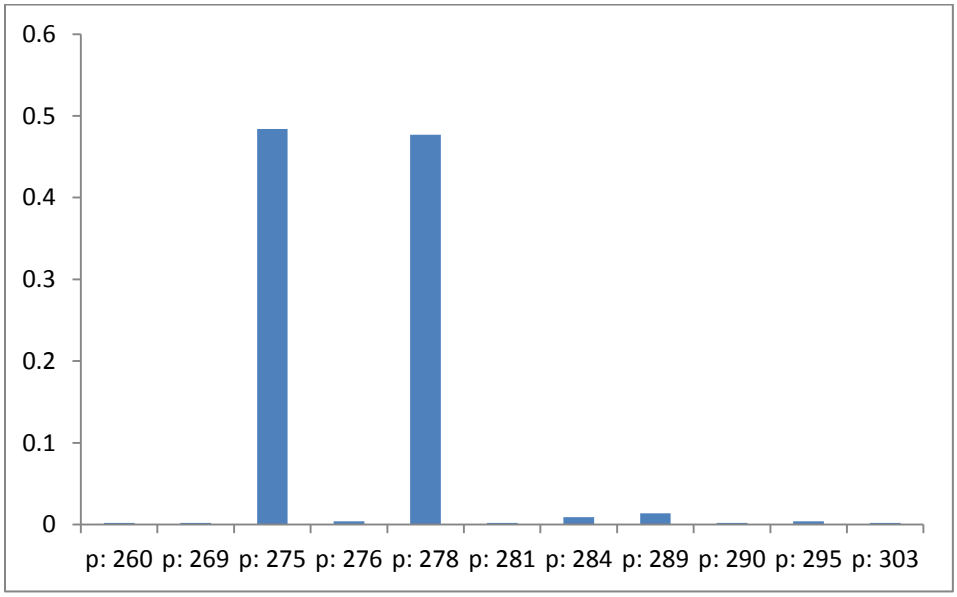


Fig. H1: Allele frequencies for Locus Sdu10

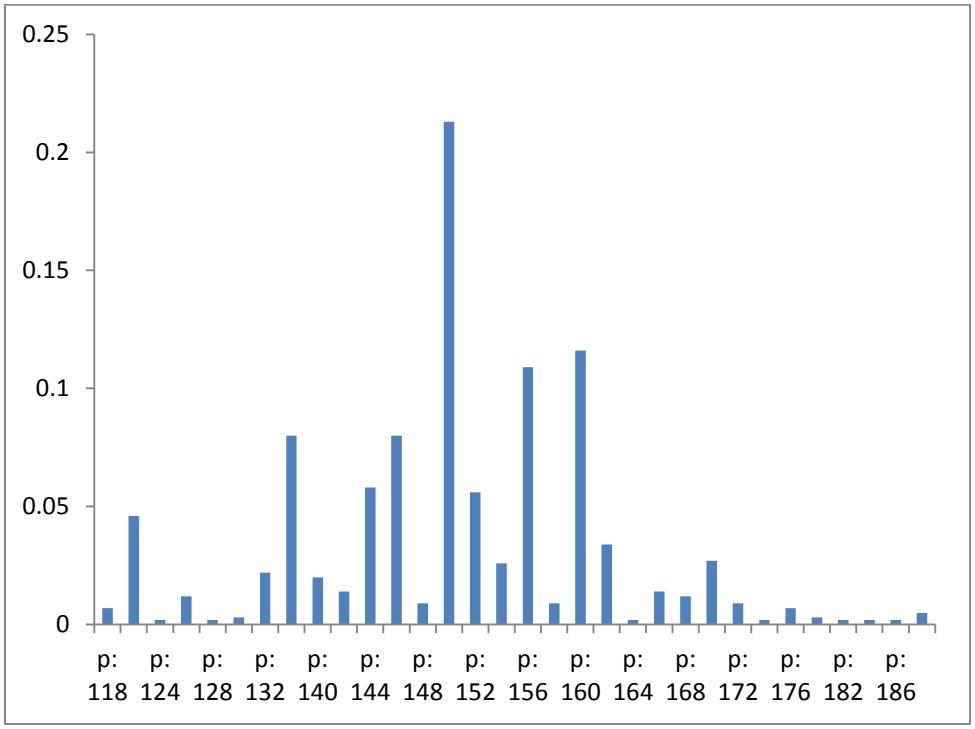


Fig. H2: Allele frequencies for Locus SduCA107

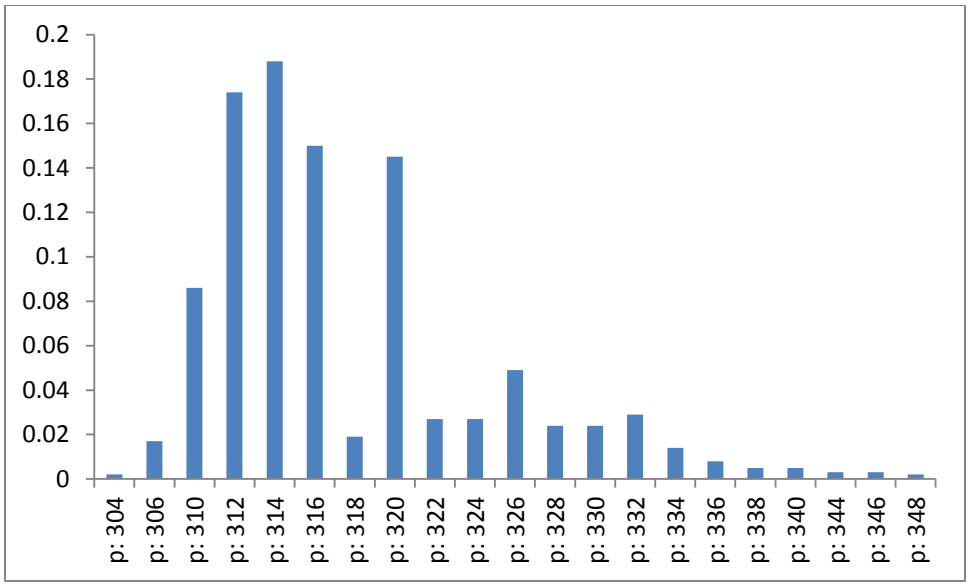


Fig. H3: Allele frequencies for Locus Sdu29

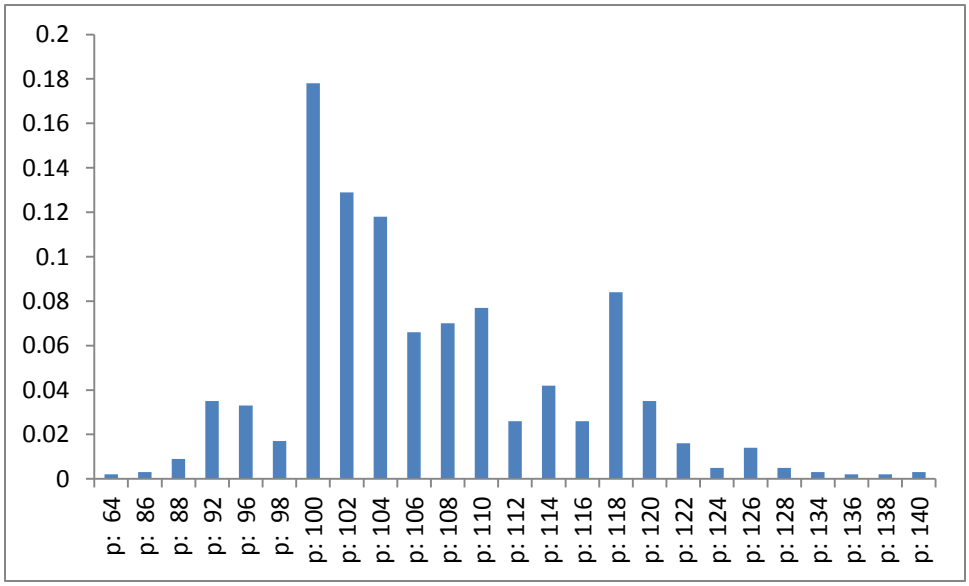


Fig. H4: Allele frequencies for Locus Sdu32

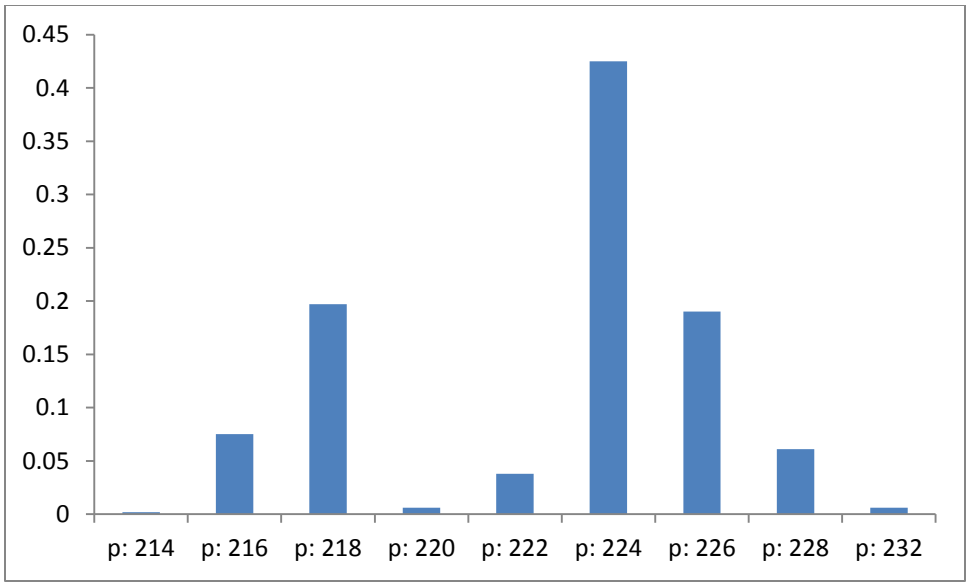


Fig. H5: Allele frequencies for Locus Sdu46

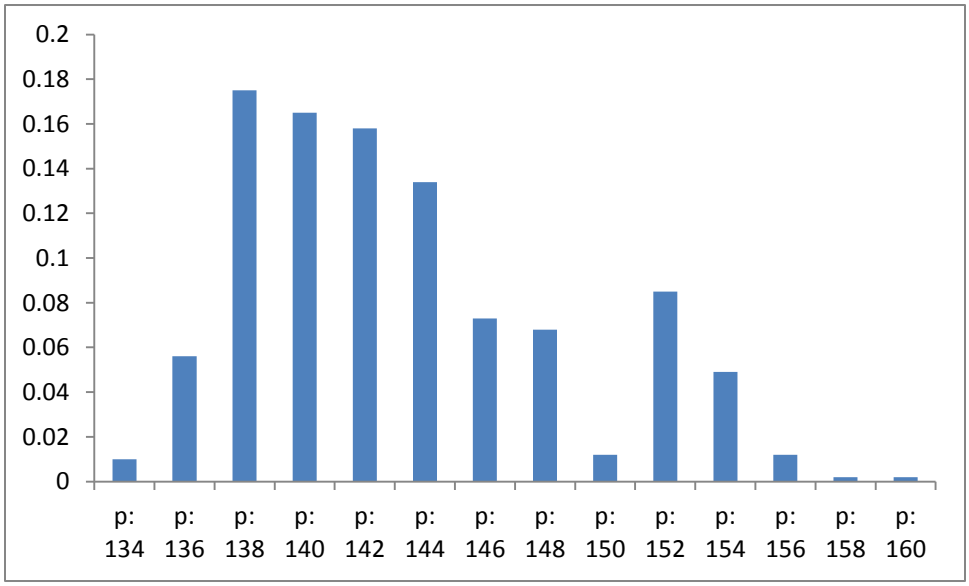


Fig. H6: Allele frequencies for Locus SduCA4j

Appendix I: Likelihood plots generated by MIGRATE

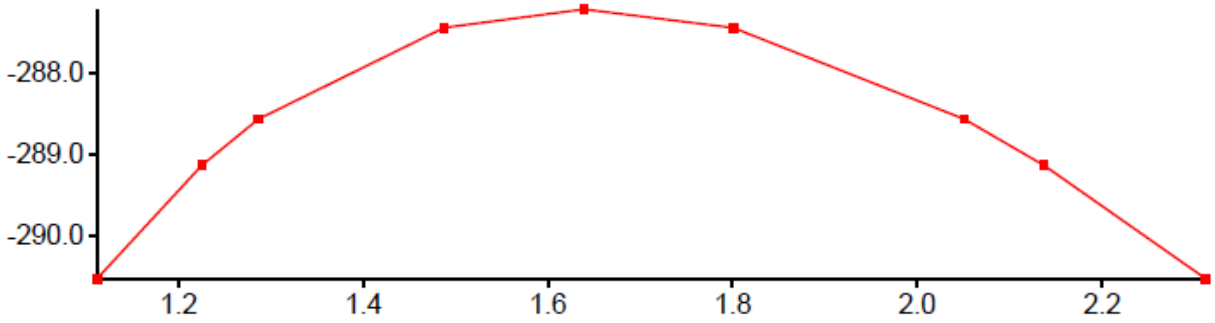


Fig. I1: The likelihood plots for M from Yzerfontein to Noord Blinder

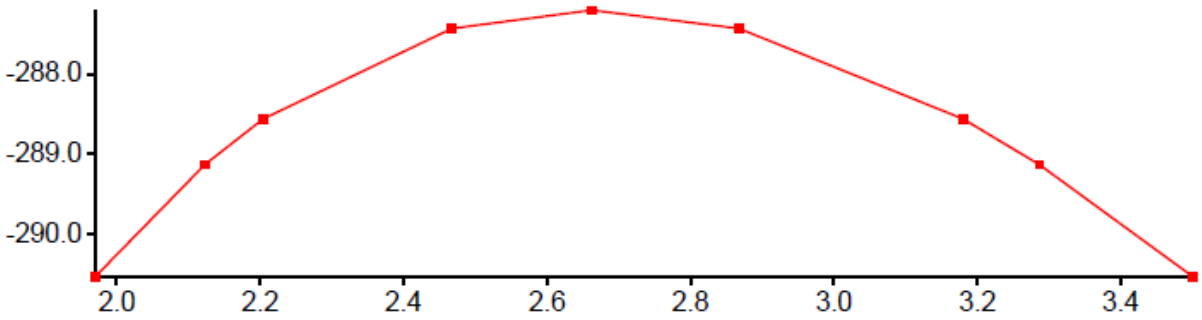


Fig. I2: The likelihood plots for M from Noord Blinder to Yzerfontein

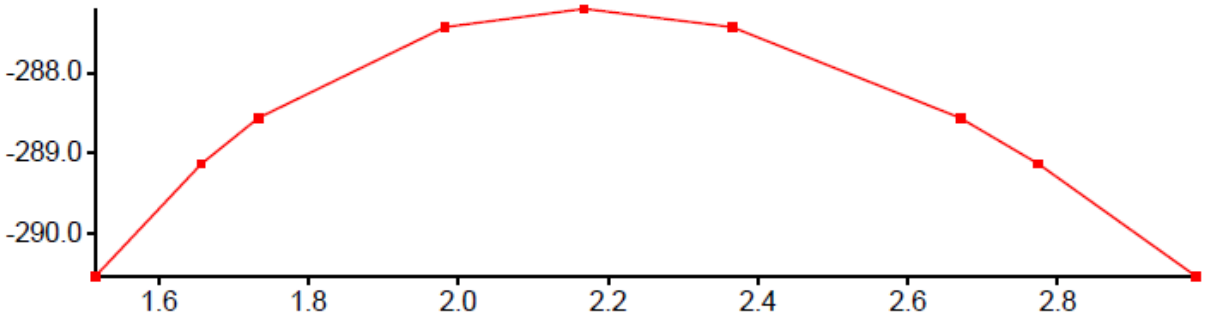


Fig. I3: The likelihood plots for M from Cape Point to Yzerfontein

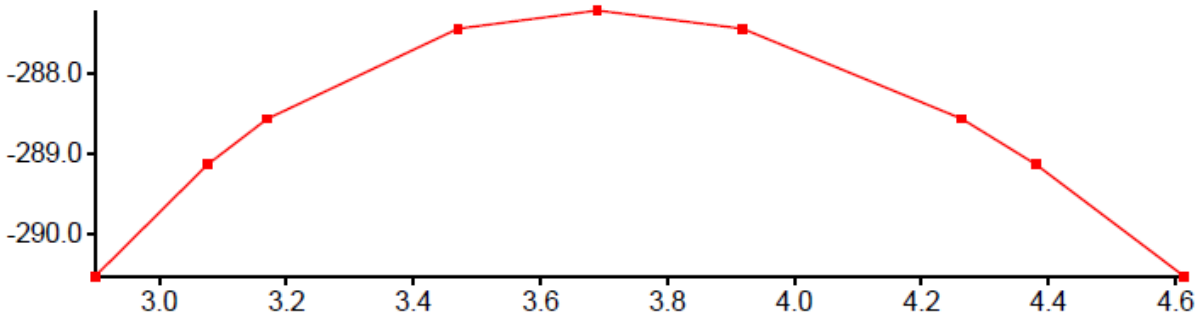


Fig. I4: The likelihood plots for M from Yzerfontein to Cape Point

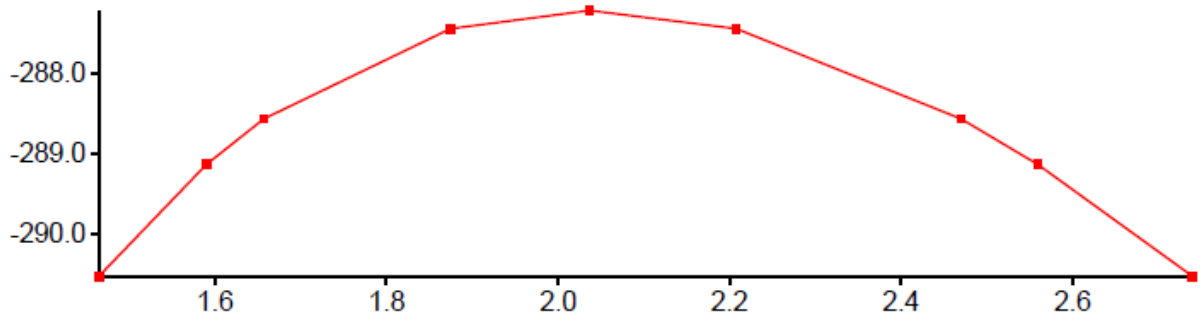


Fig. I5: The likelihood plots for M from Struis Bay to Cape Point

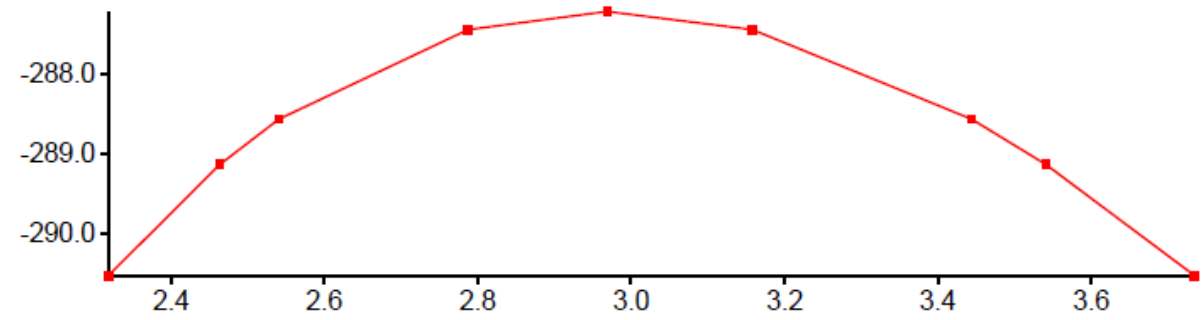


Fig. I6: The likelihood plots for M from Cape Point to Struis Bay

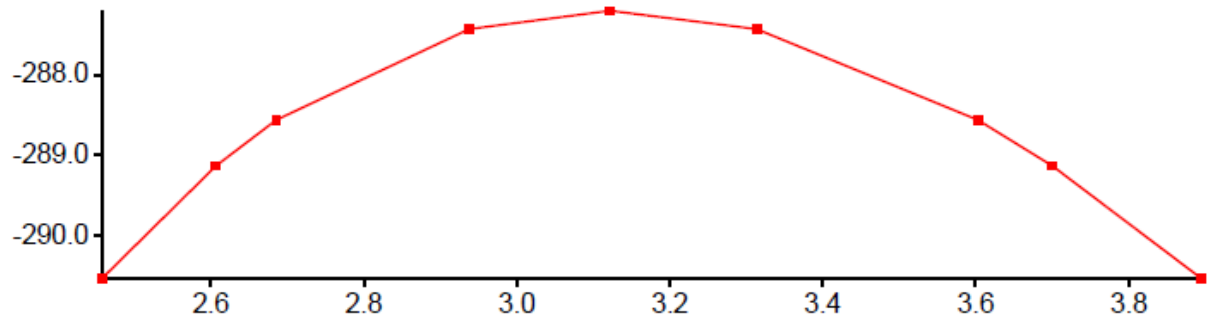


Fig. I7: The likelihood plots for M from Port Elizabeth to Struis Bay

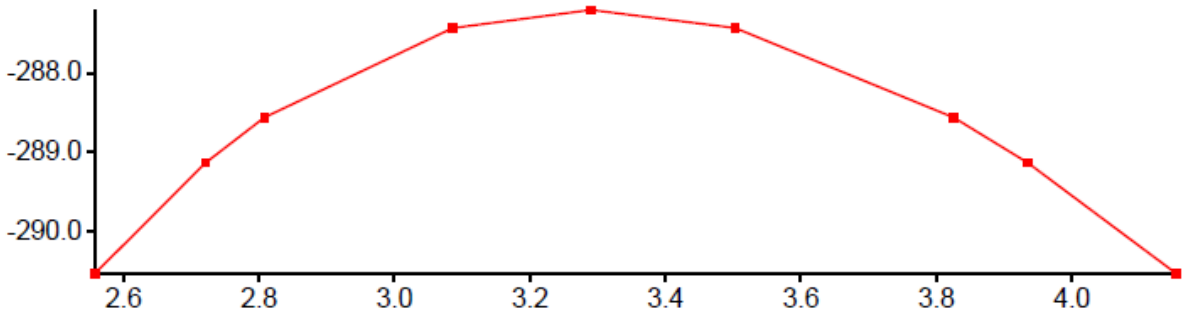


Fig. I8: The likelihood plots for M from Struis Bay to Port Elizabeth

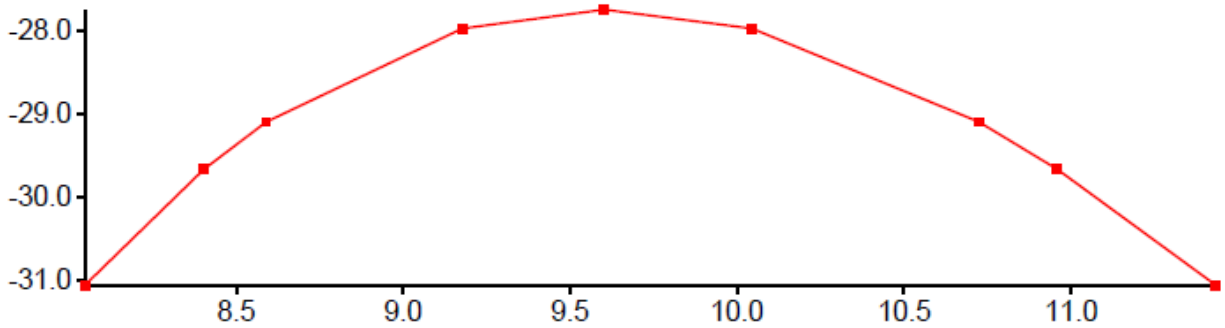


Fig. I9: The likelihood plots for M from South Africa to South Pacific

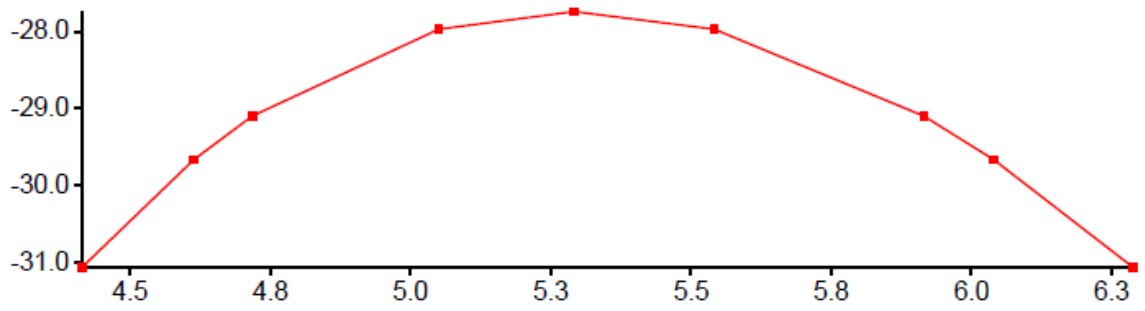


Fig. I10: The likelihood plots for M from South Pacific to South Africa

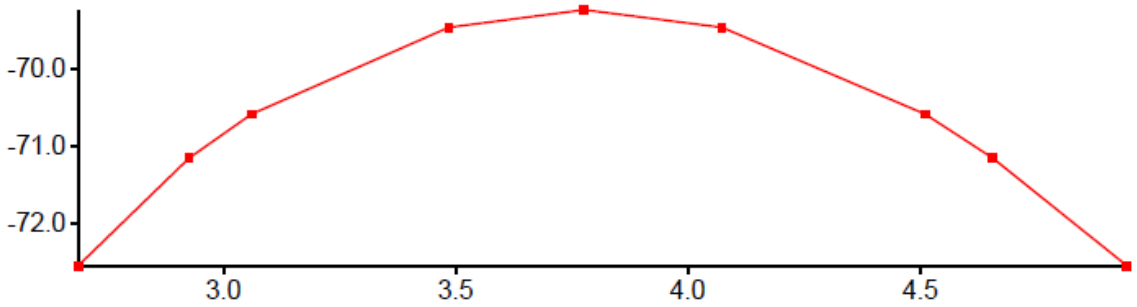


Fig. I11: The likelihood plots for M from NZL to AUS

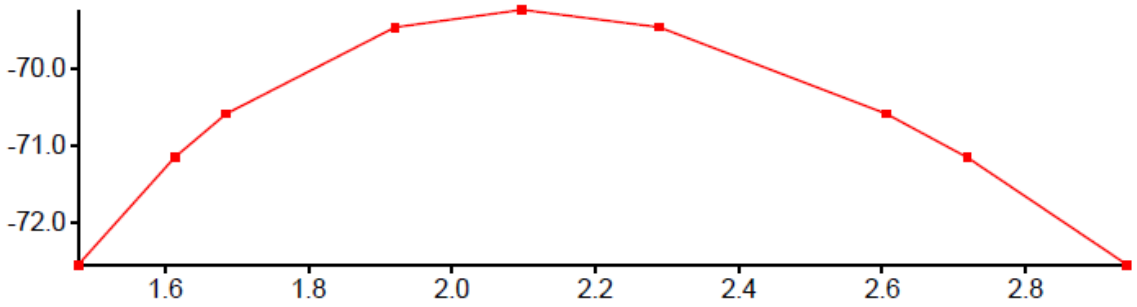


Fig. I12: The likelihood plots for M from AUS to NZL

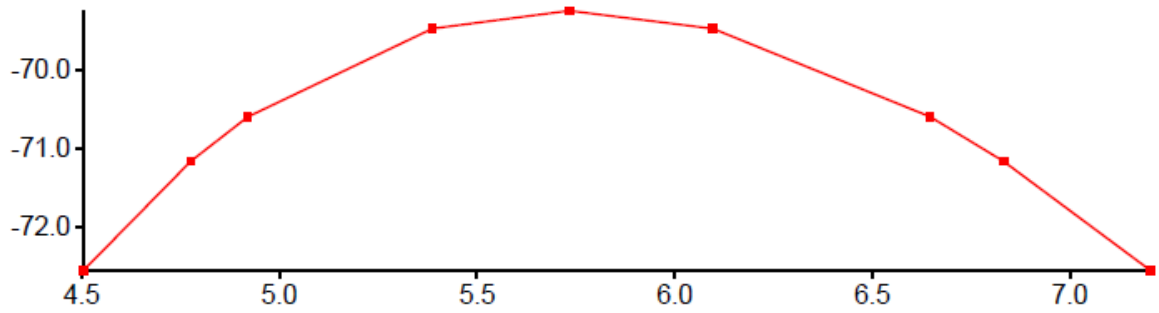


Fig. I13: The likelihood plots for M from NZL to CH

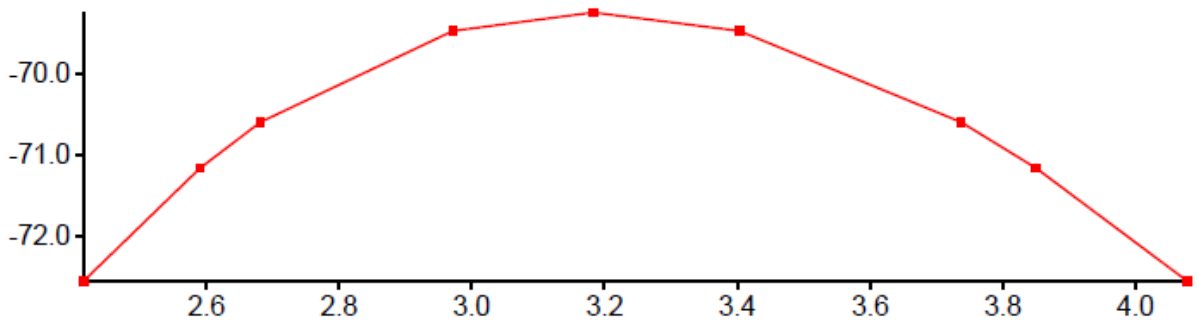


Fig. I14: The likelihood plots for M from CH to NZL

Chapter 5: General Conclusion

5.1 Thesis summary

The multidisciplinary approach of this study allowed for a better understanding of the macro- and micro-evolutionary trends within the genus *Seriola* and specifically the species *S. lalandi*, with valuable insight gained by the phylogenetic, phylogeographic, and population genetic analyses.

On a macro-evolutionary scale, phylogenetic analyses including representatives of all nine species of this globally distributed genus increased our understanding of the speciation and biogeography of *Seriola*, by generating a molecular phylogeny based on two mitochondrial (*Cytb* and *COI*) and two nuclear genes (*RAG1* and *Rhod*; presented in Chapter 2). This was, to our knowledge, the first phylogenetic analysis of this genus to investigate relationships among all known extant species. Although the sequence data used in this study did not fully resolve phylogenetic relationships within the genus, important evolutionary patterns could be identified. All species were found to be monophyletic and the species' relationships obtained during the current study have been essential in clarifying some of the taxonomic confusion of this genus. The phylogenetic patterns obtained were indicative of three main lineages: a ((*S. fasciata* and *S. peruana*), *S. carpenteri*) clade, a (*S. dumerili* and *S. rivoliana*) clade and a (*S. lalandi* and *S. quinqueradiata*) clade. In terms of biogeography, closure of the Tethys Sea (12 - 20 MYA) coincides with divergence of the ((*S. fasciata* and *S. peruana*), *S. carpenteri*) clade from the rest of the *Seriola* species; while the uplifting of the Isthmus of Panama (± 3 MYA) played an important role in speciation between *S. fasciata* and *S. peruana*. In the evolutionary history of the

remainder of the *Seriola* species, climate and water temperature are proposed to have played important roles.

With regards to the micro-evolutionary scale, phylogeographic analyses based on two mitochondrial (*Cytb* and *COI*) and one nuclear gene (*RAG1*) (Chapter 3) provided a more thorough investigation into the global population structure of one of the *Seriola* species, *S. lalandi*; the main commercial carangid in South Africa. This study is the first to include samples from across the global distribution of S. *lalandi* and the results revealed three geographically and genetically distinct groups corresponding to the previously identified subspecies: North-eastern Pacific clade - *S. lalandi dorsalis* (California yellowtail; Gill, 1863), North-western Pacific clade - *S. lalandi aureovittata* (Asian yellowtail; Schlegel, 1844) and southern hemisphere clade - *S. lalandi lalandi* (Southern yellowtail; Cuvier and Valenciennes, 1833). Rapid demographic growth characterised many of the populations (supported by a star-like mtDNA genealogy in the haplotype networks, Tajima's *D* and Fu's *Fs* statistics and the mismatch distribution analyses) and can be explained by population expansion and retraction events during Pleistocene glaciation periods. Furthermore, the two groups recovered within the southern hemisphere (South Africa and southern Pacific), are well-defined by population differentiation analyses (F_{ST} and AMOVA) but less distinct in the phylogenetic- and network analysis.

Population genetic analyses based on six microsatellite markers revealed the fine-scale population structure of *S. lalandi* within the southern hemisphere and South Africa specifically (Chapter 4). This is also the first study to investigate the population structure of this commercially important species in South Africa. In Chapter 4 the main findings were: 1) South

African populations are genetically distinct from those of the South Pacific ocean (AMOVA, FCA and STRUCTURE results). 2) High gene flow occurs within the South Pacific (AMOVA and MIGRATE results). 3) Potentially two groups are found within the South African samples. AMOVA, FCA and pairwise F_{ST} results suggest significant differentiation between populations from the west- and south coast of South Africa; suggesting a potential genetic break in the Cape Point region (BARRIER analysis). However, STRUCTURE and the migration results (MIGRATE and BAYESASS) also suggest a high degree of gene flow between these regions.

5.2 Future research directions

While this is the first study to address some important questions on the phylogeny of *Seriola* and the phylogeography and population structure of the commercially important species *S. lalandi*, the findings of this thesis, as in any scientific investigation, have given rise to further research questions.

5.2.1 What is the relationship among the three main clades found in Seriola *with regards to each other and the remaining two* Seriola *species* (S. zonata *and* S. hippos)*?*

While three main lineages were found in the phylogeny of *Seriola*, the relationships between these clades / groups remain unclear and so does the placement of *S. zonata* and *S. hippos*. Thus, the robustness of the phylogenetic study presented in Chapter 2 may be questioned. Since *Seriola* is a relative old genus (55 MYA) with many extinct taxa and only a few extant species (nine), resolving the inner nodes proved to be problematic. Additional nuclear DNA fragments (such as *ITS1*, *ITS2*), may serve to resolve the deeper nodes of the phylogenetic tree. Nuclear genes are particularly suited to resolve deeper nodes in phylogenetic analysis (Nazari *et al*., 2007). In most

species, nuclear DNA fragments have a slower mutation rate than mtDNA, providing estimates of genetic divergence over longer evolutionary timescales (Wilson *et al*., 1985; Ferris and Berg, 1987; Birky *et al*., 1989; Meyer, 1993; Shaklee and Bentzen, 1998; Waples, 1998).

The inclusion of taxonomically closer related species from the tribe Naucratini as outgroup taxa may further clarify the relationship among *Seriola* species. The tribe Naucratini consist of the genus *Seriola* and the monotypic genus *Elagatis*. The outgroups used in the current study were from the sister tribe Carangini. Furthermore, more insight on the life histories, morphology and habitat preference of all extant species is needed to provide deeper insight on their evolutionary history and ecological interactions.

5.2.2 Do the three S. lalandi *clades resemble putative subspecies or three separate species?*

The three geographical clades generated in Chapter 3 correspond to the previously described *S. lalandi* subspecies. Traditionally subspecies status is usually given to a group of organisms that are geographically distinct from others within the species, but are still capable of interbreeding with members of the same species; as is the case with the recognised *S. lalandi* subspecies. The genetic distinctness of the three clades / subspecies may call for the elevation of these subspecies to distinct species status. However, detailed comparative morphology and ecological studies are needed to further evaluate their taxonomic status. Regardless of their taxonomic status these three grouping can be treated as different stocks.

5.2.3 How does the west coast S. lalandi *population differ from those along the rest of the South African coast?*

Even though based on frequentist statistics obtained in Chapter 4 (AMOVA, FCA, pairwise F_{ST}), the west coast population seemed to be potentially distinct from the rest of the South African populations, Bayesian clustering implemented in STRUCTURE revealed a high level of admixture along the South African coast. While this is the first study to give insight into the genetic structure of *S. lalandi* populations along the South African coast, the results obtained were only based on six microsatellite markers. These markers were obtained by cross-species amplification of markers designed for *S. dumerili* (Renshaw *et al*., 2006, 2007; Porta *et al*., 2009) and thus not specific for *S. lalandi* which might have contributed to the low resolving power. To infer fine-scale population structure, a larger number of microsatellite markers or even single nucleotide polymorphism (SNP) data are usually needed. Of the 30 microsatellites tested for cross-species amplification in the current study, only the mentioned six microsatellite markers could be used for genotyping. Thus cross-species marker transferability has proven less than optimal and as no SNP markers are yet available for *S. lalandi*, additional marker development specific for *S. lalandi* is required. In a recent study by Whatmore *et al*. (2013) on the genetic parameters for some economically important traits in *S. lalandi* in Australia , six microsatellites were developed specifically for this species and may be useful for future population studies.

Additional sampling areas along the coast of KwaZulu-Natal and the west coast up to and including Namibia are also needed to fully explore the population structure across the entire South African distribution range of this species. By expanding the spatial scope of this study a more thorough assessment of biogeographic barriers can be done. Furthermore, investigations

into the social and reproductive behaviour of *S. lalandi* will further help to address questions on its population structure. For instance, do some individuals show preference to certain spawning sites (philopatry) or do some individuals spawn at different times during the year. Both these behaviours can influence the population structure of *S. lalandi* along the South African coast.

5.3 Implications for the management and conservation of *Seriola lalandi* globally and in particular in South Africa

For the management and conservation of species, two concepts, evolutionary significant units (ESU) and management units (MU), are considered important indicators. ESUs are groups of organisms that have been isolated for a sufficient period of time to become genetically distinct from conspecific groups and are genetically identified as having monophyletic mtDNA lineages (Ryder, 1986; Moritz, 1994). On the other hand, MUs are groups in which local population dynamics are determined predominantly by birth- and death rates rather than migration (Moritz *et al*., 1995) and can be identified as populations with significant differences in allele distribution (Moritz, 1994). Under these criteria the three main *S. lalandi* groupings (North-eastern Pacific clade, North-western Pacific clade and southern hemisphere clade) can be classified as three individual ESUs, while within the southern hemisphere clade the southern Pacific- and South African population can be treated as different MUs.

The findings of this study are of great importance to the fisheries and aquaculture industry of *S. lalandi* in South Africa. Information on the levels of genetic differences between biogeographic areas or provinces in South Africa (as described in Chapter 4) are vital in constructing management, conservation and recovery programs. By uncovering the pattern and extent of the

genetic structure present in the wild populations, one can identify which populations possibly have unique genetic compositions. These populations can be seen as individual genetic stocks and it is important to manage and conserve unique genetic stocks separately. Until now government authorities and commercial fisheries in South Africa have treated *S. lalandi* as a single reproductive stock. However, the current study revealed that there are potentially two genetically distinct 'stocks'; the west coast north of Yzerfontein differs significantly from stocks from the rest of South Africa. According to literature, populations with significant genetic differentiation can usually be recognised as separate MUs (Carvalho and Hauser, 1994; Moritz, 1994). It is therefore recommended that, although the genetic differentiation between the west- and south coast of South African *S. lalandi* is subtle, these populations / regions should be considered separately in terms of management.

Aquaculture with yellowtail is still in the development and planning stage. A marine hatchery has been established in Gansbaai near Cape Agulhas to produce yellowtail fingerlings (Hutchings *et al*., 2010). Furthermore, a pilot study on sea-cage farming of yellowtail at Port Elizabeth was a great success (DST, 2011). Several areas along the west- (Saldanha Bay; WCADI, 2012), south- (Mossel Bay; Hutchings *et al*., 2010; WCADI, 2012) and east (Port Elizabeth and Algoa Bay; Bloom and van Zyl, 2012) coast of South Africa have been proposed for aquaculture of *S. lalandi*. Results of the current study could be informative in the selection and management of the broodstock in these aquaculture programs. The broodstock must be selected from the area where the farm is located. The high genetic variability that occurs in the wild populations could assist in producing high quality broodstock. Furthermore, the application of microsatellite markers (such as the six which was utilised in the current study) has allowed

rapid progress in studies of genetic variability, inbreeding, parentage assignments, species- and strain identification, and the construction of high-resolution linkage maps for aquaculture species. These genetic markers could also identify genes involved in quantitative trait loci (QTL) for marker-assisted selection (Lui and Cordes, 2004). For instance, Whatmore *et al.* (2013) used 10 microsatellite markers to investigate the genetic parameters for economically important traits in South Australian *S. lalandi*, and found that the selection for high growth rate would result in increase in fillet weight. Therefore by using genetic markers and -principles in the management of aquaculture programs, commercial output and sustainability can be improved.

From 2008, a large quantity of very small yellowtail began appearing in seafood retail stores and restaurants in the Western Cape. Retailers were told that it was caught as by-catch off the coast of Namibia. However, after an investigation the Southern African Sustainable Seafood Initiative (SASSI) raised the suspicion that these fish products may have been imported from aquaculture farms in Asia, probably Japan (SASSI, 2008). However, given the fact that there is no minimum legal size limit for *S. lalandi* in South Africa, the uncertainty of the origin of the product still remains. The results from the current study could potentially also assist to resolve this seafood fraud issue. There are distinct genetic difference between *S. lalandi* populations from South Africa and those of Japan and by employing the molecular markers and analyses described in Chapter 3, the origin of these products can be traced. In genetic studies on fish products in the South African market, evidence was found that several products were mislabelled and substituted for other fish species (von der Heyden *et al*., 2010; Cawthorn *et al*., 2012). For instance, some of the samples labelled Dorado (*Coryphaena hippurus*) were in fact *S. lalandi.* Furthermore, some of the kingklip (*Genypterus capensis*) fillets sold on the South African marked come from New

Zealand (von der Heyden *et al*., 2010). More noteworthy, the study by Cawthorn *et al*. (2012) on fishery product mislabelling in the South African market found that five of nine retail outlets sold products labelled as yellowtail that were in fact *Seriola quinqueradiata* (Japanese amberjack). Thus the findings of this current study can help in identification of the region of origin and the species of marketed yellowtail products in the South African market.

In conclusion, this study is the first to explore the evolutionary history of the genus *Seriola* and is also the first large-scale genetic study of the important commercial species, *S. lalandi*, not only in southern Africa, but also globally. This study has provided essential genetic knowledge in terms of genetic stock structure of this species as well as identifying possible management units to preserve the genetic diversity of this economically important fisheries species.

References

Aboim MA, Menezes GM, Schlitt T, Rogers AD (2005) Genetic structure and history of populations of the deep-sea fish *Helicolenus dactylopterus* (Delaroche, 1809) inferred from mtDNA sequence analysis. *Molecular Ecology* **14**: 1343-1354.

Adachi J, Hasegawa M (1996) Model of amino acid substitution in proteins encoded by mitochondrial DNA. *Journal of Molecular Evolution* **42**: 459-468.

Adams SM, Lindmeier JB, Duvernell DD (2006) Microsatellite analysis of the phylogeography, Pleistocene history and secondary contact hypotheses for the killifish, *Fundulus heteroclitus*. *Molecular Ecology* **15**: 1109-1123.

Adey WH, Lindstrom SC, Hommersand MH, Muller KM (2008) The biogeographic origin of Arctic endemic seaweeds: a thermogeographic view. *Journal of Phycology* **44:** 1384-1394.

Allendorf FW, Seeb LW (2000) Concordance of genetic divergence among sockeye salmon populations at allozyme, nuclear DNA, and mitochondrial DNA markers. *Evolution* **54**: 640-651.

Alva-Campbell R, Floeter SR, Robertson DR, Bellwood DR, Bernardi G (2010) Molecular phylogenetics and evolution of *Holacanthus* angelfishes (Pomacanthidae). *Molecular Phylogenetics and Evolution* **56:** 456-461.

Alvarado-Bremer JR, Mejuto J, Baker AJ (1995) Mitochondrial DNA control region sequences indicate extensive mixing of swordfish (*Xiphias gladius* L.) populations in the Atlantic Ocean. *Canadian Journal of Fisheries and Aquatic Sciences* **52**: 1720-1732.

Alvarado-Bremer JR, Stequert B, Robertson NW, Ely B (1998) Genetic evidence for inter-oceanic subdivision of bigeye tuna (*Thunnus obesus* Lowe) populations. *Marine Biology* **132**: 547-557.

Alvarado-Bremer JR, Vinas J, Mejuto J, Ely B, Pla C (2005) Comparative phylogeography of Atlantic bluefin tuna and swordfish: the combined effects of vicariance, secondary contact, introgression, and population expansion on the regional phylogenies of two highly migratory pelagic fishes. *Molecular Phylogenetics and Evolution* **36**: 169-187.

Angers B, Bernatchez L. (1998) Combined use of SMM and non-SMM methods to infer fine structure and evolutionary history of closely related brook charr (*Salvelinus fontinalis*, Salmonidae) populations from microsatellites. *Molecular Biology and Evolution* **15**: 143-159.

Arbogast BS, Edwards SV, Wakeley J, Beerli P, Slowinski JB (2002) Estimating divergence times from molecular data on phylogenetic and population genetic timescales. *Annual Review of Ecology and Systematics* **33**: 707-740.

Avise JC (1998) Conservation genetics in the marine realm. *The Journal of Heredity* **89:** 377-382.

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

Ballard JWO, Kreitman M (1995) Is mitochondrial DNA a strictly neutral marker? *Trends in Ecology and Evolution* **10**: 485-488.

Balloux F, Lugon-Moulin N (2002) The estimation of population differentiation with microsatellite markers. *Molecular Ecology* **11:** 155-165.

Bandelt HJ, Forster P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* **16**: 37-48.

Banguera-Hinestroza E, Bjørge A, Reid RJ, Jepson P, Hoelzel AR (2010) The influence of glacial epochs and habitat dependence on the diversity and phylogeography of a coastal dolphin species: *Lagenorhynchus albirostris*. *Conservation Genetics* **11**: 1823-1836.

Banford HM, Bermingham E, Collette BB, McCafferty SS (1999) Phylogenetic systematics of the *Scomberomorus regalis* (Teleostei: Scombridae) species complex: molecules, morphology, and biogeography of Spanish mackerels. *Copeia* **1999**: 596-613.

Banford HM, Bermingham E, Collette BB (2004) Molecular phylogenetics and biogeography of transisthmian and amphi-Atlantic needlefishes (Belonidae: *Strongylura* and *Tylosurus*): perspectives on New World marine speciation. *Molecular Phylogenetics and Evolution* **31**: 833-851.

Barber PH, Bellwood DR (2005) Biodiversity hotspots: evolutionary origins of biodiversity in wrasses (*Halichoeres*: Labridae) in the Indo-Pacific and new world tropics. *Molecular Phylogenetics and Evolution* **35**: 235-253.

Barber PH, Erdmann MV, Palumbi SR (2006) Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the Coral Triangle. *Evolution* **60**: 1825-1839.

Bard E, Rickaby REM (2009) Migration of the subtropical front as a modulator of glacial climate. *Nature* **460:** 380-384.

Bargelloni L, Alarcon JA, Alvarez MC (2003) Discord in the family *Sparidae* (Teleostei): divergent phylogeographic patterns across the Atlantic–Mediterranean divide. *Journal of Evolutionary Biology* **16**: 1149-1158.

Barton NH, Hewitt GM (1985) Analysis of hybrid zones. *Annual Review of Ecology and Systematics* **16**: 113-148.

Bay LK, Choat JH, van Herwerden L, Robertson DR (2004) High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past? *Marine Biology* **144**: 757-767.

Beerli P, Felsenstein J (2001) Maximum likelihood estimation of a migration matrix and effective population sizes in n subpopulations by using a coalescent approach. *Proceedings of the National Academy of Sciences, USA* **98:** 4563-4568.

Begg GA, Waldman JR (1999) An holistic approach to fish stock identification. *Fisheries Research* **43**: 35-46.

Begg GA, Friedland KD, Pearce JB (1999) Stock identification and its role in stock assessment and fisheries management: an overview. *Fisheries Research* **43**: 1-8.

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

Bekkevold D, Andre C, Dahlgren TG, Clausen LA, Torstensen E, Mosegaard H, Carvolho GR, Christensen TB, Norlinder E, Ruzzante DE (2005) Environmental correlates of population differentiation in Atlantic herring. *Evolution* **59**: 2656-2668.

Bektas Y, Belduz AO (2008) Molecular phylogeny of Turkish *Trachurus* sp. (Perciformes: Carangidae) inferred from mitochondrial DNA analyses. *Journal of Fish Biology* **73**: 1228-1248.

Belkhir K, Borsa P, Goudet J, Chikhi L, Bonhomme F (2000) GENETIX, logiciel sous Window TM pour la génétique des populations. Laboratoire Génome et Populations, Montpellier, France.

Bensasson D, Zhang D-X, Hartl DL, Hewitt GM (2001) Mitochondrial pseudogenes: Evolution's misplaced witnesses. *Trends in Ecology and Evolution* **16**: 314-321.

Berg SL (1947) *Classification of fishes, Both recent and fossil*. JW Edwards publishers.

Bermingham E, McCafferty SS, Martin AP (1997) Fish biogeography and molecular clocks: perspectives from the Panamanian Isthmus. In: *Molecular systematics of fishes*. Ed. by Kocher TD, Stepien CA. Academic Press, New York.

Bester-van der Merwe AE, Roodt-Wilding R, Volckeart FAM, D'Amato ME (2011) Historical isolation and hydrodynamically constrained gene flow in declining populations of the South African abalone, *Haliotis midae*. *Conservation Genetics* **12**: 543-555.

Birky CW, Fuerst P, Maruyama T (1989) Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. *Genetics* **121**: 613-627.

Birky CW, Maruyama T, Fuerst P (1983) An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. *Genetics* **103**: 513-527.

Bloom J, van Zyl JH (2012) Baseline socio-economic assessment for the development of aquaculture development zones for mariculture fish farming in Algoa Bay. CapeEAPrac (Pty) Ltd

Bolton JJ (2010) The biogeography of kelps (Laminariales, Phaeophyceae): a global analysis with new insights from recent advances in molecular phylogenetics. *Marine Research* **64:** 263-279.

Bonhomme F, Naciri F, Bahri-Sfar L, Lemaire C (2002) Comparative analysis of genetic structure of two species of marine fish *Dicentrarchus labrax* and *Dicentrarchus punctatus*. *Comptes Rendus Biologies* **325**: 213-220.

Booke HE (1999) The stock concept revisited: perspectives on its history in fisheries. *Fisheries Research* **43**: 9-11.

Borrell YJ, Pineda H, McCarthy I, Vazquez E, Sanchez JA, Lizana GB (2004) Correlations between fitness and heterozygosity at allozyme and microsatellite loci in the Atlantic salmon, *Salmo salar* L. *Heredity* **92**: 585-593.

Bowen BW, Grant WS (1997) Phylogeography of the sardines (*Sardinops* spp.): Assessing biogeographic models and population histories in temperate upwelling zones. *Evolution* **51**: 1601-1610.

Bowen BW, Karl SA (2007) Population genetics and phylogeography of sea turtles. *Molecular Ecology* **16**: 4886-4907.

Bowen BW, Bass AL, Soares L, Toonen RJ (2005) Conservation implications of complex population structure: lessons from the loggerhead turtle (*Caretta caretta*). *Molecular Ecology* **14**: 2389-2402.

Boyd IL, McCafferty DJ, Walker TR (1997) Variation in foraging effort by lactating Antarctic fur seals: response to simulated increased foraging costs. *Behavioral Ecology and Sociobiology* **40**: 135-144.

Branch GM, Branch M (1995) *The Living Shores of Southern Africa***.** Cape Town: Struik Publishers.

Branch GM, Griffiths CL, Branch ML, Beckley LE (1994) *Two Oceans: a guide to the marine life of Southern Africa.* David Philip Publishers (Pty) Ltd, JHB, SA.

Briggs JC (1961) The East Pacific Barrier and the distribution of marine shore fishes. *Evolution* **15**: 545-554.

Briggs JC (1974) *Marine zoogeography*. McGraw-Hill, New York.

Briggs JC (2010) Marine biology: the role of accommodation in shaping marine biodiversity. *Marine Biology* **157**: 2117-2126.

Briggs JC, Bowen BW (2012) Marine biogeographic provinces: a realignment with particular reference to fish distributions. *Journal of Biogeography* **39:** 12-30.

Briggs JC, Bowen BW (2013) Marine shelf habitat: biogeography and evolution. *Journal of Biogeography* **40**: 1023-1035.

Brown WM, George M Jr, Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. *Proceedings of the National Academy of Sciences, USA* **76**: 1967-1971.

Buonaccorsi VP, McDowell JR, Graves JE (2001) Reconciling patterns of inter-ocean molecular variance from four classes of molecular markers in blue marlin (*Makaira nigricans*). *Molecular Ecology* **10**: 1179-1196.

Buonaccorsi VP, Reece KS, Morgan LW, Graves JE (1999) Geographic distribution of molecular variance within the blue marlin (*Makaira nigricans*): a hierarchical analysis of allozyme, single-copy nuclear DNA, and mitochondrial DNA markers. *Evolution* **53:** 568-579.

Burnett WJ, Benzie JAH, Beardmore JA, Ryland JS (1994) High genetic variability and patchiness in a common Great Barrier Reef zoanthid (*Palythoa caesia*). *Marine Biology* **121**: 153-160.

Burridge CP, Versace VL (2007) Population genetic structuring in *Acanthopagrus butcheri* (Pisces: Sparidae): Does low gene flow among estuaries apply to both sexes? *Marine Biotechnology* **9**: 33-44.

Cardenas L, Hernandez CE, Poulin E, Magoulas A, Kornfield I, Ojeda FP (2005) Origin, diversification, and historical biogeography of the genus *Trachurus* (Perciformes: Carangidae). *Molecular Phylogenetics and Evolution* **35**: 496-507.

Cardenas L, Silva AX, Magoulas A, Cabezas J, Poulin E, Ojeda FP (2009) Genetic population structure in the Chilean jack mackerel, *Trachurus murphyi* (Nichols) across the South-eastern Pacific Ocean. *Fisheries Research* **100**: 109-115.

Carvalho GR, Hauser L (1994) Molecular genetics and the stock concept in fisheries. *Reviews in Fish Biology and Fisheries* **4**: 326-350.

Carvalho GR, Hauser L (1998) Advances in the molecular analysis of fish population structure. *Italian Journal of Zoology* **65**: 21-33.

Carvalho-Costa LF, Piorski NM, Willis SC, Galetti PM, Orti G (2011) Molecular systematics of the neotropical shovelnose catfish genus *Pseudoplatystoma* Bleeker 1862 based on nuclear and mtDNA markers. *Molecular Phylogenetics and Evolution* **59**: 177-194.

Case RAJ, Hutchinson W F, Hauser L, Van Oosterhout C, Carvalho GR (2005) Macro- and micro-geographic variation in pantophysin (Pan I) allele frequencies in NE Atlantic cod *Gadus morhua*. *Marine Ecology Progress Series* **301:** 267-278.

Castella V, Ruedi M, Excoffier L (2001) Contrasted patterns of mitochondrial and nuclear structure among nursery colonies of the bat *Myotis myotis*. *Journal of Evolutionary Biology* **14**: 708-720.

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

Cawthorn DM, Steinman HA, Witthuhn RC (2012) DNA barcoding reveals a high incidence of fish species misrepresentation and substitution on the South African market. *Food Research International* **46**: 30-40.

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

Chai X, Li X, Lu R, Clarke S (2009) Karyotype analysis of the yellowtail kingfish *Seriola lalandi lalandi* (Perciformes: Carangidae) from South Australia. *Aquaculture Research* **40**: 1735-1741.

Chakraborty A, Aranishi F, Iwatsuki Y (2006) Genetic differences among three species of the genus *Trichiurus* (Perciformes: Trichiuridae) based on mitochondrial DNA analysis. *Ichthyological Research* **53**: 93-96.

Chan IKK, Chan DKO, Lee SC, Tsukamoto K (1997) Genetic variability of the Japanese eel *Anguilla japonica* (Temminck and Schlegel) related to latitude. *Ecology of Freshwater Fish* **6**: 45-49.

Charlesworth B, Morgan MT, Charlesworth D (1993) The effect of deleterious mutations on neutral molecular variation. *Genetics* **134**: 1289-1303.

Chen C, Durand E, Forbes F, François O (2007) Bayesian clustering algorithms ascertaining spatial population structure: A new computer program and a comparison study. *Molecular Ecology Resources* **7:** 747-756.

Chen SP, Liu T, Li ZF, Gao TX (2008) Genetic population structuring and demographic history of red spotted grouper (*Epinephelus akaara*) in South and East China Sea. *African Journal of Biotechnology* **7**: 3554-3562.

Chen W, Ruiz-Carus R, Ortí G (2007) Relationships among four genera of mojarras (Teleostei: Perciformes: Gerreidae) from the western Atlantic and their tentative placement among percomorph fishes. *Journal of Fish Biology* **70:** 202-218.

Chen Z, Dupre DJ, Le Gouill C, Rola-Pleszczynski M, Stankova J (2002) Agonist-induced internalization of the platelet-activating factor receptor is dependent on arrestins but independent of G-protein activation. Role of the C terminus and the (D/N)PXXY motif. *Journal of Biological Chemistry* **277**: 7356-7362.

Chevolot M, Hoarau G, Rijnsdorp AD, Stam WT, Olsen JL (2006) Phylogeography and population structure of thornback rays (*Raja clavata* L., Rajidae). *Molecular Ecology* **15**: 3693-3705.

Chiang HC, Hsu CC, Wu GCC, Chang SK, Yang HY (2008) Population structure of bigeye tuna (*Thunnus obesus*) in the Indian Ocean inferred from mitochondrial DNA. *Fisheries Research* **90**: 305-312.

Chin NKM, Brown MT Heads MJ (1991). The biogeography of Lessoniaceae, with special reference to *Macrocystis* C. Agardh (Phaeophyta: Laminariales). *Hydrobiologia* **215**: 1-11.

Chistiakov DA, Hellemans B, Haley CS, Law AS, Tsigenopoulos CS, Kotoulas G, Bertotto D, Libertini A, Volckaert FA (2005) A microsatellite linkage map of the European sea bass *Dicentrarchus labrax* L. *Genetics* **170**: 1820-1826.

Chlaida M, Laurent V, Kifani S, Benazzou T, Jaziri H, Planes S (2009) Evidence of a genetic cline for *Sardina pilchardus* along the Northwest African coast. *ICES Journal of Marine Science* **66**: 264-271.

Chow S, Okamoto H, Miyabe N, Hiramatsu K, Barut N (2000) Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. *Molecular Ecology* **9**: 221-227.

Chow S, Suzuki N, Brodeur RD, Ueno Y (2009) Little population structuring and recent evolution of the Pacific saury (*Cololabis saira*) as indicated by mitochondrial and nuclear DNA sequence data. *Journal of Experimental Marine Biology and Ecology* **369**: 17-21.

Cimmaruta R, Bondanelli P, Nascetti G (2005) Genetic structure and environmental heterogeneity in the European hake *Merluccius merluccius*. *Molecular Ecology* **14**: 2577-2591.

Cnaania A, Hallerman EM, Ron M, Weller JI, Indelman M, Kashi Y, Gall GAE, Hulata G (2003) Detection of a chromosomal region with two quantitative trait loci, affecting cold tolerance and fish size, in an F2 tilapia hybrid. *Aquaculture* **223**: 117-128.

Costedoat C, Chappaz R, Barascud B, Guillard O, Gilles A (2006) Heterogeneous colonization pattern of European Cyprinids, as highlighted by the dace complex (Teleostei: Cyprinidae). *Molecular Phylogenetics and Evolution* **41**: 127-148.

Cowen RK, Sponaugle S (2009) Larval dispersal and marine population connectivity. *Annual Review of Marine Science* **1**: 443-466.

Coyer JA, Smith GJ, Andersen RA (2001) Evolution of *Macrocystis* spp. (Phaeophyceae) as determined by ITS1 and ITS2 sequences. *Journal of Phycology* **37:** 574-585.

Craig MT, Eble JA, Bowen BW, Robertson DR (2007) High genetic connectivity across the Indian and Pacific Oceans in the reef fish *Myripristis berndti* (Holocentridae). *Marine Ecology Progress Series* **334**: 245-254.

Crame JA (1993) Bipolar molluscs and their evolutionary implications. *Journal of Biogeography* **20**: 145-161.

Cunningham KM, Canino MF, Spies IB, Hauser L (2009) Genetic isolation by distance and localized fjord population structure in Pacific cod (*Gadus macrocephalus*): limited effective dispersal in the northeastern Pacific Ocean. *Canadian Journal of Fisheries and Aquatic Sciences* **66**: 153-166.

Cutler KB, Edwards RF, Taylor FW, Cheng H, Adkins J, Gallup CD, Cutler MP, Burr GS, Bloom AL (2003) Rapid sea-level fall and deep-ocean temperature change since the last interglacial period. *Earth and Planetary Science Letters* **206**: 253-271.

David P, Perdieu MA, Jarne P (1997) Fine-grained spatial and temporal population genetic structure in the marine bivalve *Spisula ovalis*. *Evolution* **51**: 1318-1322.

Dercourt J, Zonenshain LP, Ricou LE, Kazmin VG, Le Pichon X, Knipper AL, Grandjacquet C, Sbortshikov IM, Geyssant J, Lepvrier C, Pechersky DH, Boulin J, Sibuet JC, Savostin LA, Sorokhtin O, Westphal M, Bazhenov ML, Lauer JP, Biju-Duval B, Le Pichon X, Monin AS (1986) Geological evolution of the Tethys belt from the Atlantic to the Pamirs since the Lias. *Tectonophysics* **123**: 241-315.

DeWoody JA, Walker D, Avise JC (2000) Genetic parentage in large half-sib clutches: theoretical estimates and empirical appraisals. *Genetics* **154**: 1907-1912.

Díaz-Jaimes P, Uribe-Alcocer M, Rocha-Olivares A, García-de-León FJ, Nortmoon P, Durand JD (2010) Global phylogeography of the dolphinfish (*Coryphaena hippurus*): The influence of large effective population size and recent dispersal on the divergence of a marine pelagic cosmopolitan species. *Molecular Phylogenetics and Evolution* **57**: 1209-1218.

Diggles BK (2002) Import risk assessment: Juvenile yellowtail kingfish (*Seriola lalandi*) from Spencer Gulf aquaculture, South Australia. National Institute of Water and Atmospheric Research (NIWA) Ltd, Kilbirnie, Wellington, New Zealand.

Dizon AE, Lockyer C, Perrin WF, DeMaster DP, Sisson J (1992) Rethinking the stock concept: a phylogeographic approach. *Conservation Biology* **6**: 24-36.

Dodson JJ, Tremblay S, Colombani F, Carscadden JE, Lecomte F (2007) Trans-Arctic dispersals and the evolution of a circumpolar marine fish species complex, the capelin *Mallotus villosus*. *Molecular Ecology* **16**: 5030-5043.

Doiuchi R, Nakabo T (2006) Molecular phylogeny of the stromateoid fishes (Teleostei: Perciformes) inferred from mitochondrial DNA sequences and compared with morphology-based hypotheses. *Molecular Phylogenetics and Evolution* **39**: 111-123.

Donald KM, Kennedy M, Spencer HG (2005) The phylogeny and taxonomy of austral monodontine topshells (Mollusca: Gastropoda: Trochidae), inferred from DNA sequences. *Molecular Phylogenetics and Evolution* **37**: 474-483.

Doyle JJ, Doyle JL (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* **19**: 11-15.

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

Drummond AJ, Ho SYW, Phillips MJ, Rambaut A (2006) Relaxed phylogenetics and dating with confidence. *PLOS Biology* **4**: e88.

Drummond AJ, Suchard MA, Dong X, Rambaut A (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29**: 1969-1973.

Dudgeon CL, Broderick D, Ovenden JR (2008) IUCN classification zones concord with, but underestimate, the population genetic structure of the zebra shark *Stegostoma fasciatum* in the Indo-West Pacific. *Molecular Ecology* **18**: 248-261.

Duque C, Aro H (1990) Neogene stratigraphy, paleoceanography and paleobiography in northwest South America and the evolution of the Panama Seaway. *Palaeogeography, Palaeoclimatology, Palaeoecology* **77**: 203-234.

Durand JD, Collet A, Chow S, Guinand B, Borsa P (2005) Nuclear and mitochondrial DNA markers indicate unidirectional gene flow of Indo-Pacific to Atlantic bigeye tuna (*Thunnus obesus*) populations, and their admixture off southern Africa. *Marine Biology* **147**: 313-322.

Durrett R, Buttel L, Harrison R (2000) Spatial models for hybrid zones. *Heredity* **84**: 9-19.

Edmands S, Moberg PE, Burton RS (1996) Allozyme and mitochondrial DNA evidence of population subdivision in the purple sea urchin, *Strongylocentrotus purpuratus*. *Marine Biology* **126**: 443-450.

Eizirik E, Kim JH, Menotti-Raymond M, Crawshaw PG Jr, O'Brien SJ, Johnson WE (2001) Phylogeography, population history and conservation genetics of jaguars (*Panthera onca*, Mammalia, Felidae). *Molecular Ecology* **10**: 65-79.

Ekman S (1953) *Zoogeography of the sea*. Sidgwick and Jackson, London.

Ekrem T, Willassen E, Stur E (2010) Phylogenetic utility of five genes for dipteran phylogeny: A test case in the Chironomidae leads to generic synonymies. *Molecular Phylogenetics and Evolution* **57**: 561-571.

Ely B, Viñas J, Alvarado-Bremer J, Black D, Lucas L, Covello K, Labrie AV, Thelen E (2005) Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) and the skipjack tuna (*Katsuwonis pelamis*). *BMC Evolutionary Biology* **5**: 19.

Eschmeyer WN, Herald ES, Hammann H (1983) *A field guide to Pacific coast fishes of North America*. Houghton Mifflin Company, Boston.

Estoup A, Jarne P, Cornuet JM (2002) Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Molecular Ecology* **11**: 1591-1604.

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

Evans BS, Sweijd NA, Bowie RCK, Cook PA, Elliott NG (2004) Population genetic structure of the perlemoen *Haliotis midae* in South Africa: evidence of range expansion and founder events. *Marine Ecology Progress Series* **270**: 163-172.

Evans RD, van Herwerden L, Russa GR, Frischa AJ (2010) Strong genetic but not spatial subdivision of two reef fish species targeted by fishers on the Great Barrier Reef. *Fisheries Research* **102**: 16-25.

Excoffier L (2004) Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. *Molecular Ecology* **13**: 853-864.

Excoffier L, Laval G, Schneider S (2005) Arlequin (version 3.0): an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* **1**: 47-50.

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* **131**: 479-491.

FAO (2012) FAO Fisheries and Aquaculture – FishStat plus. www.fao.org.

Fauvelot C, Bernardi G, Planes S (2003) Reductions in the mitochondrial DNA diversity of coral reef fish provide evidence of population bottlenecks resulting from Holocene sea-level change. *Evolution* **57**: 1571-1583.

Felsenstein J (1981) Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution* **17**: 368-376.

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.

Ferris SD, BergWJ (1987) The utility of mitochondrial DNA in fish genetics and fishery management. In: *Population genetics and fishery management*. Ed. by Ryman N, Utter F. University Washington Press, Seattle.

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

Floeter SR, Rocha LA, Robertson DR, Joyeux JC, Smith-Vaniz WF, Wirtz P, Edwards AJ, Barreiros JP, Ferreira CEL, Gasparini JL, Brito A, Falcon JM, Bowen BW, Bernardi G (2008) Atlantic reef fish biogeography and evolution. *Journal of Biogeography* **35**: 22-47.

Flores JA, Gersonde R, Sierro FJ (1999) Pleistocene fluctuations in the Agulhas Current Retroflection based on the calcareous plankton record. *Marine Micropaleontology* **37:** 1-22.

Fowler AJ, Ham JM, Jennings PR (2003) Discriminating between cultured and wild yellowtail kingfish (*Seriola lalandi*) in South Australia. SARDI Aquatic Sciences Publication, Adelaide, South Australia.

Francois O, Ancelet S, Guillot G (2006) Bayesian clustering using hidden Markov random field in spatial population genetics. *Genetics* **174:** 805-816.

Fraser CI, Nikula R, Spencer HG, Waters JM (2009) Kelp genes reveal effects of subantarctic sea ice during the Last Glacial Maximum. *Proceedings of the National Academy of Sciences, USA* **106**: 3249-3253.

Fraser DJ, Bernatchez L (2005) Allopatric origins of sympatric brook charr populations: colonization history and admixture. *Molecular Ecology* **14**: 1497-1509.

Frimodt C (1995) *Multilingual illustrated guide to the world's commercial coldwater fish*. Fishing News Books, Osney Mead, Oxford, England.

Fu YX (1997) Statistical tests of neutrality of mutations against population growth and background selection. *Genetics* **147**: 915-925.

Fuji T, Nishida M (1997). High sequence variability in the mitochondrial DNA control region of the Japanese flounder *Paralichthys olivaceus*. *Fisheries Science* **63**: 906-910.

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

Gatesy J, O'Grady P, Baker RH (1999) Corroboration among data sets in simultaneous analysis: hidden support for phylogenetic relationships among higher level artiodactyl taxa. *Cladistics* **15**: 271-313.

Gilbert-Horvath EA, Larson RJ, Garza JC (2006) Temporal recruitment patterns and gene flow in kelp rockfish (*Sebastes atrovirens*). *Molecular Ecology* **15**: 3801-3815.

Gillanders BM, Ferrell DJ, Andrew NL (1997) Determination of ageing in kingfish *(Seriola lalandi*) in New South Wales. Fisheries Research Development Corporation. Australia.

Gillanders BM, Ferrell DJ, Andrew NL (2001) Estimates of movement and life history parameters of yellowtail kingfish (*Seriola lalandi*): how useful are data from a cooperative tagging programme? *Marine and Freshwater Research* **52**: 179-192.

Gladden JGB, Ferguson MM, Friesen MK, Clayton JW (1999) Population structure of North American beluga whales (*Delphinapterus leucas*) based on nuclear DNA microsatellite variation and contrasted with the population structure revealed by mitochondrial DNA variation. *Molecular Ecology* **8**: 347-363.

Golani D, Orsi-Relini L, Massutí E, Quignard JP (2002) In: *CIESM atlas of exotic species in the Mediterranean: Fishes.* Ed. by Briand F. CIESM Publishers, Monaco.

Gold RJ, Richardson LR, Turner TF (1999) Temporal stability and spatial divergence of mitochondrial DNA haplotype frequencies in red drum (*Sciaenops ocellatus*) from coastal regions of the western Atlantic Ocean and Gulf of Mexico. *Marine Biology* **133**: 593-602.

Goodman SJ (1998) Patterns of extensive genetic differentiation and variation among European harbour seals (*Phoca vitulina vitulina*) revealed using microsatellite DNA polymorphisms. *Molecular Biology and Evolution* **15**:104-118.

Gomon M, Bray D, Kuiter R (2008) *Fishes of Australia's southern coast*. Sydney: Reed New Holland.

González-Wangüemert M, Cánovas F, Pérez-Ruzafa A, Marcos C, Alexandrino P (2010) Connectivity patterns inferred from the genetic structure of white seabream (*Diplodus sargus* L.). *Journal of Experimental Marine Biology and Ecology* **383**: 23-31.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3) (updated from Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate F-statistics). *Journal of Heredity* **86**: 485-486.

Goudet J, Raymond M, de Meeüs T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics* **144**: 1933-1940.

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.

Grant WS, Leslie RW (2001) Inter-ocean dispersal is an important mechanism in the zoogeography of hakes (Pisces: *Merluccius* spp.). *Journal of Biogeography* **28**: 699-721.

Grant WS, Leslie RW (2005) Bayesian analysis of allozyme markers indicates a single genetic population of kingklip *Genypterus capensis* off South Africa. *African Journal of Marine Science* **27**: 479-486.

Grant WS, Robin AC, Leslie W, Cherry MI (1992) Population genetics of the brown mussel *Perna perna* in southern Africa. *Journal of Experimental Marine Biology and Ecology* **165:** 45-58.

Graves JE, McDowell JR (2003) Stock structure of the world's istiophorid bill fishes: a genetic perspective. *Marine and Freshwater Research* **54**: 287-298.

Gray RD, Drummond AJ, Greenhill SJ (2009) Language phylogenies reveal expansion pulses and pauses in Pacifica Settlement. *Science* **323**: 479-483.

Griffiths CL, Robinson TB, Mead A (2010) Marine biodiversity in South Africa: an evaluation of current states of knowledge. *PLoS ONE* **5**: e12008.

Griffiths MH (2000) Long-term trends in catch and effort of commercial linefish off South Africa's Cape Province: snapshots of the 20th century. *South African Journal of Marine Science* **22**: 81-110.

Grigg RW, Hey R (1992) Paleoceanography of the tropical eastern Pacific Ocean. *Science* **255**: 172-178.

Gruenthal KM, Burton RS (2005) Genetic diversity and species identification in the endangered white abalone (*Haliotis sorenseni*). *Conservation Genetics* **6**: 929-939.

Guindon S, Gascuel O (2003) A simple, fast and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systems Biology* **52**: 696-704.

Hall TA (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95⁄98⁄NT. *Nucleic Acids Symposium Series* **41**: 95-98.

Hansen B, Østerhus S (2000) North Atlantic-Nordic Seas exchanges. *Progress in Oceanography* **45:** 109-208.

Hare MP, Avise JC (1996) Molecular genetic analysis of a stepped multilocus cline in the American oyster (*Crassostrea virginica*). *Evolution* **50**: 2305-2315.

Harlin-Cognato AD, Markowitz T, Wuersig B, Honeycutt RL (2007) Multi-locus phylogeography of the dusky dolphin *(Lagenorhynchus obscurus*): passive dispersal via the west-wind drift or response to prey species and climate change? *BMC Evolutionary Biology* **7**: 131.

Hart MW, Marko PB (2010) It's about time: Divergence, demography, and the evolution of developmental modes in marine invertebrates. *Integrative and Comparative Biology* **50**: 643-661.

Hasselman DJ, Ricard D, Bentzen P (2013) Genetic diversity and differentiation in a wide ranging anadromous fish, American shad (*Alosa sapidissima*), is correlated with latitude. *Molecular Ecology* **22**: 1558-1573.

Hedgecock D (1994) Does variance in reproductive success limit effective population size? In: *Genetics and Evolution of Aquatic Organisms*. Ed. by Beaumont AR. Chapman and Hall, London.

Heemstra P, Heemstra E (2004) Coastal fishes of southern Africa. NISC Publishers and SAIAB, Grahamstown, South Africa.

Hellberg ME, Burton RS, Neigel JE, Palumbi SR (2002) Genetic assessment of connectivity among marine populations. *Bulletin of Marine Science* **70**: 273-290.

Hemmer-Hansen J, Nielsen EE, Frydenberg J, Loeschcke V (2007a) Adaptive divergence in a high gene flow environment: Hsc70 variation in the European flounder (*Platichthys flesus* L.). *Heredity* **99**: 592-600.

Hemmer-Hansen J, Nielsen EEG, Gronkjaer P, Loeschcke V (2007b) Evolutionary mechanisms shaping the genetic population structure of marine fishes; lessons from the European flounder (*Platichthys flesus* L.). *Molecular Ecology* **16**: 3104-3118.

Hendry AP, Day T (2005) Population structure attributable to reproductive time: isolation by time and adaptation by time. *Molecular Ecology* **14**: 901-916.

Hewitt GM (2000) The genetic legacy of the Quaternary ice ages. *Nature* **405:** 907-913.

Hewitt GM (2004) Genetic consequences of climatic oscillations in the Quantenary. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**: 183-195.

Hoarau G, Piquet AMT, van der Veer HW, Stam WT, Olsen JL (2004) Population structure of plaice (*Pleuronectes platessa* L.) in northern Europe: a comparison of resolving power between microsatellites and mitochondrial DNA data. *Journal of Sea Research* **51**: 183-190.

Hobbs JPA, Frisch AJ, Allen GR, Van Herwerden L (2009) Marine hybrid hotspot at Indo-Pacific biogeographic border. *Biology Letters* **5**: 258-261.

Hoelzel AR, Shivji MS, Magnussen J, Francis MP (2000) Low worldwide genetic diversity in the basking shark (*Cetorhinus maximus*). *Biology Letters* **2**: 639-642.

Hrbek T, Seekinger J, Meyer A (2007) A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. *Molecular Phylogenetics and Evolution* **43**, 986-998.

Huelsenbeck JP, Imennov NS (2002) Geographic origin of human mitochondrial DNA: accommodating phylogenetic uncertainty and model comparison. *Systematic Biology* **51**: 155-165.

Hueter R, Heupel M, Heist E, Keeney D (2004) The implications of philopatry in sharks for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science* **35**: 239-247.

Hueter RE, Heupel MR, Heist EJ, Keeney DB (2005) Evidence of philopatry in sharks and implications for the management of shark fisheries. *Journal of Northwest Atlantic Fishery Science* **35**: 239-247.

Hurt C, Anker A, Knowlton N (2009) A multilocus test of simultaneous divergence across the Isthmus of Panama using snapping shrimp in the genus *Alpheus*. *Evolution* **63**: 514-530.

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

Hutchings K, Porter S, Clark BM, Sink K (2010) Identification of potential marine aquaculture development zones for fin fish cage culture. Department of Agriculture, Forestry and Fisheries. South Africa.

Hutchings L, Beckley LE, Griffiths MH, Roberts MJ, Sundby S, van der Lingen C (2002) Spawning on the edge spawning grounds and nursery areas around the southern African coastline. *Marine and Freshwater Research* 53: 307-318.

Hutchinson WF, Carvalho GR, Rogers SI (2001) Marked genetic structuring in localized spawning populations of cod *Gadus morhua* in the North Sea and adjoining waters, as revealed by microsatellites. *Marine Ecology Progress Series* **223**: 251-260.

Hyde JR, Vetter RD (2007) The origin, evolution, and diversification of the rockfishes of the genus *Sebastes*. *Molecular Phylogenetics and Evolution* **44**: 790-811.

Ida H, Murofushi S, Fujiwara S, Fujino K (1978) Preparation of fish chromosomes by *in vitro* colchicine treatment. *Japanese Journal of Ichthyology* **24**: 281-284.

Imbrie J, Boyle E, Clemens S, Duffy A, Howard W, Kukla G, Kutzbach J, Martinson D, McIntyre A, Mix A, Molfino B, Morley J, Peterson L, Pisias N, Prell W, Raymo M, Shackleton N, Toggweiler JR (1992). One the structure and origin of major glaciation cycles. *Paleoceanography* **7**: 701-738.

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* **23**: 1801-1806.

Javonillo R, Malabarba LR, Weitzman SH, Burns JR (2010) Relationships among major lineages of characid fishes (Teleostei: Ostariophysi: Characiformes), based on molecular sequence data. *Molecular Phylogenetics and Evolution* **54**: 498-511.

Joensen H, Steingrund P, Fjallstein I, Grahl-Nielsen O (2000) Discrimination between two reared stocks of cod (*Gadus morhua*) from the Faroe Islands by chemometry of the fatty acid composition in the heart tissue. *Marine Biology* **136**: 573-580.

Johns G, Avise J (1998) A comparative summary of genetic distances in the vertebrates from the mitochondrial cytochrome b gene. *Molecular Biology and Evolution* **15**: 1481-1490.

Johnson JA, Toepfer JE, Dunn PO (2003) Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens. *Molecular Ecology* **12**: 3335-3347.

Johnson MS, Black R (1982) Chaotic genetic patchiness in an intertidal limpet, *Siphonaria* sp. *Marine Biology* **70**: 157-164.

Johnson MS, Black R (1984) Pattern beneath the chaos: the effect of recruitment on genetic patchiness in an intertidal limpet. *Evolution* **38**: 1371-1383.

Johnson MS, Black R (1998) Effects of isolation by distance and geographical discontinuity on genetic subdivision of *Littorina cingulata*. *Marine Biology* **132**: 295-303.

Jordan DS (1908) The law of geminate species. *American Naturalist* **42**: 73-80.

Kailola PJ, Williams MJ, Steward PC, Reichelt RE, McNee A, Griece C (1993) Australian Fisheries Resources. Bureau of Recource Science and Fisheries Development Cooperation. Canberra.

Kanda N, Goto M, Kato H, McPhee MV, Pastene LA (2007) Population genetic structure of Bryde's whales (Balaenoptera brydei) at the inter-oceanic and transequatorial levels. *Conservation Genetics* 8: 853-864.

Kanno M, Suyama Y, Li Q, Kijima A (2006). Microsatellite analysis of Japanese sea cucumber, *Stichopus (Apostichopus) japonicus*, supports reproductive isolation in color variants. *Marine Biotechnology* **6**: 672-685.

Karaiskou N, Apostolidis A, Triantafyllidis A, Kouvatsi A, Triantaphyllidis C (2003) Genetic identification and phylogeny of three species of the genus *Trachurus* based on mtDNA analysis. *Marine Biotechnology* **5**: 493-504.

Karl SA, Bowen BW, Avise JC (1992) Global population genetic structure and male mediated gene flow in the green turtle (*Chelonia mydas*): RFLP analysis of anonymous nuclear loci. *Genetics* **131**: 163-173.

Karl SA, Toonen RJ, Grant WS, Bowen BW (2012) Common misconceptions in molecular ecology: echoes of the modern synthesis. *Molecular Ecology* **21**: 4171-4189.

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

Kimura M, Ohta T (1971) *Theoretical Aspects of Population Genetics*. Princeton Univ. Press, Princeton, NJ.

Kinitz T, Quack M, Paulus M, Veith M, Bergek S, Strand J, Tuvikene A, Soirinsuo A, Hochkirch A (2013) Strong isolation-by-distance in the absence of genetic population structure in the eelpout (*Zoarces viviparus*, Linnaeus 1758). *Ecological Indicators* **27**: 116-122.

Kirby MX, Jones DS, MacFadden BJ (2008) Lower miocene stratigraphy along the Panama Canal and its bearing on the Central American peninsula. *PloS ONE* **3**: e2791.

Klopper AW (2005) Intraspecific genetic variation in the percoid teleosts, *Argyrosomus japonicus* (Temminck and Schlegel, 1843) and *Pomadasys commersonnii* (Lacepede, 1801), as inferred from the mitochondrial control region. Unpublished MSc thesis, University of Pretoria, South Africa.

Knowlton N, Weigt LA (1998) New dates and new rates for divergence across the Isthmus of Panama. *Proceedings of the Royal Society B: Biological Sciences* **265**: 2257-2263.

Knowlton N, Weight LA, Solorzano LA, Mills DK, Bermingham E (1993) Divergence in proteins, mitochondrial DNA, and reproductive compatibility across the isthmus of Panama. *Science* **260**: 1629-1632.

Knutsen H, Jorde PE, André C, Stenseth NC (2003) Fine-scaled geographic population structuring in a highly mobile marine species: the Atlantic cod. *Molecular Ecology* **12**: 385-394.

Koehn RK (1969) Esterase heterogeneity: dynamics of a polymorphism. *Science* **163**: 943-944.

Koehn RK, Williams GC (1978) Genetic differentiation without isolation in American eel, *Anguilla rostrata*. *Evolution* **32**: 624-637.

Koehn RK, Hall JG, Zera AJ (1980) Parallel variation of genotype-dependent aminopeptidase-I activity between *Mytilus edulis* and *Mercenaria mercenaria*. *Marine Biology Letters* **1**: 245-254.

Kordos LM, Burton RS (1993) Genetic differentiation of Texas Gulf Coast populations of the blue crab *Callinectes sapidus*. *Marine Biology* **117**: 227-233.

Lakra WS, Goswami M, Gopalakrishnan A, Singh DP, Singh A, Nagpure NS (2010) Genetic relatedness among fish species of genus *Channa* using mitochondrial DNA genes. *Biochemical Systematics and Ecology* **38:** 1212-1219.

Larmuseau MHD, Raeymaekers JAM, Ruddick KG, Van Houdt JKJ, Volckaert FAM (2009) To see in different seas: spatial variation in the rhodopsin gene of the sand goby *(Pomatoschistus minutus*). *Molecular Ecology* **18**: 4227-4239.

Larson RJ, Julian RM (1999) Spatial and temporal genetic patchiness in marine populations and their implications for fisheries management. *CalCOFI Rep* 40:94-99.

Lehman TN, Besansky J, Hawley WA (1997) Microgeographic structure of *Anopheles gambiae* in western Kenya based on mtDNA and microsatellite loci. *Molecular Ecology* **6**: 243-253.

Lessios HA (2008) The Great American Schism: divergence of marine organisms after the rise of the Central American Isthmus. *Annual Review of Ecology Evolution and Systematics* **39**: 63-91.

Lessions HA, Robertson DR (2006) Crossing the impassable: genetic connections in 20 reef fishes across the eastern Pacific barrier. *Proceedings of the Royal Society B: Biological Sciences* **273**: 2201-2208.

Lessios HA, Kane J, Robertson DR (2003) Phylogeography of the pantropical sea urchin *Tripneustes*: contrasting patterns of population structure between oceans. *Evolution* **57**: 2026-2036.

Levin LA (2006) Recent progress in understanding larval dispersal: new directions and digressions. *Integrative and Comparative Biology* **46**: 282-297.

Li G, Hedgecock D (1998) Genetic heterogeneity, detected by PCR-SSNP, among samples of larval Pacific oysters (*Crassostrea gigas*) supports the hypothesis of large variance in reproductive success. *Canadian Journal of Fisheries and Aquatic Sciences* **55**: 1025-1033.

Lim LS, Ang KC, Mahani MC, Shahrom AW, Md-Zain BM (2010) Mitochondrial DNA polymorphism and phylogenetic relationships of Proto Malays in Peninsular Malaysia. *International Journal of Biological Sciences* **10**: 71-83.

Lin HC, Hastings PA (2011) Evolution of a Neotropical marine fish lineage (Subfamily Chaenopsinae, Suborder Blennioidei) based on phylogenetic analysis of combined molecular and morphological data. *Molecular Phylogenetics and Evolution* **60**: 236-248.

Lindberg DR (1991) Marine biotic exchange between the northern and southern hemispheres. *Paleobiology* **17**: 308-324.

Livi S, Sola L, Donatella Crosetti D (2011) Phylogeographic relationships among worldwide populations of the cosmopolitan marine species, the striped gray mullet (*Mugil cephalus*), investigated by partial cytochrome b gene sequences. *Biochemical Systematics and Ecology* **39**: 121-131.

Liu H, Wang M, Xia CH, Du X, Flannery JG, Ridge KD, Beutler B, Gong X (2010) Severe retinal degeneration caused by a novel rhodopsin mutation. *Investigative Ophthalmology and Visual Science* **51**: 1059-1065.

Liu JY, Brown CL, Yong TB (2010) Phylogenetic relationships of mullets (Mugilidae) in China Seas based on partial sequences of two mitochondrial genes. *Biochemical Systematics and Ecology* **38**: 647-655.

Liu JX, Gao TX, Wu S F, Zhang YP (2007). Pleistocene isolation in the Northwestern Pacific marginal seas and limited dispersal in a marine fish, *Chelon haematocheilus* (Temminck and Schlegel, 1845). *Molecular Ecology* **16**: 275-288.

Liu ZJ, Cordes FJ (2004) DNA marker technologies and their applications in aquaculture genetics. *Aquaculture* **238**: 1-37.

Longhurst A (1998). *Ecological Geography of the Sea*. San Diego: Academic Press.

López MD, Alcocer MU, Jaimes PD (2010) Phyleography and historical demography of the Pacific Sierra mackerel (*Scomberomorus sierra*) in the Eastern Pacific. *BMC Genetics* **11**: 34.

Lu CP, Chen CA, Hui CF, Tzeng TD, Yeh SY (2006) Population genetic structure of the swordfish, *Xiphias gladius* (Linnaeus, 1758), in the Indian Ocean and West Pacific inferred from the complete DNA sequence of the mitochondrial control region. *Zoological Studies* **45**: 269-279.

Lundy CJ, Rico C, Hewitt GM (2000) Temporal and spatial genetic variation in spawning grounds of European hake (*Merluccius merluccius*) in the Bay of Biscay. *Molecular Ecology* **9**: 2067-2079.

Lutjeharms JRE (2006) *The Agulhas Current*, 1st edn. Springer, Berlin.

Luttikhuizen PC, Drent J, Baker AJ (2003) Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in a marine bivalve with pelagic larval dispersal. *Molecular Ecology* **12**: 2215-2229.

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

Machado-Schiaffino G, Campo D, Garcia-Vazquez E (2009) Strong genetic differentiation of the Austral hake (*Merluccius australis*) across the species range. *Molecular Phylogenetics and Evolution* **53**: 351-356.

Maes GE, Volckaert FAM (2002) Clinal genetic variation and isolation by distance in the European eel *Anguilla anguilla* (L.). *Biological Journal of the Linnean Society* **77**: 509-521.

Maes GE, Pujolar JM, Hellemans B, Volckaert FAM (2006) Evidence for isolation by time in the European eel (*Anquilla anguilla* L.). *Molecular Ecology* **15**: 2095-2107.

Manni F, Guérard E, Heyer E (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: how barriers can be detected by "Monmonier's algorithm". *Human Biology* **76**: 173-190.

Manooch CM, Potts JC (1997) Age, growth and mortality of greater amberjack from the southeastern United States. *Fisheries Research* **30**: 229-240.

Mao Y, Gao T, Yanagimoto T, Xiao Y (2011) Molecular phylogeography of *Ruditapes philippinarum* in the Northwestern Pacific Ocean based on COI gene. *Journal of Experimental Marine Biology and Ecology* **407**: 171-181.

Marko PB (2002) Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama. *Molecular Biology and Evolution* **19**: 2005-2021.

Marko PB, Hart MW (2011) The complex analytical landscape of gene flow inference. *Trends in Ecology and Evolution* **26**: 448-456.

Martínez P, González EG, Castilho R, Zardoya R (2005) Genetic diversity and historical demography of Atlantic bigeye tuna (*Thunnus obesus*). *Molecular Phylogenetics and Evolution* **39**: 404-416.

Matthee CA, Cockcroft AC, Gopal K, Von der Heyden S (2007) Mitochondrial DNA variation of the west-coast rock lobster, *Jasus lalandii*: Marked genetic diversity differences among sampling sites. *Marine and Freshwater Research* **58**: 1130-1135.

May JL, Maxwell JGH (1986) Trawl fish from temperate waters of Australia. CSIRO Division of Fisheries Research, Tasmania.

Maynard-Smith J, Haigh J (1974) The hitch-hiking effect of a favourable gene. *Genetics Research* **23**: 23-35.

Mayr E (1963) *Animal Species and Evolution*. Cambridge: Belknap Press of Harvard University Press.

McCartney MA, Lessios HA (2002) Quantitative analysis of gametic incompatibility between closely related species of neotropical sea urchins. *Biological Bulletin* **202**: 166-181.

McGregor GA (1995) Is the northern region the kingfish capital of the Pacific? Part 1: the fish. Seafood N.Z.

McPherson AA, O'Reilly PT, Taggart CT (2004) Genetic differentiation, temporal stability, and the absence of isolation by distance among Atlantic herring populations. *Transactions of the American Fisheries Society* **133**: 434-446.

Menezes MR, Ikeda M, Taniguchi N (2006) Genetic variation in skipjack tuna *Katsuwonus pelamis* (L.) using PCR-RFLP analysis of the mitochondrial DNA D-loop region. *Journal of Fish Biology* **68**: 156-161.

Merritt RB (1972) Geographic distribution and enzymatic properties of lactate dehydrogenase allozymes in the fathead minnow, *Pimephales promelas*. *American Naturalist* **106**: 173-184.

Meyer A (1993) *Evolution of mitochondrial DNA in fishes*, 2 edn. Elsevier Science Publishers B. V., Amsterdam.

Meylan AB, Bowen BW, Avise JC (1990) A genetic test of the natal homing versus social facilitation models for green turtle migration. *Science* **248**: 724-727.

Miller KG, Kominz MA, Browning JV, Wright JD, Mountain GS, Katz ME, Sugarman PJ, Cramer BS, Christie-Blick N, Pekar SF (2005) The Phanerozoic record of global sea-level change. *Science* **310**: 1293-1298.

Miller PA, Fitch AJ, Gardner M, Hutson KS, Mair G (2011) Genetic population structure of yellowtail kingfish (*Seriola lalandi*) in temperate Australasian waters inferred from microsatellite markers and mitochondrial DNA. *Aquaculture* **319**: 328-336.

Miura O, Torchin ME, Bermingham E (2010) Molecular phylogenetics reveals differential divergence of coastal snails separated by the Isthmus of Panama. *Molecular Phylogenetics and Evolution* **56**: 40-48.

Moberg PE, Burton RS (2000) Genetic heterogeneity among adult and recruit red sea urchins, *Strongylocentrotus franciscanus*. *Marine Biology* **136**: 773-784.

Monmonier MS (1973) Maximum-difference barriers: an alternative numerical regionalization method. *Geographical Analysis* **5**: 245-261.

Moritz C (1994) Defining “Evolutionarily Significant Units” for conservation. *Trends in Ecology and Evolution* **9**: 373-375.

Mourier J, Mills SC, Planes S (2013) Population structure, spatial distribution and life-history traits of blacktip reef sharks *Carcharhinus melanopterus*. *Journal of Fish Biology* **82**: 979-993.

Muths D, Grewe P, Jean C, Bourjea J (2009) Genetic population structure of the Swordfish (*Xiphias gladius*) in the southwest Indian Ocean: sex-biased differentiation congruency between markers and its incidence in a way of stock assessment. *Fisheries Research* **97**: 263-269.

Naish T, Powell R, Levy R, Wilson G, Scherer R, Talarico F, Kressek L, Niessen F, Pompilio M, Wilson T, Carter L, DeConto R, Huybers P, McKay R, Pollard D, Ross J, Winter D, Barret P, Browne G, Cody R, Cowen E, Crampton J, Dunbar G, Dunbar N, Florindo F, Gebhardt C, Graham I, Hannah M, Hanaraj D, Harwood D, Helling D, Henrys S, Hinnov L, Kuhn G, Kyle P, Läufer A, Maffioli P, Magens D, Mandernack K, McIntosh W, Millan C, Morin R, Ohneiser C, Paulsen T, Persico D, Raine I, Reed J, Riesselman C, Sagnotti L, Schmitt D, Sjunneskog C, Strong P, Taviani M, Vogel S, Wilch T, Williams T (2009) Obliquity-paced Pliocene West Atlantic ice sheet oscillations. *Nature* **458**: 322-328.

Nakada M (2008) Capture-based aquaculture of yellowtail. In *Capture-Based Aquaculture. Global Overview*. FAO Fisheries Technical Paper. FAO, Rome.

Natoli A, Peddemors VM, Hoss M (2004) Population structure and speciation in the genus *Tursiops* based on microsatellite and mitochondrial DNA analyses. *Journal of Evolutionary Biology* **17**: 363-375.

Natoli A, Peddemors VM, Aguilar A, Vaquero C, Fernàndez-Piqueras P, Hoelzel AR (2006) Phylogeography and alpha taxonomy of the common dolphin (*Delphinus* sp.). *Journal of Evolutionary Biology* **19**: 943-954.

Nazari V, Zakharov EV, Sperling FAH (2007). Phylogeny, historical biogeography, and taxonomic ranking of Parnassiinae (Lepidoptera, Papilionidae) based on morphology and seven genes. *Molecular Phylogenetics and Evolution* **42**: 131-156.

Nei M (1987) *Molecular Evolutionary Genetics.* Columbia University Press, New York.

Neethling M, Matthee CA, Bowie RCK, von der Heyden S (2008) Evidence for panmixia despite barriers to gene flow in the southern African endemic, *Caffrogobius caffer* (Teleostei: Gobiidae). *BMC Evolutionary Biology* **8**: 325.

Nelson J (2006) *Fishes of the world* (Fourth ed.). John Wiley and Sons, Inc., New Jersey.

Neubauer P, Jensen OP, Hutchings JA, Baum JK (2013) Resilience and recovery of overexploited marine populations. *Science* **340**: 347-349.

Nielsen EE, Kenchington E (2001) A new approach to prioritizing marine fish and shellfish populations for conservation. *Fish and Fisheries* **2**: 328-243.

Nielsen EE, Gronkjær P, Meldrup D, Paulsen H (2005) Retention of juveniles within a hybrid zone between North Sea and Baltic Sea Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* **62**: 2219-2225.

Nielsen EE, Hansen MM, Ruzzante DE, Meldrup D, Gronkjaer P (2003) Evidence of a hybrid-zone in Atlantic cod (*Gadus morhua*) in the Baltic and the Danish Belt Sea revealed by individual admixture analysis. *Molecular Ecology* **12**: 1497-1508.

Nielsen EE, Nielsen PH, Meldrup D, Hansen MM (2004) Genetic population structure of turbot (*Scophthalmus maximus* L.) supports the presence of multiple hybrid zones for marine

fishes in the transition zone between the Baltic Sea and the North Sea. *Molecular Ecology* **13**: 585-595.

Nikula R, Spencer HG, Waters JM (2012) Passive rafting is a powerful driver of transoceanic gene flow. *Biology Letters* **9**: 20120821.

Nomura K, Ozaki A, Morishima K, Yoshikawa Y, Tanaka H, Unuma T, Ohta H, Arai K (2011) A genetic linkage map of the Japanese eel (*Anguilla japonica*) based on AFLP and microsatellite markers. *Aquaculture* **310**: 329-342.

Norris AT, Bradley DG, Cunningham EP (2000) Parentage and relatedness determination in farmed Atlantic salmon (*Salmo salar*) using microsatellite markers. *Aquaculture* **182**: 73-83.

Norton OB (2005) The population structure of two estuarine fish species, *Atherina breviceps* and *Gilchristella aestuaria* along the southern African coastline. Unpublished MSc thesis, Rhodes University, Grahamstown, South Africa.

Nugroho E, Ferrell DJ, Smith P, Taniguchi N (2001) Genetic divergence of kingfish from Japan, Australia and New Zealand inferred by microsatellite DNA and mitochondrial DNA control region markers. *Fisheries Science* **67**: 843-850.

O'Connell M, Wright, JM (1997) Microsatellite DNA in fishes. *Reviews in Fish Biology and Fisheries* **7**: 331-363.

Ochiai A, Tanaka M (1986) *Ichthyology* (II). Kouseisyakouseiku, Tokyo

Ohara E, NishimuraT, NagakuraY, SakamotoT, Mushiake K, Okamoto N (2005) Genetic linkage maps of two yellowtails (*Seriola quinqueradiata* and *Seriola lalandi*). *Aquaculture* **244**: 41-48.

Olsen JL, Stam WT, Coyer JA, Reusch TB, Billingham M, Boström C, Calvert E, Christie H, Granger S, la Lumière R, Milchakova N, Oudot-Le Secq MP, Procaccini G, Sanjabi B, Serrao E, Veldsink J, Widdicombe S, Wyllie-Echeverria S (2004) North Atlantic phylogeography and large-scale population differentiation of the seagrass *Zostera marina* L. *Molecular Ecology* **13**: 1923-1941.

Oosthuizen A, Jiwaji M, Shaw P (2004) Genetic analysis of the *Octopus vulgaris* population on the coast of South Africa. *South African Journal of Science* **100**: 603-607.

Oosthuizen CJ (2007) Genetic variation within the Cape Stumpnose, *Rhabdosargus holubi* Steindachner (Teleostei: Sparidae). Unpublished MSc thesis, University of Pretoria, South Africa.

ORI (2012) Tagging news vol. 25 Oceanographic Research Institute. South Africa.

Palero F, González-Candelas F, Marta Pascual M (2011) MICROSATELIGHT - Pipeline to expedite microsatellite analysis. *Journal of Heredity* **102**: 247-249.

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

Palumbi SR (1997) Molecular biogeography of the Pacific. *Coral Reefs* **16**: 47-52.

Palumbi SR (2003) Population genetics, demographic connectivity and the design of marine protected areas. *Ecological Applications* **13**:146-158.

Palumbi SR (2004) Marine reserves and ocean neighbourhoods: the spatial scale of marine populations and their management. *Annual Review of Environment and Resources* **29:** 31-68.

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

- In some respects, these sharks behave more like whales and dolphins than other fish. *Nature* **412**: 139-140.

Patterson C, Owen HG (1991) Indian isolation or contact? A response to Briggs. *Systematic Zoology* **40**: 96-100.

Peeters FJC, Acheson R, Brummer GJA, de Ruijter WPM, Schneider RR, Ganssen GM, Ufkes E, Kroon D (2004) Vigorous exchange between the Indian and Atlantic Oceans at the end of the past five glacial periods. *Nature* **430**: 661-665.

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

Penney AJ (1990) Yellowtail migration patterns and their management implications. In: *Marine recreational fishing: resource usage, management and research.* Ed. by Van der Elst RP. South Africa Natural Science Progress Report.

Pepin P (1991) The effect of temperature and size on development and mortality rates of the pelagic early life history stages of marine fish. *Canadian Journal of Fisheries and Aquatic Sciences* **48**: 503-518.

Peterson RG, Stramma L (1991) Upper-level circulation in the South Atlantic Ocean. *Progress in Oceanography* **26**: 1-73.

Pinsky ML, Jensen OP, Ricard D, Palumbi SR (2011). Unexpected patterns of fisheries collapse in the world's oceans. *Proceedings of the National Academy of Sciences, USA* **108**: 8317-8322.

Planes S, Lenfant P (2002) Temporal change in the genetic structure between and within cohorts of a marine fish, *Diplodus sargus*, induced by a large variance in individual reproduction success. *Molecular Ecology* **11**: 1511-1524.

Pogson GH, Taggart CT, Mesa KA, Boutilier RG (2001) Isolation by distance in the Atlantic cod, *Gadus morhua* , at large and small geographic scales. *Evolution* **55**: 131-146.

Pol D (2004) Empirical problems of the hierarchical likelihood ratio test for model selection. *Systematic Biology* **53**: 949-962.

Poortenaar C, Jeffs A, Heath P, Hooker S (2003) Commercial opportunities for Kingfish aquaculture in Northland. NIWA, Auckland, New Zealand.

Porta JM, P Novel P, Martinez-Rodriguez G, Alvarez MC, Porta J (2009) Isolation and characterization of microsatellites from *Seriola dumerili* (Risso 1810). *Aquaculture Research* **40**: 249-251.

Portnoy DS, McDowell JR, Heist EJ, Musick JA, Graves JE (2010) World phylogeography and male-mediated gene flow in the sandbar shark, *Carcharhinus plumbeus. Molecular Ecology* **19**: 1994-2010.

Posada D (2008) JModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25:** 1253-1256.

Posada D, Buckley TR (2004) Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology* **53**: 793-808.

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817-818.

Poulin E, Cárdenas CA, Hernándes CE, Kornfield I, Ojeda FP (2004) Resolution of the taxonomic status of Chilean and Californian jack mackerels using mitochondrial DNA sequence. *Journal of Fish Biology* **65**: 1160-1164.

Poulsen NA, Nielsen EE, Schierup MH, Loeschke V, Grønkjær P (2006) Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhau*). *Molecular Ecology* **15**: 321-331.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* **155:** 945-959.

Pujolar JM, Bevacqua D, Andrello A, Capoccioni F, Ciccotti E, De Leo GA, Zane L (2011) Genetic patchiness in European eel adults evidenced by molecular genetics and population dynamics modeling. *Molecular Phylogenetics and Evolution* **58**: 198-206.

Pujolar JM, Maes GE, Volckaert FAM (2006) Genetic patchiness among recruits of the European eel *Anguilla anguilla*. *Marine Ecology Progress Series* **307**: 209-217.

Pujolar JM, Zane L, Congiu L (2012) Phylogenetic relationships and demographic histories of the Atherinidae in the Eastern Atlantic and Mediterranean Sea re-examined by Bayesian inference. *Molecular Phylogenetics and Evolution* **63**: 857-865.

Qiu F, Kitchen A, Beerli P, Miyamoto M (2013) A possible explanation for the population size discrepancy in tuna (genus *Thunnus*) estimated from mitochondrial DNA and microsatellite data. *Molecular Phylogenetics and Evolution* **66**: 463-468.

Quartly GD, Srokosz MA (1993) Seasonal variations in the region of the Agulhas eetroflection: Studies with Geosat and FRAM. *Journal of Physical Oceanography* **23:** 2107-2124.

Quigley DT (2007) Carangidae in Irish and Northern European Waters. *Sherkin Comment* vol 43.

Rabassa J, Coronato AM, Salemme M (2005) Chronology of the Late Cenozoic Patagonian glaciations and their correlation with biostratigraphic units of the Pampean region (Argentina). *Journal of South American Earth Sciences* **20**: 81-103.

Rambaut A, Drummond AJ (2007) Tracer v1.4.1. University of Edinburgh. Edinburgh, UK http://beast.bio.ed.ac.uk/Tracer.

Rannala B (2007) BayesAss Edition 3.0 User's Manual. Accessed online 27 November 2012 at: http://www.rannala.org/?page_id=245.

Ravago-Gotanco RG, Juinio-Meñez MA (2010) Phylogeography of the mottled spinefoot *Siganus fuscescens*: Pleistocene divergence and limited genetic connectivity across the Philippine archipelago. *Molecular Ecology* **19**: 4520-4534.

Reed DL, Carpenter KE, deGravelle MJ (2002) Molecular systematics of the Jacks (Perciformes: Carangidae) based on mitochondrial cytochrome b sequences using parsimony, likelihood, and Bayesian approaches. *Molecular Phylogenetics and Evolution* **23**: 513-524.

Reed DL, deGravelle MJ, Carpenter KE (2001) Molecular systematics of *Selene* (Perciformes: Carangidae) based on cytochrome b sequences. *Molecular Phylogenetics and Evolution* **21**: 468-475.

Reece JS, Bowen BW, Joshi K, Goz V, Larson A (2010) Phylogeography of two moray eels indicates high dispersal throughout the indo-pacific. *Journal of Heredity* **101**: 391-402

Rensaw MA, Patton JC, Rexroad CE, Gold JR (2006) PCR primers for trinucleotide and tetranucleotide microsatellites in greater amberjack, *Seriola dumerili*. *Molecular Ecology Notes* **6**: 1162-1164.

Rensaw MA, Patton JC, Rexroad CE, Gold JR (2007) Isolation and characterization of dinucleotide microsatelltes in greater amberjack, *Seriola dumerili*. *Conservation Genetics* **8**: 1009-1011.

Riginos C, Nachman MW (2001) Population subdivision in marine environments: the contributions of isolation by distance, discontinuous habitat, and biogeography to genetic differentiation in a blennioid fish, *Axoclinus nigricaudus*. *Molecular Ecology* **10**: 1439-1453.

Robertson DR, Allen GR (1996) Zoogeography of the shorefish fauna of Clipperton Atoll. *Coral Reefs* **15**: 121-131.

Robertson DR, Grove JS, McCosker JE (2004) Tropical transpacific shore fishes. *Pacific Scientific* **58**: 507-565.

Robinchaud D, Rose GA (2001) Multiyear homing of Atlantic cod to a spawning ground. *Canadian Journal of Fisheries and Aquatic Sciences* **58**: 2325-2329.

Rocha LA, Bass AL, Robertson DR, Bowen BW (2002) Adult habitat preferences, larval dispersal, and comparative phylogeography of three Atlantic surgeon fishes (Teleostei:Acanthuridae). *Molecular Ecology* **11**: 243-252.

Rocha LA, Craig MT, Bowen BW (2007) Phylogeography and the conservation of coral reef fishes. *Coral Reefs* **26**: 501-512.

Rocha LA, Robertson DR, Roman J, Bowen BW(2005) Ecological speciation in tropical reef fishes. *Proceedings of the Royal Society B: Biological Sciences* **272**: 573-579.

Rog F, Steininger FF (1984) Neogene Paratethys, Mediterranean and Indo-Pacific seaways. Implications for the paleo-biogeography of marine and terrestrial biotas. In: *Fossils and climate*. Ed. by Brenchley P. Wiley, New York.

Rogers A (1995) Genetic evidence for a Pleistocene population explosion. *Evolution* **49**: 608-615.

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

Roman J, Palumbi SR (2004) A global invader at home: population structure of the green crab, *Carcinus maenas*. *Molecular Ecology* **13**: 2891-2898.

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

Ronquist F, Huelsenbeck JP (2003) MrBayes: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572-1574.

Ropson J, Brown DC, Powers DA (1990) Biochemical genetics of *Fundulus heteroclitus* (L.). VI. Geographical variation in the gene frequencies of 15 loci. *Evolution* **44**: 16-26.

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes* **4**: 137-138.

Rousset F (1996) Equilibrium values of measures of population subdivision for stepwise mutation processes. *Genetics* **142**: 1357-1362.

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

Ruan X, Wang W, Kong J, Yu F, Huang X (2010) Genetic linkage mapping of turbot (*Scophthalmus maximus* L.) using microsatellite markers and its application in QTL analysis. *Aquaculture* **308**: 89-100.

Ruzzante DE, Taggart CT, Cook D (1996) Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort contribution and genetic stability. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 2695-2705.

Ruzzante DE, Taggart CT, Lang S, Cook D (2000) Mixed-stock analysis of Atlantic cod near the Gulf of St. Lawrence based on microsatellite DNA. *Ecological Applications* **10**: 1090-1109.

Ryder OA (1986) Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution* **1:** 9-10.

Sachs JP, Anderson RF, Lehman SJ (2001) Glacial surface temperatures of the southeast Atlantic Ocean. *Science* **293**: 2077-2079.

Sakakura Y, Tsukarnoto K (1996) Onset and development of cannibalistic behaviour in early life stages of yellowtail. *Journal of Fish Biology* **48**: 16-29.

Sandoval-Castillo J, Rocha-Olivares A, Villavicencio-Garayzar C, Balart E (2004) Cryptic isolation of Gulf of California Shovelnose Guitarfish evidenced by mitochondrial DNA. *Marine Biology* **145**: 938-988.

Sato S, Kojima H, Ando J, Ando H, Wilmot RL, Seeb LW, Efremov V, LeClair L, Buchholz W, Jin DH, Urawa S, Kaeriyama M, Urano A, Abe S (2004) Genetic population structure of chum salmon in the Pacific Rim inferred from mitochondrial DNA sequence variation. *Environmental Biology of Fishes* **69**: 37-50.

Savin SM, Douglas RG, Stehli FG (1975) Tertiary marine paleotempertures. *Geological Society of America Bulletin* **86**: 1499-1510.

Schneider S, Excoffier L (1999) Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics* **152**: 1079-1089.

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

Schubart CD, Gondáles-Gordillo JI, Reyns N, Liu HC, Cuesta JA (2001) Are Atlantic and Indo-Pacific populations of the rafting crab, *Plagusia depressa*, distinct? New evidence from larval morphology and mtDNA. *Raffles Bulletin of Zoology* **49**: 301-310.

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

Schultz JK, Pyle RL, DeMartini EE, Bowen BW (2006) Genetic connectivity among color morphs and Pacific archpelagos for the flame angelfish, *Centropyge loriculus*. *Marine Biology* **151**: 167-175

Sekino M, Saito K, Yamada T, Kumagai A, Hara M, Yamashita Y (2003) Microsatellite-based pedigree tracking in the Japanese flounder *Paralichthys olivaceus* hatchery strain: implications of hatchery management related to stock enhancement program. *Aquaculture* **221**: 255-263.

Shaklee JB, Bentzen P (1998) Genetic identification of stocks of marine fish and shellfish. *Bulletin of Marine Science* **62**: 589-621.

Shannon LV, (1985) The Benguela ecosystem Part 1: Evolution of the Benguela, physical features and processes. *Oceanography and Marine Biology* **23**: 105-182.

Shimodaira H (2001) Multiple comparisons of log-likelihoods and combining nonnested models with applications to phylogenetic tree selection. *Communications in Statistics - Theory and Methods* **30**: 1751-1772.

Shimodaira H, Hasegawa M (1999) Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**: 1114-1116.

Shipley P (2003) MICRO-CHECKER, Version 2.2.3 Department of Biological Sciences, University of Hull, Hull, UK.

Smith PJ, Robertson SG, Horn PL, Bull B, Anderson OF, Stanton BR, Okec CS (2002) Multiple techniques for determining stock relationships between orange roughy, *Hoplostethus atlanticus*, fisheries in the eastern Tasman Sea. *Fisheries Research* **58**: 119-140.

Smith SDA (2002) Kelp rafts in the southern ocean. *Global Ecology and Biogeography* **11**: 67-69.

Smith-Vaniz WF (1984) Carangidae: Relationships In: *Ontogeny and Systematics of Fishes*. Ed. by Moser HG, Richards WJ, Cohen DM, Fahay MP, Kendall Jr AW, Richardson SL. American Society Ichthyology and Herpetology Specs.

Sola L, Cipelli O, Gornung E, Rossi AR, Andaloro F, Crosetti D (1997) Cytogenetic characterization of the greater amberjack, *Seriola dumerili* (Pisces: Carangidae), by different staining techniques and fluorescence *in situ* hybridization. *Marine Biology* **128**: 573-577.

Starling S (1988) The Australian Fishing Book. Hong Kong: Bacragas Pty. Ltd.

Steininger FF, Rögl FR (1984) Paleogeography and palinspastic reconstruction of the Neogene of the Mediterranean and Paratethys. In: *The geological evolution of the eastern Mediterranean*. Ed. by Dixon JE, Robertson AHF. Blackwell Scientific Publications, Oxford.

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

Stopar K, Ramšak A, Trontelj P, Malej A (2010) Lack of genetic structure in the jellyfish *Pelagia noctiluca* (Cnidaria: Scyphozoa: Semaeostomeae) across European seas. *Molecular Phylogenetics and Evolution* **57**: 417-428.

Storz JF (2002) Contrasting patterns of divergence in quantitative traits and neutral DNA markers: analysis of clinal variation. *Molecular Ecology* **11**: 2537-2551.

Stramma L, Lutjeharms JRE (1997) The flow field of the subtropical gyre in the South Indian. *Journal of Geophysical Research* **102**: 5513-5530.

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

Swofford DL, Olsen G (1990) Phylogeny reconstruction. In: *Molecular systematics*. Ed. by Hillis DM, Moritz C. Sinauer Associates, Sunderland, Massachusetts.

Tajima F (1989) Statistical methods to test for nucleotide mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585-595.

Tamura K, Nei M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10**: 512-526.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) version 4.0. *Molecular Biology and Evolution* **24**: 1596-1599.

Tang ZH, Ding ZL, White PD, Dong XX, Ji JL, Jiang HC, Luo P, Wang X (2011) Late Cenozoic central Asian drying inferred from a palynological record from the northern Tian Shan. *Earth and Planetary Science Letters* **302**: 439-447.

Tang RWK, Yau C, Ng WC (2010) Identification of stomatopod larvae (Crustacea: Stomatopoda) from Hong Kong waters using DNA barcodes. *Molecular Ecology Resources* **10**: 439-448.

Techow NMSM, O'Ryan C, Phillips RA, Gales R, Marin M, Patterson-Fraser D, Quintana F, Ritz MS, Thompson DR, Wanless RM, Weimerskirch H, Ryan PG (2010) Speciation and phylogeography of giant petrels *Macronectes*. *Molecular Phylogenetics and Evolution* **54**: 472-487.

Teske PR, Forget FRG, Cowley PD, von der Heyden S, Beheregaray LB (2010) Connectivity between marine reserves and exploited areas in the philopatric reef fish *Chrysoblephus laticeps* (Teleostei: Sparidae). *Marine Biology* **157**: 2029-2042.

Teske PR, McQuaid CD, Froneman PW, Barker NP (2006) Impacts of marine biogeographic boundaries on phylogeographic patterns of three South African estuarine crustaceans. *Marine Ecology Progress Series* **314**: 283-293.

Teske PR, Papadopoulos I, Barker NP, McQuaid CD (2013) Dispersal barriers and stochastic reproductive success do not explain small-scale genetic structure in a broadcast spawning marine mussel. *Marine Ecology Progress Series* **482**:133-140.

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**: Art #514.

Teske PR,Winker H, McQuaid CD, Barker NP (2009) A tropical/subtropical biogeographic disjunction in southeastern Africa separates two Evolutionarily Significant Units of an estuarine prawn. *Marine Biology* **156**: 1265-1275.

Theisen TC, Bowen BW, Lanier W, Baldwin JD (2008) High connectivity on a global scale in the pelagic wahoo, *Acanthocybium solandri* (tuna family Scombridae). *Molecular Ecology* **17**: 4233-4247.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**: 4876-4882.

Tillett BJ, Meekan MG, Field IC, Thorburn DC, Ovenden JR (2012) Evidence for reproductive philopatry in the bull shark *Carcharhinus leucas*. *Journal of Fish Biology* **80**: 2140-2158.

Tolley KA, Groeneveld JC, Gopal K, Matthee CA (2005) Mitochondrial DNA panmixia in spiny lobster *Palinurus gilchristi* suggests a population expansion. *Marine Ecology Progress Series* **297**: 225-231.

Tripathy NK, Das CC (1988) Karyotypes of five Indian Perciform fishes. *Copeia* **1988**: 231-233.

Uthicke JA, Benzie H (2003) Gene flow and population history in high dispersal marine invertebrates: mitochondrial DNA analysis of *Holothuria nobilis* (Echinodermata: Holothuroidea) populations from the Indo-Pacific. *Molecular Ecology* **12**: 2635-2648.

Utter FM, Ryman N (1993) Genetic markers and mixed stock fisheries. *Fisheries* **18**: 11-21.

Van Oppen M, Gates R (2006) Conservation genetics and the resilience of reef-building corals. *Molecular Ecology* **15**: 3863-3883.

Vasemägi A (2006) The adaptive hypothesis of clinal variation revisited: Single-locus clines as a result of spatially restricted gene flow. *Genetics* **173**: 2411-2414.

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

Veríssimo A, McDowell JR, Graves JE (2010) Global population structure of the spiny dogfish *Squalus acanthias*, a temperate shark with an antitropical distribution. *Molecular Ecology* **19**: 1651-1662.

Vermeij GJ (1978) *Biogeography and Adaptation: Patterns of Marine Life*. Harvard University Press, Cambridge.

Vermeij GJ (1987a) *Evolution and Escalation: An Ecological History of Life*. Princeton University Press, Princeton, NJ.

Vermeij GJ (1987b) The dispersal barrier in the tropical Pacific: implication for molluscan speciation and extinction. *Evolution* **41**: 1046-1058.

Veron JEN (1995) *Corals in Space and Time.* UNSW Press, Sydney

Viñas J, Alavarado-Bremer JR, Pla C (2004) Inter-oceanic genetic differentiation among albacore (*Thunnus alalunga*) populations. *Marine Biology* **145**: 225-232.

Vinson C, Gomes G, Schneider H, Sampaio I (2004) Sciaenidae fish of the Caeté River estuary, northern Brazil: Mitochondrial DNA suggests explosive radiation for the Western Atlantic assemblage. *Genetics and Molecular Biology* **27**: 174-180.

von der Heyden S (2009) Why do we need to integrate population genetics into South African marine protected area planning? *African Journal of Marine Science* **31**: 263-269.

von der Heyden S, Lipiriski MR, Matthee CA. (2007) Species specific genetic markers for identification of early life history stages of Cape hakes, *Merluccius capensis* and *M. paradoxus*, in the southern Benguela Current. *Journal of Fish Biology* **70**: 262-268.

von der Heyden S, Prochazka K, Bowie RCK (2008) Significant population structure and asymmetric gene flow patterns amidst expanding populations of *Clinus cottoides*

(Perciformes, Clinidae): application of molecular data to marine conservation planning in South Africa. *Molecular Ecology* **17**: 4812-4826.

von der Heyden S, Barendse J, Seebregts AJ, Matthee CA (2010a) Misleading the masses: detection of mislabelled and substituted frozen fish products in South Africa. *Journal of Marine Science* **67**: 176-185.

von der Heyden S, Lipinski Mr, Matthee CA (2010b) Remarkably low mtDNA control region diversity in an abundant demersal fish. *Molecular Phylogenetics and Evolution* **55**: 1183-1188.

von der Heyden S, Bowie RC, Prochazka K, Bloomer P, Crane ML, Bernardi G (2011) Phylogeographic patterns and cryptic speciation across oceanographic barriers in South African intertidal fishes. *Journal of Evolutionary Biology* **24**: 2505-2519.

Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. *Journal of Biogeography* **27**: 1153-1167.

Wang FH, Wang GX, Li XY, Huang JL, Zheng JK (2008) Heredity, physiology and mapping of a chlorophyll content gene of rice (*Oryza sativa* L.). *Journal of Plant Physiology* **165**: 324-330.

Wang S, Xu P, Thorsen J, Zhu B, de Jong PJ, Waldbieser G, Kucuktas H, Liu Z (2007) Characterization of a BAC library from channel catfish *Ictalurus punctatus*: indications of high levels of chromosomal reshuffling among teleost genomes. *Marine Biotechnology* **9**: 701-711.

Waples RS (1998) Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *The Journal of Heredity* **89**: 438-450.

Waples RS, Gaggiotti O (2006) What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology* **15**: 1419-1439.

Wares JP (2002) Community genetics the Northwestern Atlantic intertidal. *Molecular Ecology* **11**: 1131-1144.

Ward RD (2000) Genetics in fisheries management. *Hydrobiologia* **420**: 191-201.

Ward RD (2006) The importance of identifying spatial population structure in restocking and stock enhancement programmes. *Fisheries Research* **80**: 9-18.

Ward RD, Woodwark M, Skibinski DOF (1994) A comparison of genetic diversity levels in marine, freshwater and anadromous fishes. *Journal of Fish Biology* **44**: 213-232.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **360**: 1847-1857.

Waters JM, Roy MS (2003) Global phylogeography of the fissiparous sea-star *Coscina sterias*. *Marine Biology* **142**: 185-191.

Waters JM, Roy MS (2004) Out of Africa: the slow train to Australasia. *Systematic Biology* **53**: 18-24.

Watts RJ, Johnson MS, Black R (1990) Effects of recruitment on genetic patchiness in the urchin *Echinometra mathaei* in western Australia. *Marine Biology* **105**: 145-152.

WCADI (2012) Western Cape Aquaculture market analysis and development programme/strategy. Western Cape Aquaculture Development Initiative.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* **38**: 1358-1370.

Wenburg JK, Bentzen P (2001) Genetic and behavioral evidence for restricted gene flow among coastal cutthroat trout populations. *Transactions of the American Fisheries Society* **130**: 1049-1069.

Whatmore P, Nguyen N, Miller A, Lamont R, Powell D, D'Antignana T, Bubner E, Elizur A, Knibb W (2013) Genetic parameters for economically important traits in yellowtail kingfish *Seriola lalandi*. *Aquaculture* **400**: 77-84.

White BN (1994) Vicariance biogeography of the open-ocean Pacific. *Progress in Oceanography* **34**: 257-284.

Widmer A, Lexer C (2001) Glacial refugia: sanctuaries for allelic richness, but not for gene diversity. *Trends in Ecology and Evolution* **16**: 267-269.

Williams ST, Knowlton N (2001) Mitochondrial pseudogenes are pervasive and often insidious in the snapping shrimp genus *Alpheus*. *Molecular Biology and Evolution* **18**:1484-1493.

Wilson AB, Eigenmann Veraguth I (2010) The impact of Pleistocene glaciation across the range of a widespread European coastal species. *Molecular Ecology* **19**: 4535-4553.

Wilson AC, Cann RL, Carr SM, George M, Gyllensten UB, Helm-Bychowski KM, Higuchi RG, Palumbi SR, Prager EM, Sage RD, Stoneking M (1985) Mitochondrial DNA and two perspectives on evolutionary genetics. *Biological Journal of the Linnean Society* **26**: 401-405.

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* **163**: 1177-1191.

Wirth T, Bernatchez L (2001) Genetic evidence against panmixia in the European eels. *Nature* **409**: 1037-1040.

Wolvaardt C (2007) Initiation of Company Coverage – IPO, Western Kingfish Ltd.

Worm B, Barbier EB, Beaumont N, Duffy JE, Folke C, Halpern BS, Jackson JBC, Lotze HK, Micheli F, Palumbi SR, Sala E, Selkoe K A, Stachowicz JJ, Watson R (2006) Impacts of biodiversity loss on ocean ecosystem services. *Science* **314**: 787-790.

Wright S (1943) Isolation by distance. *Genetics* **28**: 114-138.

Wu GC, Chiang H, Chou Y, Wong Z, Hsu CC, Chen C, Yanga H (2010) Phylogeography of yellowfin tuna (*Thunnus albacares*) in the Western Pacific and the Western Indian Oceans inferred from mitochondrial DNA. *Fisheries Research* **105**: 248-253.

Xu Q, Liu R, Liu Y (2009) Genetic population structure of the swimming crab, *Portunus trituberculatus* in the East China Sea based on mtDNA 16S rRNA sequences. *Journal of Experimental Marine Biology and Ecology* **371**: 121-129.

Yamaguchi A, Onishi Y, Omata A, Kawai M, Kaneda M, Ikeda T (2010) Population structure, egg production and gut content pigment of large grazing copepods during the spring phytoplankton bloom in the Oyashio region. *Deep-Sea Research II* **57**: 1679-1690.

Yokoyama Y, Lambeck K, De Deckker P, Johnston P, Fifield LK (2000) Timing of the Last Glacial Maximum from observed sea-level minima. *Nature* **406**: 713-716.

Yue GH, Li Y, Lim LL, Orban L (2004) Monitoring the genetic diversity of three Asian arowana (*Scleropages formosus*) captive stocks using AFLP and microsatellites. *Aquaculture* **237**: 89-102.

Yue GH, Zhu ZY, Lo LC, Wang CM, Lin G, Feng F, Pang HY, Li J, Gong P, Liu HM, Tan J, Chou R, Lim H, Orban L (2009) Genetic variation and population structure of Asian seabass (*Lates calcarifer*) in the Asia-Pacific region. *Aquaculture* **293**: 22-28.

Zachos J, Pagani M, Sloan L, Thomas E, Billups K (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292**: 686-693.

Zardi GI, McQuaid CD, Teske PR, Barker NP (2007) Unexpected genetic structure of indigenous (*Perna perna*) and invasive (*Mytilus galloprovincialis*) mussel populations in South Africa. *Marine Ecology Progress Series* **337**: 135-144.

Zardoya R, Castilho R, Grande C, Favre-Krey L, Caetano S, Marcato S, Krey G, Patarnello T, (2004) Differential population structuring of two closely related fish species, the mackerel (*Scomber scombrus*) and the chub mackerel (*Scomber japonicus*), in the Mediterranean Sea. *Molecular Ecology* **13:** 1785-1798.