

**REGIONAL DIFFERENTIATION OF THREE GOATFISHES
(*PARUPENEUS SPP.*) WITHIN THE WESTERN INDIAN OCEAN**

by

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the degree of **MASTER OF SCIENCE (ZOOLOGY)**
in the Faculty of Science and Agriculture
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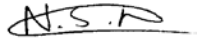
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Yours sincerely

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ABSTRACT

Goatfishes inhabit inshore reefs and corals and are commercially important across their distribution in the Western Indian Ocean (WIO). The biogeography of these species in the WIO has not been explored with regards to their levels of diversity and relationships among regions. The genetic connectivity and differentiation of three goatfishes of the genus *Parupeneus* (*P. barberinus*, *P. macronemus* and *P. rubescens*) was studied using two mitochondrial genes (ND2 and 16S rRNA) and one nuclear gene (RAG1) using specimens from East and southern Africa, islands around the Mascarene plateau, Oman, Maldives and the Red Sea. Haplotype diversities, networks and AMOVA were used to measure genetic variance among localities and defined regional groups. There were high haplotype ($HD > 0.9$) and low nucleotide diversities (< 0.006) among all species for all gene regions, suggesting high levels of genetic differentiation among different areas, except for the mtDNA 16S data for *P. macronemus* and *P. rubescens*. For all three species, the F_{ST} population pairwise values revealed significant differentiation in all datasets for most population pairwise comparisons with the Maldives and genetic connectivity with haplotypes being shared among other localities. The 16S and RAG1, AMOVA for *P. barberinus* revealed a significant ($P < 0.05$) strong genetic structure among groups, for example $P = 0.00$ was estimated in the 16S data for four groups (the Maldives, WIO islands, Kenya and eastern mainland). This study found evidence for regional differentiation within the WIO for these three species supporting the presence of genetic breaks among areas. This differentiation could be either due to the historical isolation among areas or due to geographic and oceanic barriers such as the Mascarene Plateau and the Agulhas Current eddies in the Mozambique Channel. The effects of oceanographic features and physical barriers in the species distribution range and the dispersal potential based on the life history features of the species can have an

influence on the genetic structuring of a population. It is also important to note that the length of the pelagic larval phase is just one factor affecting dispersal in marine organisms that can also explain the difference in genetic population structure. Unfortunately there is no specific information on the larval dispersal of these three goatfish. Therefore, studies are needed to be conducted on the specific biology and life history strategies of each *Parupeneus* species. These results suggest the importance of other factors, such as currents, and larval retention that may cause strong differentiation. These factors should also be considered when observing larval dispersal and its effect on population genetic structure. This study support the hypotheses that physical factors, processes (geographic barriers and oceanographic characteristics) and life history parameters need to be studied to understand the genetic differentiation of these *Parupeneus* reef fishes.

KEYWORDS: Biogeography, Genetic structure, Mitochondrial DNA, Nuclear DNA, *Parupeneus* spp., Western Indian Ocean

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CHAPTER ONE

GENERAL INTRODUCTION

1.0. BACKGROUND

The Western Indian Ocean (WIO) is a province of the Indian Ocean that has been characterised as a biogeographic sub-region (Gullström *et al.* 2002; Briggs and Bowen 2012). It is a region of great diversity that supports a wide array of different fishery types due to the variety of habitats (van der Elst *et al.* 2005). The majority of the fish species found in the WIO are coral reef and shore fishes with a large portion of widespread species that frequently range eastwards to the western Pacific Ocean (Allen 1985; Mora 2004). This region therefore presents a useful model to study the physical and biological factors, including processes shaping patterns of marine species, diversity and biogeography. It has been noted that patterns of differentiation and genetic variability in marine taxa are a result of interrelationships of the physical effects of marine environments and the life history features of species (Hauser and Ward 1998).

The WIO is a distinct biogeographical province of the Indo-West Pacific (IWP), with high levels of regional endemism around island states and at locations along the continent (Cohen 1973; Randall 1995). A number of the studies that have been done with a focus on the biogeography of the WIO have recognised the South Western Indian Ocean (SWIO) as an area of endemism (Hoareau 2013; Zacarias 2013). For example; at least 2 200 species have been recorded (Smith and Heemstra 1986; Heemstra and Heemstra 2005; van der Elst *et al.* 2005.) which comprise about 15% of the total marine fishes in the world. The SWIO is located south of the highly productive Arabian Sea (Fonseca *et al.* 2007). This unexpected high level of endemism off South

Africa can be attributed to the unique environment of the southern tip of continental Africa (van der Elst *et al.* 2005). Only this coastal region in the WIO has a temperate climate, with distinctly different environmental conditions in association with the Agulhas Current Large Marine Ecosystem (Beckley *et al.* 2002). Northern regions such as the Red Sea and the Arabian Gulf are also areas of high endemism with their restricted opening and relative isolation from other WIO regions (Briggs and Bowen 2012). Santini and Winterbottom (2002) demonstrated the separation of IWP regional faunas including the WIO, as a result of a series of vicariant events that may have caused the splitting of these regions and a separation between adjacent regions. Despite the years of research on SWIO marine fish fauna, the origin(s) and relationship(s) of most of this fauna remains unknown. However, more focused species specific studies are needed to develop a fuller understanding of the origins and relationships and interaction of the WIO faunas.

The target species for this study are the goatfishes (Family Mullidae) of the genus *Parupeneus* Bleeker 1868. The Mullidae is a widespread family in the WIO region and contains about 70 species in six genera (Randall 2004; Uiblein and Heemstra 2010; Rajan *et al.* 2012). This family is characterised by their conspicuous barbels on the chin that differ from similar organs of other fish groups, which are used as a very efficient tool for searching for food and finding location (Kim *et al.* 2001; Uiblein 2007). These barbels have also been found to vary considerably in structure, size and sensory equipment within this family (Gosline 1984; McCormick 1994; Rajan *et al.* 2012). Goatfishes are relatively common and of high importance in many coastal areas (Uiblein 2007). Some genera within this family have been suggested to be particularly speciose, with *Parupeneus* being the most diverse consisting of about 27 species (Uiblein and Heemstra 2010).

This genus is also widely distributed in the tropical Indo-Pacific (Figure 1.1). The characteristic feature of this genus is its incomplete dentition consisting of a single row of snout and widely spaced teeth on both jaws with the vomer and palatines being devoid of teeth (Thomas 1969). The preorbital region of the head of mullids is elongated and allows them to dig in the substratum to access prey items (Caldwell 1962). Gosline (1984) stated that *Parupeneus* has the most pronounced and considerably extended snout compared to other mullid genera. The lateral line scales of *Parupeneus* are usually 28-30 with the scales being present on the caudal but absent on anal and dorsal fins and the preorbital fins. Goatfishes are also distinguished by their two well-separated dorsal fins and a forked caudal fin. Species of *Parupeneus* have a spinous dorsal fin with eight spines (the first one very small and soft with one spine) and eight rays with the pectoral fin having 14-18 rays (Randall and Kulbicki 2006).

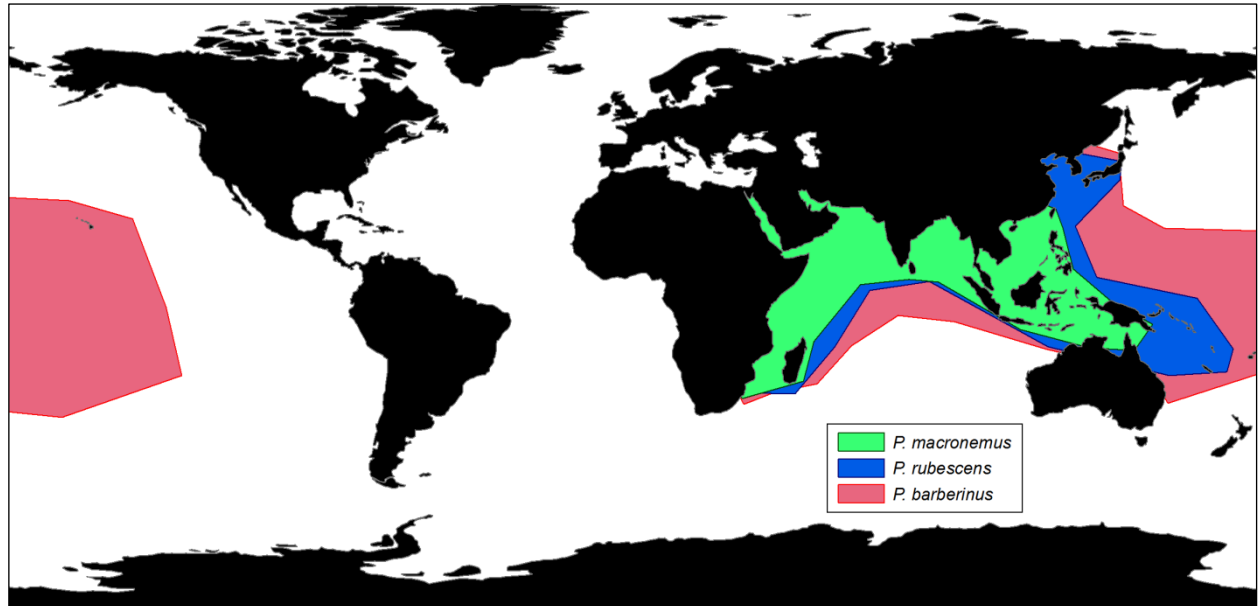


Figure 1.1. Geographical distribution of the three selected goatfishes (*Parupeneus* spp.) in the Indo-Pacific.

In recent years, much attention has been given to the study of the food and feeding habits of fishes including goatfishes (Chacko 1949; Kuthalingam 1955, 1956; Rabindranath 1966; Ismen 2005, 2006). Goatfishes have also been the subject of rather intense biological and taxonomic studies (Lee 1974; Gosline 1984; Golani 1994), and this is also because of their economic value. Goatfishes have also been highlighted as a group that has a high value for ecosystem monitoring and management that requires intensified systematic and ecological research (Uiblein 2007; Rajan *et al.* 2012). Although almost all species descriptions of goatfishes so far have been based exclusively on morphological data, this morphological variation may however be higher than the differentiation found at the genetic level in some cases (Stepien *et al.* 1994).

For example, Rajan *et al.* (2012) examined three species of a circumtropical complex of goatfishes that are very similar in colour and morphology to compare their relationships, using allozyme and meristic data. These species namely *Mulloidichthys vanicolensis* (Valenciennes 1831) from the Indo-Pacific, *M. dentatus* (Gill 1862) from the eastern Pacific, and *M. martinicus* (Cuvier 1829) from the western Atlantic could be distinguished using several allozyme differences as well as their gill-raker counts. However, allozyme data from several widely separated populations of *M. vanicolensis* (including isolated islands with high endemism in other groups) suggested little genetic divergence, consistent with high levels of dispersal and gene flow. Morphological data suggested greater divergence between populations of *M. vanicolensis* from the WIO and the Pacific Ocean than is apparent from allozyme data. The aim and goal of the current study was to explore the relationships, interaction and evolutionary history among different geographic regions in the WIO for each of the three selected widespread goatfish species.

1.1. LITERATURE REVIEW

1.1.1. Study area

The WIO region is a large ocean region with a surface area of about 30 million km² (van der Elst *et al.* 2005) and of which more or less than 6.3% is the shelf area. It includes the western part of the Indian Ocean including the Red Sea, Persian Gulf and Arabian Sea. It extends over continental coastlines with diverse habitats, various oceanic and continental islands, ocean basins and regions, and a complex system of major ocean current circulations (Lutjeharms 2006). This region has some of the most dynamic and variable large marine ecosystems in the world, with most of its fishes being part of the Indo-Pacific fauna (Branch *et al.* 1994; Peschack 2005). The WIO coastline is characterised by estuaries, coastal lagoons, mangrove forests, coral reefs, sea-grass beds, mud flats, algal beds, barrier islands, sandy and rocky beaches (Smith and Heemstra 1986; Ngoile and Linden 1997).

The ocean current systems in the WIO (Figure 1.2) are strongly influenced by monsoon winds which often reverse the direction of water movement according to the season (Ngoile and Linden 1997). These ocean currents include the South Equatorial Current (SEC), Equatorial Counter Current (ECC), Mozambique and Agulhas Currents (AC), East African Coastal Current (EACC) and the Somali Current (SC) (Ngoile and Linden 1997; Lutjeharms 2006). The WIO current systems and eddies have played a major role in facilitating connectivity through dispersal and creating isolation among biogeographical regions (Thandar 1989; Donohe and Toole 2003; Samyn and Tallon 2005). For example, the influence of strong eddies in the Mozambique Channel and strong anti-cyclonic gyres have some influence on the genetic structure of marine species (Lambardi *et al.* 2008). Bourjea *et al.* (2007) studied the phylogeography of the green turtle, *Chelonia mydas* (Linnaeus 1758), in the SWIO to survey the patterns of mitochondrial

DNA (mtDNA) variation of nesting green turtles at ten different rookeries. The author's analyses showed significant population differentiation between sites in the South Mozambique Channel (MC) and the remaining nesting sites that were sampled in the North. They suggested that this particular genetic pattern along the MC is attributed to a recent colonisation from the Atlantic Ocean and is maintained by oceanic current conditions in the north and southern Mozambique Channel that influence early stages in the green turtle life cycle.

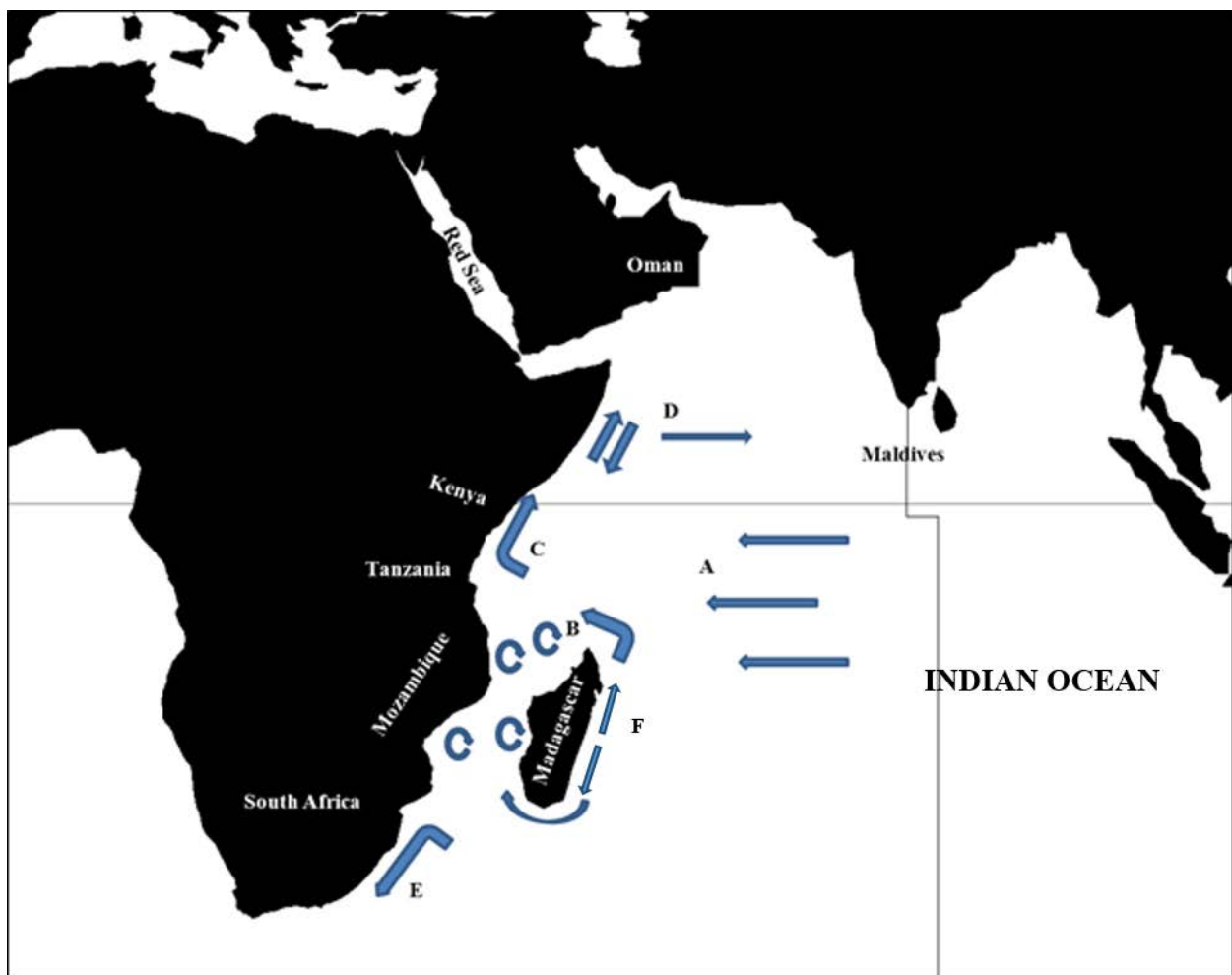


Figure 1.2. Map of the Western Indian Ocean (WIO) with the main oceanographic features: A = South Equatorial Current (SEC), B = eddies and gyres in the Mozambique Channel, C = East African Coastal Current (EACC), D = Somali Current, E = Agulhas Current and F = Madagascar current. Modified from Obura (2012).

The SEC is a key ocean current which draws water from the Pacific Ocean and the Indonesian region and flows into the WIO throughout the year from east to west just south of the equator until it reaches the coast of Tanzania (Bock 1978; McClanahan 1988). Once the coast of Tanzania is reached, the SEC divides into the MC to form the southward flowing AC, and the northward flow to join the South Equatorial Counter Current (SECC). The EACC is another current that flows northward along the Tanzania and Kenya coasts throughout the year. This current is influenced by monsoon or trade winds of the Indian Ocean, flowing south-westward along the Somali coast (during the northern hemisphere winter) and north-eastward (in summer) as the SC (Bock 1978). The seasonally reversing monsoon winds cause ocean current reversal twice a year in the WIO (Wakwabi and Nguli 1995).

The monsoon winds are the dominant influence on wind direction, temperature and rainfall, with northeast trade winds blowing from November to March and southeast winds from April to October (Figure 1.3) (Ngoile and Linden 1997). The northeast monsoon season (NEM) is a period of calm weather, elevated temperatures and lower salinities, whereas the southeast monsoon (SEM) is characterised by rough seas, cool weather and high salinities (Locham *et al.* 2010). The influence of this seasonality on biological and physical factors, and therefore connectivity, is recognised for the Eastern African coastal regions (Kenya, Tanzania, Mozambique and South Africa), including the Islands states (Madagascar and Mauritius) of the WIO (Ngoile and Linden 1997; Locham *et al.* 2010). The Somali Current is an ocean boundary current that flows along the coast of Somalia and Oman. This current possesses seasonal cold water upwelling off Somalia and off southern Oman (Currie *et al.* 1973; Glynn 1993) and could be an effective barrier on the movement of species during these periods.

The Eastern Madagascar Current is the oceanic flow near Madagascar which is an important source that also successively feeds the Agulhas Current. This current is the southern branch of the South Equatorial Current which separates as it reaches the eastern coast of Madagascar (Tomczak and Godfrey 1994). Its flow is complicated by large cyclonic and anti-cyclonic eddies (De Ruijter *et al.* 2003) which may also have an impact on the movement of marine organisms such as fish and connectivity. The Agulhas Current which is found in the south is known as the western boundary current of the SWIO. It flows down the east and southern coasts of Africa (Gordon 1985) along the edge of the continental shelf, with its main stream being 60-100 km wide. The waters of the Agulhas Current are clear and poor in nutrients, while the seas surface temperature can reach highs of 28°C. When the current finally reaches the Agulhas bank in South Africa, it turns back on itself and begins to flow in a north-easterly direction. At this point oceanographers call it the Agulhas return Current (Quartly and Srokosz 1993). Although this complex system of ocean currents and eddies is expected to have an impact on the genetic structure of WIO marine fishes, the full extent of these is unknown due to the scarcity of research studies in the area (Quartly and Srokosz 2004; Bourjea *et al.* 2007; Visram *et al.* 2010; Obura 2012).

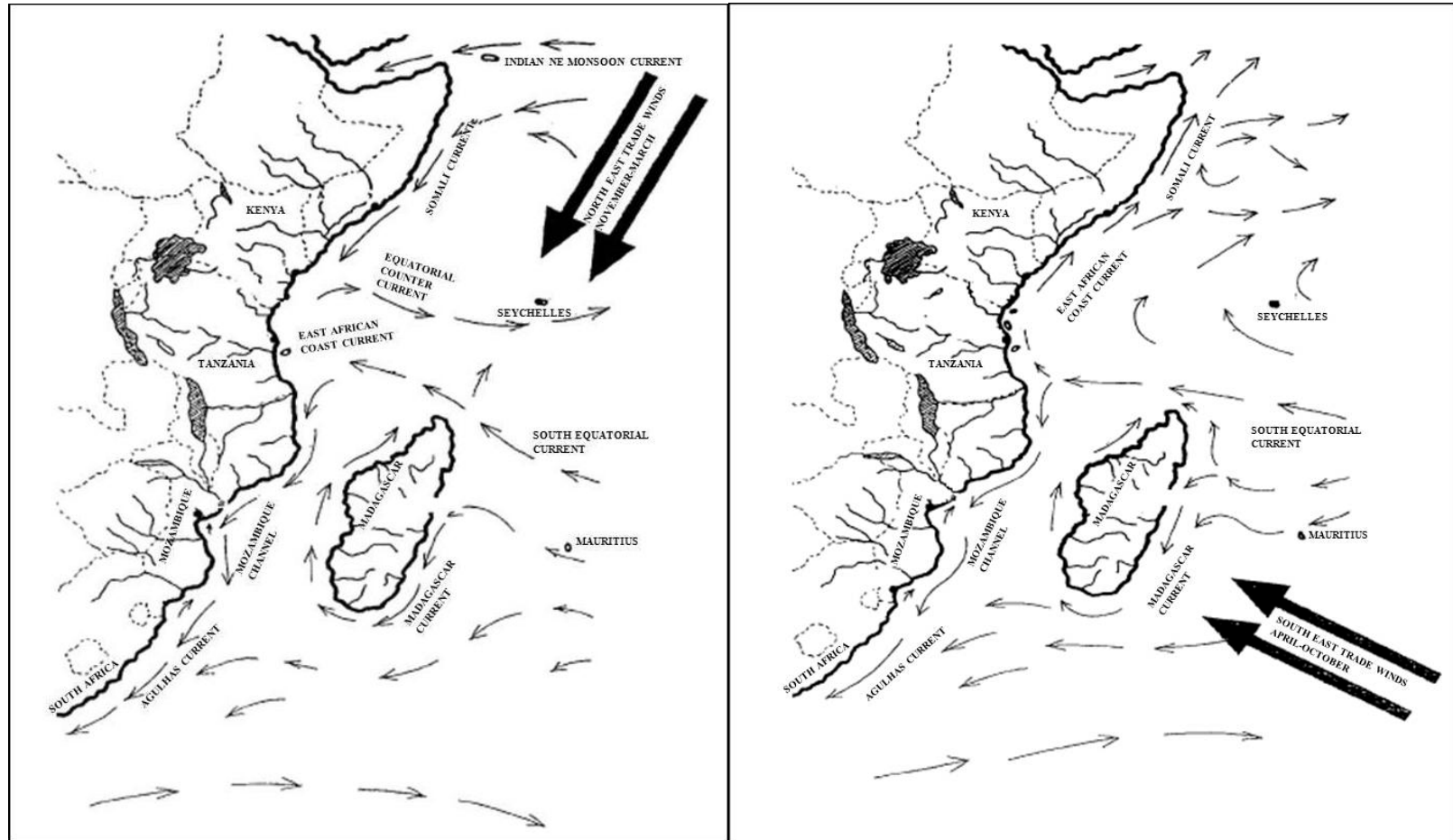


Figure 1.3. Maps illustrating identified currents in the WIO during Northeast and Southeast monsoon winds. The maps were obtained from Ngoile and Linden (1997).

1.1.2. General biogeography within the WIO

Many fish species in the WIO are coral and shore fishes, with the majority having distributions that extend eastward to the southern and eastern Pacific coral reef regions (Briggs and Bowen 2012; DiBattista *et al.* 2013). The extreme complexity of WIO coral reef ecosystems implies that biogeographic studies should be conducted at various spatial and temporal scales in order to assess the widest possible range of variability in the distribution of organisms (Letourneur *et al.* 2008). Indeed, numerous studies have revealed that the distribution and organisation of marine organisms such as fishes are not uniform in space or over time, but display various gradients or other types of spatio-temporal structuring (Williams 1991; Adjeroud *et al.* 1998; Kulbicki *et al.* 2012). At relatively wide spatial scales, the distribution of reef fish is usually dependent on substrate variables such as the percentage of the main type of dominant coral forms (Bell and Galzin 1984; Chabanet *et al.* 2005). At a larger spatial scale, fish distribution may be linked to (dis)similarities among reef areas (Kulbicki 1998; Miloslavich *et al.* 2013) or prevalent oceanographic processes and their effects on the dispersion of larvae along currents, and the swimming capacities of larvae. These would all result in different patterns of settlement and recruitment of new individuals into reef systems or areas (Lecchini and Galzin 2003; Leis *et al.* 2003).

The WIO possesses diverse marine fauna that have originated from the IWP (Briggs 1999). However, regional species inventories are fragmented (Randall 1998; Turpie *et al.* 2000) and this diversity is less compared to that of the Indo-Malayan region (Santini and Winterbottom 2002). Various studies of marine diversity in this region have supported the perception that regional faunas in the Indo-Pacific and WIO have originated and are maintained by dispersal or migration from the Indo-Malayan centre of origin (Randall 1998; Briggs 1999; Santini and Winterbottom 2002). The reduction in species diversity from this centre of origin was distinguished by Randall (1998), Briggs (1992, 1999) and Briggs and Bowen (2012), as well

as the presence of older taxa at the periphery to this region with reduced genetic diversity (Briggs 1999; Santini and Winterbottom 2002). A similar pattern in coral reef species was also found by Pandolfi (1992) in this region. The origin of this rich diversity has been attributed to the establishment of barriers and subsequent vicariance (Bowen *et al.* 2001). Santini and Winterbottom (2002) and DiBattista *et al.* (2013) separated WIO fauna after identifying and using a series of vicariant events where the Red Sea, Arabian Basin, Natal Basin, Mascarene Plateau, and the Chagos Ridge were isolated into different biogeographic regions. However, there is a need to verify these as vicariant hypotheses often require the recognition of historical events separating species, and this is poorly supported by the scarcity of fossil records in the WIO (Bellwood and Wainwright 2002).

1.1.3. Habitat and biology of goatfishes

Most goatfishes inhabit inshore areas and are commercially important throughout their distribution (Golani and Ritte 1999). They are distributed worldwide in tropical, subtropical and temperate habitats generally between the upper littoral and upper slope (Uiblein 2007), although some species may be found down to the depth of 500 m (Golani 2001). There are therefore marked differences among goatfish species with respect to preferred habitat type and depth (Uiblein 2007). Like the majority of marine species, goatfishes spawn planktonic eggs from which feeding larvae hatch (Riginos and Victor 2001; Rajan *et al.* 2012). Goatfishes spawn at select points by releasing many buoyant eggs into the water along the outer reef edge to which they migrate from their normal inshore feeding areas (Essipov 1934; Colin and Clavijo 1978; 1988; Santana *et al.* 2006). The postlarvae will then float in the surface waters until they reach around 5 or 6 cm in length, when they take on the adult bottom-feeding lifestyle. In marine organisms, a pelagic larval stage increases the opportunities for long-distance dispersal and is thus typical for species that display little genetic differentiation among reef habitats (Reece *et al.* 2011). The pelagic larval duration

(PLD) is therefore an important factor in determining the dispersal potential of a fish species and, therefore, the amounts of genetic interchange among geographically distant populations (Riginos and Victor 2001).

Most studies on the Mullidae have focused on the taxonomic and distributional aspects (e.g. in the Red Sea) (Wahbeh and Ajiad 1985a; Golani 1998; Khalaf and Kochzius 2002). Few studies on their biology that have been reported (Hashem 1973; El-Absey 1977; Wahbeh and Ajiad 1985a; Pavlov *et al.* 2011). Nevertheless, goatfishes represent an essential component of food chains, and they are regarded as indicators of the changes of biotopes (Uiblein 2007).

1.2. STUDY TAXA

Three species of the genus *Parupeneus* were selected for this study. These are the dash-and-dot goatfish *Parupeneus barberinus* (Lacepède 1801), the long-barbel goatfish *P. macronemus* (Lacepède 1801), and the rosy goatfish *P. rubescens* (Lacepède 1801). Previous descriptions of these goatfish species have been based exclusively on morphological data. While *P. rubescens* is distinct from the others, *P. barberinus* and *P. macronemus* are very similar in appearance (Day 1878). Smith (1949) indicated that the differences between the last dorsal and anal fin rays which are quite elongate in *P. macronemus* are useful in separating these two species. Lachner (1960) contested that the characters given by Smith (1949) to distinguish between these two species were completely inaccurate. It was observed that the relative position of the black spot distinguishes the two species, with the spot being located at the middle base of the caudal fin in *P. barberinus*, and at the middle of the caudal peduncle and being slightly larger in *P. macronemus* (Thomas 1969). The two species can also be differentiated by the number of gill rakers that range from 25-30 in *P. barberinus* and 32-37 in *P. macronemus*. The unclear description and inaccurate illustration of these species leads to confusion, therefore, genetic research will provide significant understanding of their

differentiation. The genetic analyses of these goatfishes will be useful in closing the gap in the knowledge on goatfish systematics when there is taxonomic uncertainty for widespread groups.

1.2.1. Dash-and-dot goatfish *Parupeneus barberinus* (Lacepède 1801)

The dash-and-dot goatfish (Figure 1.4) is one of the most abundant species of *Parupeneus* (Myers 1991). The body colour is white with a black to dark brown stripe (dash) reaching from the upper lip through the eye and along the body, followed by a black spot (dot) at the base of the caudal fin, hence the name dash and dot goatfish. Above the stripe, the body has a yellowish tint and usually has red stripes and spots when collected from deeper waters (Randall 2004). It is the largest species of the family Mullidae with a recorded maximum length of 60 cm (Lieske and Myers 1994). The dash-and-dot goatfish is distributed in the Indian and western Pacific oceans from the Gulf of Aden and Oman, south on the east coast of Africa to Mossel Bay in South Africa, east to the Islands of Micronesia, Line Islands, Marquesas Islands and from southern Japan to Australia (Figure 1.3; Randall 2004). It is also widely distributed in the sheltered shallow waters of the Gulf of Aqaba (Ben-Tuvia 1968). This goatfish inhabits large sand patches as well as sand and rubble areas of reef flats, lagoon and seaward reefs to a depth of about 100 m (Randall 2004). The species forages on sand dwelling invertebrates like polychaete worms and crustaceans (Sano *et al* 1984). The composition of food in *P. barberinus* suggests an omnivorous diet with emphasis on the carnivorous side (Wahbeh and Ajiad 1985b).

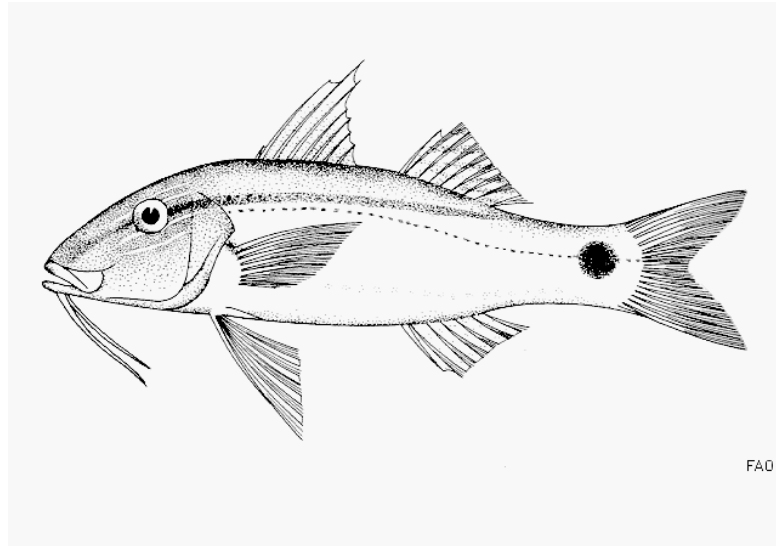


Figure 1.4. *Parupeneus barberinus* (Dash-and-dot goatfish). The FAO image obtained from De Bruin *et al.* (1995).

1.2.2. Long-barbel goatfish *Parupeneus macronemus* (Lacepède 1801)

Parupeneus macronemus (Figure 1.5) is distributed in the Indo-West Pacific from the Red Sea and Persian Gulf south to KwaZulu-Natal in South Africa, extending east to Indonesia and the Philippines (Randall 2004). This goatfish is either solitary or occurs in pairs over shallow coastal reefs and can reach a maximum length of 40 cm (Kuiter and Tono-zuka, 2001). The long-barbel goatfish inhabits lagoon and seaward reefs to over 25 m and is found on sandy or weedy bottoms rarely at depths of more than 40 m (Randall 2004). This species has two very long slender chin barbels that reach or nearly touch the posterior end of the head. The body colour is reddish brown, paler ventrally and posteriorly with a dark brown stripe passing from the front of the snout, through the eyes, across the upper end of gill openings, and ending beneath the rear base of the second dorsal fin. A roundish black spot, usually larger than the eye is located on the posterior third of the caudal peduncle. The basal third of second dorsal fin is blackish, with a narrow extension to the posterior tip of fin while the lateral edge of pelvic fins is broadly blackish (Randall 2004).

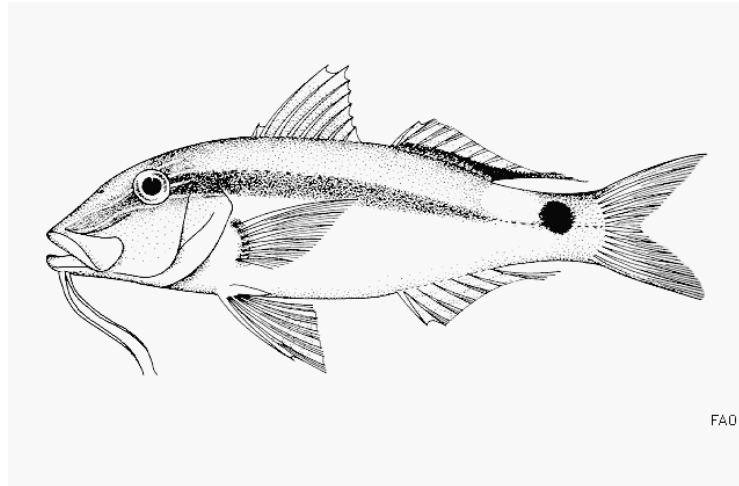


Figure 1.5. *Parupeneus macronemus* (long-barbel goatfish). The image was obtained from an FAO publication (De Bruin *et al.* 1995).

1.2.3. Rosy goatfish *Parupeneus rubescens* (Lacepède 1801)

The rosy goatfish (Figure 1.6) is distributed in the IWP from the Red Sea and Persian Gulf, south to South Africa and east to the Western Pacific and Southeast Atlantic. It is found on sandy bottoms and murky areas of coastal waters (Kumaran and Randall 1984). This species has two slender chin barbels which usually extend from the posterior to the rear margin of preopercle. The body colour is reddish to greenish brown dorsally, with shading and light red ventrally. There is also a pale-edged dark brown band from the front of the snout through the eye and continuing a short distance anteriorly on the body along the lateral line (dark brown sometimes not persisting in preservative). A large black spot is located dorsally on the posterior part of the caudal peduncle (above lateral line), preceded by a whitish spot of about equal size (this whitish area is often faint; Ben-Tuvia 1986). There are no obvious markings on the fins. The species can reach a maximum length of 43 cm (Lieske and Myers 1994).

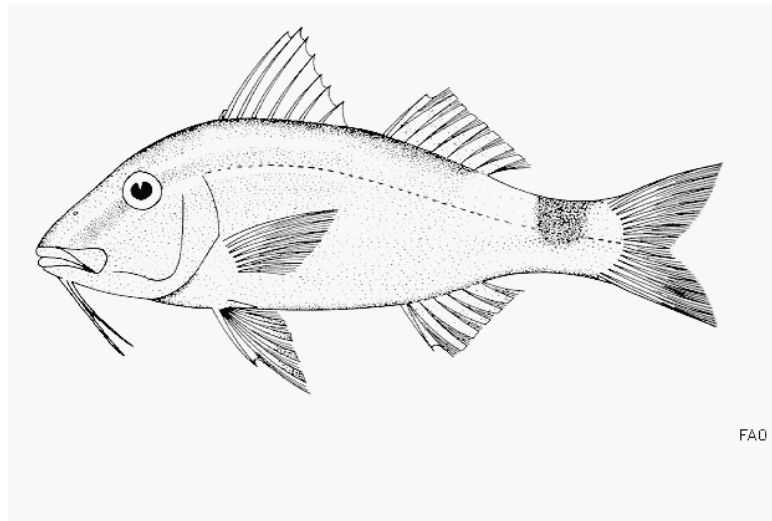


Figure 1.6. *Parupeneus rubescens* (Rosy goatfish). This image is from FAO publication (De Bruin *et al.* 1995).

1.3. BIOGEOGRAPHY

Biogeography can be briefly defined as the science that attempts to describe and interpret the geographic distributions of organisms (Avice 2004). An understanding of the biological and physical processes (at both ecological and evolutionary timeframes) that have shaped the spatial arrangements of the earth's species and biotas (Cox and Moore 1993) is a crucial goal of biogeography. Biogeographic analyses (explicit or implicit) are an important component of what many scientists, such as anthropologists, ecologists, population geneticists, systematists and others actually do (Avice 2004). Although, biogeography is an active field in ichthyology, it is rich in problems (Nelson 2006). These problems are mainly in unravelling the past and the current factors responsible for the distribution of species.

The two approaches to biogeography involve looking at historical and ecological processes. Ecological biogeography, attempts to determine how environmental factors limit the distribution of species (Whitfield 1998). Meyer and Giller (1988) described ecological

biogeography as an approach focusing on ecological processes occurring over short temporal and small spatial scales. Morrone and Crisci (1995) described historical biogeography as the branch of biogeography that deals with evolutionary and geological processes that occur over large temporal and spatial scales, and is often used to provide hypotheses for explaining discontinuous patterns of distribution of organisms. Thus historical biogeography attempts to explain the origin of present day distributional patterns and is largely based on systematics (Avice 2000). Another discussion within historical biogeography is whether to view history through taxa or areas, as a taxon has a single history but an area may have many (Parenti and Humphries 2004; Ebach and Morrone 2005). The tension within historical biogeography has been between proponents of vicariance versus dispersal hypotheses as they are often considered competing (Sanmartin 2003). The distinction between these two is that dispersal is an everyday occurrence undertaken by succeeding generations of almost all species while vicariance is an event of much greater rarity since it must involve the creation of the barrier to separate existing populations (Briggs 1987; 1995; Kruckeberg 2004). It has been implied that vicariance is an approach that must be used to examine distribution in the light of continental drift and the biogeographical regions (Nelson and Platnick 1980; Endler 1982; Wilkinson 1994).

Historical biogeographers however have often been split as to whether they believe that vicariance or dispersal is the dominant paradigm behind distributions of taxa (Avice 2004). These two processes can explain the distributions of both species and populations (Parenti and Humphries 2004) and they are the key components in the generation and conservation of biodiversity (Page 2006). Many studies have found both vicariance and dispersal as important explanations for organism distributions (Porter 2007). Both historical and ecological biogeography are very important in understanding processes affecting genetic

structure and distributions of species as genetic patterns can be used to infer processes and histories of species or populations and environments.

Most of the studies of historical biogeography found in the literature have been performed using mainly, if not exclusively, terrestrial or freshwater organisms (Santini and Winterbottom 2002). This is because these organisms are often characterised by small population sizes, narrow ranges, low dispersal capabilities, low fecundity and different life history strategies (Palumbi 1992). Very few studies have been based on marine organisms, and, of these, most were limited to a very small number of taxonomic groups (Pandolfi 1992; van Soest and Hajdu 1997). Most marine organisms and fishes with a pelagic larval and juvenile life stage have available means of dispersal such as oceanic currents than many of their terrestrial counterparts, which poses an especially interesting challenge.

The marine biodiversity of the WIO is one of the least known globally, with major gaps in the species distribution records of even well-known taxonomic groups (Wafar *et al.* 2011; Obura 2012). The study by Obura (2012) assessed the biogeographic classification of the WIO on the basis of the species biodiversity and distribution of reef-building corals. In their study, species richness of 21 locations in the WIO was estimated from species accumulation curves (Michaelis-Menten equation). In total, 369 species were included in the dataset and cluster analysis of presence/absence data was conducted, using the Bray-Curtis similarity coefficient. This study demonstrated a centre of high diversity for Indian Ocean corals in the northern Mozambique Channel. The observed diversity patterns were consistent with primary oceanographic drivers in the WIO, reflecting inflow to the SEC, maintaining the high diversity and export from this centre to the north and south, including to the Seychelles and Mascarene islands. Obura (2012) concluded that other species groups and biogeographic processes need to be evaluated further in order to improve and understand the diversity

patterns in the region. This study therefore will test whether the distribution of these selected WIO goatfishes was affected by dispersal or vicariant events and also add information about the marine biodiversity of the region.

1.4. MOLECULAR PHYLOGENETICS

Molecular phylogenetics is a comparative molecular genetic approach that uses several taxa to provide an understanding of the origins and relationships of fauna (Wang *et al.* 2001). A molecular phylogenetic approach can also help to resolve relationships among biogeographic regions and identify cryptic species when there's taxonomic uncertainty for widespread morphologically similar species (Horne *et al.* 2008). The developments of molecular markers and techniques have therefore helped invigorate the study of fish systematics, particularly of groups that have proved difficult using traditional morphological approaches (Thacker 2003).

Molecular markers are very useful in evolutionary studies as they can be interpreted more objectively than previously used methods including morphological and allozyme studies. Molecular data are categorised by function (protein-coding vs. non-coding vs. structural RNA) and genome (mitochondrial vs. nuclear DNA; Springer *et al.* 2001). Detected patterns of population genetic variation among nuclear and mitochondrial DNA (mtDNA) data sets may differ according to the response of each independent genome to the combined effects of mutations, migration, selection and effective population size (Hou *et al.* 2007). Therefore, the trend in systematics has been to use different genes in molecular studies including both nuclear and mtDNA to obtain information that provides a more complete picture of populations and phylogenies (Field *et al.* 1988; Hedges *et al.* 1990; Turbeville *et al.* 1991; Gadagkar *et al.* 2005). These two markers are commonly selected because they represent both more conservative and highly variable regions, and therefore together provide a good phylogenetic signal for analyses.

1.4.1. Mitochondrial DNA markers (mtDNA)

The invention of the polymerase chain reaction (PCR) has been useful in the determination of the complete mitochondrial genome (Miya and Nishida 2000). The mitochondrial genome is a closed circular molecule of approximately 16-20 kilobases (kb) in size and consists of 37 genes including 13 protein coding, two ribosomal RNAs (rRNA), 22 transfer RNA (tRNAs) and one major non-coding region (control region) that contains the initiation sites for mtDNA and RNA transcription (Figure 1.7; Brown *et al.* 1982; Meyer 1993; Inoue *et al.* 2000). The mtDNA genetic marker has been successfully used in studying genetic variation among fishes and is a marker of choice (Kocher *et al.* 1989). This is because mtDNA which is maternally inherited is relatively easy to isolate and undergoes little to no recombination (Hartl and Clark 1997; Kocher and Stepien 1997) and it also appears in multiple copies in the cell (Galtier *et al.* 2009; Morin *et al.* 2010). Some of its other valuable characteristics include higher mutation rates compared to nuclear DNA genes meaning that mtDNA sequences often contain high levels of informative variation, the cost and technical ease of amplification and the ability to use a variety of primers for different species where needed (Avice 2000; Rozas *et al.* 2003; Douglas and Douglas 2010).

The mitochondrial genome also has a fast evolutionary rate, shorter coalescence time (Jacobsen *et al.* 2010) and a greater sensitivity in reflecting the genetic impact of population subdivisions over large geographic scales (Inoue *et al.* 2000; Duran *et al.* 2004). The mitochondrial region is also known to be a sensitive indicator of the population processes of genetic drift, selection and demographic events such as bottlenecks in a population making it useful for population genetics studies (Wilson *et al.* 1985; Wood *et al.* 2005). Mitochondrial genes are also important tools in the research fields studying animal evolution (Moritz *et al.* 1987), such as phylogeography (Avice 2000; Morin *et al.* 2010) and biogeography (Orrel *et al.* 2002). The mtDNA gene is widely recommended because it has proven to be useful in

clarifying the relationships among populations and closely related species for phylogenetics and determining population structure (Moritz *et al.* 1987; Lavoue *et al.* 2003; Chauhan and Rajiv 2010). Animal mtDNA was the first DNA based genetic marker system that could be routinely applied to surveys of genetic variation in natural populations (Neigel 1997; Avise 2000). For example, Bay *et al.* (2004) examined the genetic composition and population structure of the common reef fish, *Chlorurus sordidus* over a large part of its Indo-central Pacific range by employing phylogenetic, population genetics and cladistic approaches. Their study used mtDNA to examine the geographic patterns of genetic differentiation in this species in order to understand the roles of historical and present day gene flow. Their results revealed that the population of *C. sordidus* displayed high levels of genetic diversity at the scale of oceans with a strong break between WIO and the Pacific Ocean.

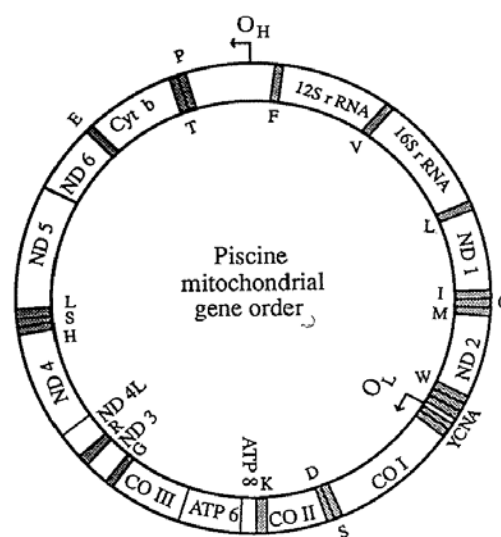


Figure 1.7. The origins of the H-and L-strands replication are indicated in the diagram and transfer RNA is shown in the shaded boxes. This mitochondrial gene order of fish was taken from Meyer (1993).

There are various mtDNA markers that have been used to investigate the genetic structure of marine species (Okumus and Ciftci 2003). Some studies have used the restriction fragment lengths polymorphism (RFLPs) as the first quantitative procedure of detecting a genetic

variation, using probes or amplifications followed by restriction digestions (Bermingham *et al.* 1999). Some general problems associated with the use of mtDNA, are generally because the sequence amplified was either too short to contain significant genetic variations or the evolutionary rate of the segment was not suitable for the specific purpose of the study (Inoue *et al.* 2000).

1.4.2. Nuclear DNA markers (nDNA)

Analyses of nuclear DNA markers, such as exons, introns and microsatellites provide both maternal and paternal information for organisms (Neigel 1997). Because nuclear introns are abundant throughout the genome, they have been used as additional sources of genetic variation for species-level phylogenies (Steiner *et al.* 2005). A large proportion of nuclear DNA markers employed for population analyses have been the non-coding regions, because they tend to be more variable than the coding regions (Zhang and Hewitt 2003). Nuclear markers can also provide an alternative perspective, as evolutionary patterns derived from mtDNA are often oversimplified and confounded by lineage sorting (Zhang and Hewitt 2003; Taylor and Hellberg 2006). Coding and non-coding sequences can be examined in the Ribosomal DNA (rDNA) and single copy DNA (Hodges *et al.* 2002). The coding and non-coding regions of nuclear DNA evolve at different rates, allowing a broad range of inferences from intra- population dynamics based on markers from independent linkage groups (Sunnucks 2000; Fujita *et al.* 2004).

Previous studies on fish have effectively applied nuclear DNA for phylogeny evaluation (e.g. Lavoue *et al.* 2003; Quenouille *et al.* 2004; Ruber *et al.* 2006). For example, a study of the damselfishes (Pomacentridae) used Bayesian phylogenetic analyses of three mitochondrial genes and 1500 bp (base pair) of the single copy nuclear encoded recombination activating gene 1 (RAG1) DNA sequences (Quenouille *et al.* 2004) to understand the relationships

within the family and advance knowledge regarding the relative efficacy of different molecular markers for phylogeny estimation. This study also utilised the geographic distribution and prevailing taxonomy to infer the likely effect of missing taxa on the stability of their phylogenetic hypothesis for the family. The study revealed high statistical support whether the data were analysed from an mtDNA, RAG1 or combined perspective and suggested that molecular studies should aim to provide more gene regions in order to obtain more characters for phylogenetic analyses.

A study by Taillebois *et al.* (2013) was done to assess past and present genetic structure of populations of two amphidromous fish (*Sicyopus zosterophorum* and *Smilosicyopus fehlmanni*,) that are widely distributed in the Central West Pacific and which have similar pelagic larval durations (about 55 days). Sections of mitochondrial cytochrome *c* oxidase subunit 1 (COI), cytochrome *b* (Cyt-*b*) and nuclear Rhodospine genes were analysed to test hypotheses of genetic connectivity. Despite the low number of samples, genetic connectivity among *S. fehlmanni* populations was high as expected, suggesting that for this species, neither the fragmentation of freshwater habitat nor the oceanic currents surrounding the Central West Pacific Ocean or the presence of the Torres Strait barrier have created significant barriers to gene exchange. Although these two gobies have similar pelagic larval duration, population genetic analyses over the sampled region revealed contrasting patterns of genetic structure. Their data, together with similar studies in that region, suggested that a Pleistocene barrier to marine dispersal (the Torres Strait) may have shaped the genetic pattern of one of the species (*S. zosterophorum*). Thus nuclear genes are useful markers for phylogenetic analyses.

1.5. AIM OF THE STUDY

The aim of this study is to examine the genetic structure and differentiation of three widespread WIO goatfish species (*Parupeneus barberinus*, *P. macronemus* and *P. rubescens*) and to determine their intraspecific relationships among geographic sub regions in the WIO.

The specific questions related to this aim are:

1. To determine the genetic connectivity and levels of genetic differentiation among different areas of the WIO for each goatfish species using a molecular phylogenetic approach. A comparative molecular genetic approach can provide insight into the levels of diversity and relationships among regions.
2. To identify the processes and/or events that may have shaped the genetic structure within species among WIO regions. Differentiation and genetic variability in marine fishes results from a complex interplay (physical factors and life history features; Hauser and Ward 1998; Kesäniemi *et al.* 2014). These selected goatfishes were examined to compare and establish whether the biogeographic distribution of the regional fauna was affected by dispersal or vicariant events. Examining the genetic structure of species should allow for estimates of the level of individual exchange between populations and the identification of barriers to gene flow in the region (Muths *et al.* 2012).

The WIO possesses a combination of continental coastlines with diverse fish faunas (Lutjeharms 2006; Obura 2012). This region therefore, presents an outstanding model for studying patterns of differentiation because there is no clarity on the origin of its diverse fauna, regional components and levels of endemism. Understanding regional biogeography in this study will be valuable as baseline data for long-term strategies in conservation and management planning. This study is expected to expose low levels of genetic differentiation

because the studies on population genetic structure in marine organisms with planktonic larvae will generally have less genetic structure than those with direct development (Ward *et al.* 1994; Bucklin and Wiebe 1998; Graves 1998; Kyle and Boulding 2000). Although the few studies which have been conducted in the WIO (Ragioneiri *et al.* 2010; Visram *et al.* 2010) have also mostly demonstrated high levels of marine connectivity between localities, genetic differences have been observed in some peripheral areas such as the studies by Visram *et al.* (2010) on the parrotfish *Scarus ghobban* and Ragioneiri *et al.* (2010) on the mangrove crab *Neosarmatium meinerti*. The present study will therefore help in the understanding of the levels of diversity and genetic differentiation of these three selected goatfishes in the WIO by also including samples from the peripheral regions such as the Red Sea, Oman and the Maldives.

CHAPTER TWO

MATERIAL AND METHODS

2.0. OVERVIEW

This chapter summarises the research procedures and materials used in this study. It also discusses the laboratory methodology and the molecular analyses including the direct sequencing of two mitochondrial DNA (mtDNA) genes, the protein coding NADH Dehydrogenase subunit 2 (ND2) and 16S ribosomal protein (16S) as well as a nuclear protein coding gene (nDNA), recombination activation gene-1 (RAG1). Statistical methodology and data analyses are also explained including the methods in DNA sequence editing and alignment procedures.

2.1. SAMPLING DESIGN AND PROCEDURES

Three species of the genus *Parupeneus* (*P. barberinus*, *P. macronemus* and *P. rubescens*) were selected because of their wide distribution in the WIO, their similar life history features and habitat preferences. These goatfishes are widely distributed across the WIO, being found in tropical and subtropical waters in isolated reef habitats. Sampling was conducted and designed in a way that was relevant to the aims of the study in order to identify patterns of connectivity and relationships among biogeographic regions and known areas of endemism of the WIO. The sampling sites were selected, where possible, from the biogeographic regions identified by Santini and Winterbottom (2002) in order to evaluate and test their biogeographic hypotheses and those of Pandolfi (1992); including Bellwood and Wainwright (2002). The sampling sites where specimens were collected included several WIO countries viz. Oman, Red Sea and the Maldives (Peripheral WIO regions); Kenya, Tanzania,

Mozambique and South Africa (Continental African coast); Madagascar (Island state) and Mauritius and Seychelles (Mascarene Plateau; see Figure 2.1). These localities cover areas of endemism and ensure that this distribution will be useful for comparing the levels of connectivity within this region and for testing biogeographic hypotheses.

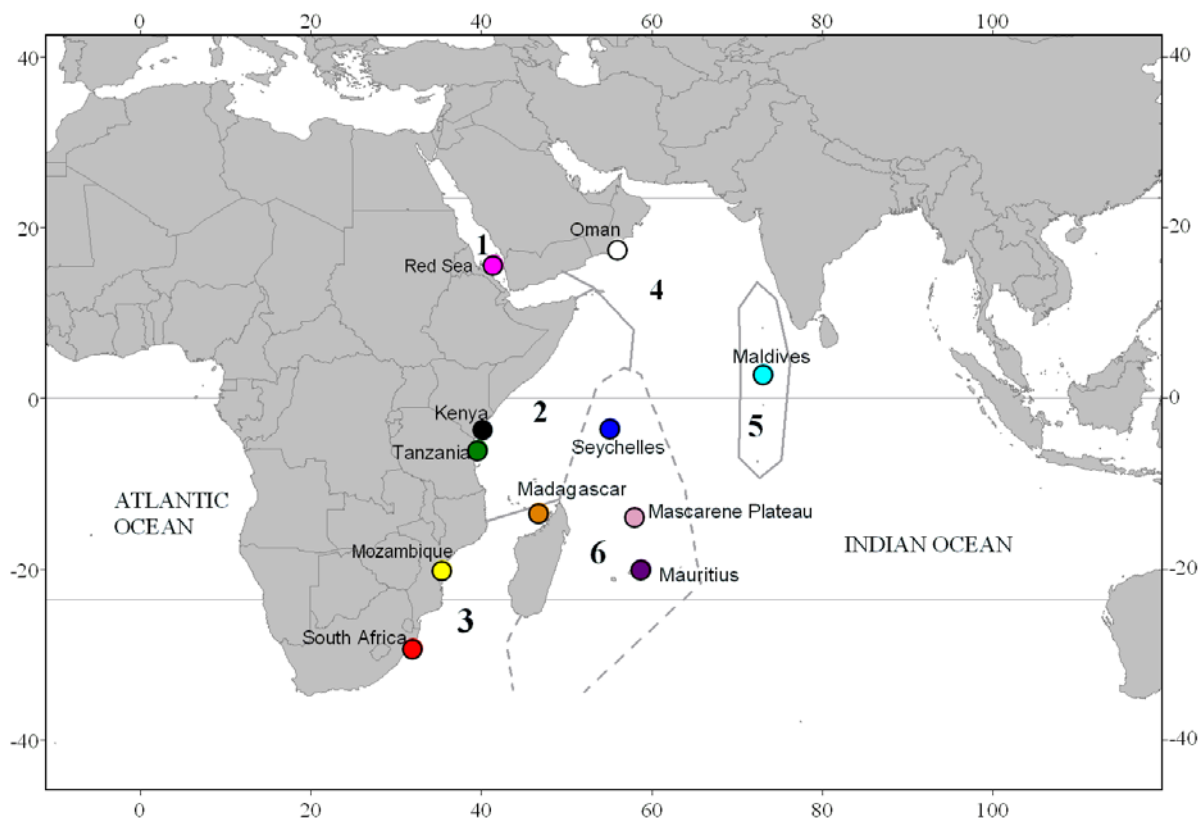


Figure 2.1. Sampling localities within the WIO region, the coloured circles indicate the different sampling localities and the numbers indicate the WIO biogeographic regions identified by Santini and Winterbottom (2002): 1 = Red Sea, 2 = Somalian Basin, 3 = Natal Basin, 4 = Arabian Basin, 5 = Chagos Plateau and 6 = Mascarene Plateau.

A variety of sampling techniques and gear were used to collect the goatfishes; including spear fishing, beach seine netting (10 x 2 m; 1.2 cm mesh size), gill netting (50 x 5 m) and hook and line. A number of individuals of the three species collected from eleven localities

(Oman, Red Sea, Maldives, Kenya, Tanzania (including Zanzibar Island), Inhaca Island in Mozambique, South Africa, Madagascar, Mauritius, Seychelles and the Mascarene Plateau) of the WIO were analysed. *Parupeneus macronemus* was abundant and well represented as it was collected from all the eleven localities compared to the other two species (*P. barberinus* and *P. rubescens*) that were not as abundant in collections (see Table 2.1). In some localities (e.g. Kenya), samples were purchased from fish markets and local fishers at landing sites for known fishing areas. Other samples were collected by collaborators which include cruises of the African Coelacanth Ecosystem Programme (ACEP), Agulhas and Somali Current Large Marine Ecosystem (ASCLME) Programme and IFREMER (Reunion). Muscle tissue and fin clips were taken from all available samples (voucher specimen) for genetic analyses and were preserved in 95% ethanol in 1.5 ml Eppendorf's. These collected DNA specimens were labeled with station and DNA tissue numbers and were lodged with the South African Institute for Aquatic Biodiversity (SAIAB) National Fish Collection where they were stored at -20°C.

Table 2.1. Sampling localities, locality abbreviations and sample size (N) for the three target species, *Parupeneus barberinus* (PB), *P. macronemus* (PM) and *P. rubescens* (PR) in the WIO.

Locality	Locality Abbrev.	Species sample sizes (N)			GPS Positions	
		PB	PM	PR	Latitude	Longitude
Oman	OM	1	2	-	21.513	55.958
Red Sea	RS	-	4	-	26.629	55.991
Maldives	ML	8	2	-	3.2234	73.218
Kenya	KE	5	17	4	-4.6478	39.381
Tanzania (Zanzibar Is.)	TZ	4	5	2	-6.1295	39.466
Inhaca Is. (Mozambique)	MZ	12	24	32	-26.047	32.851
South Africa	SA	-	4	10	-29.867	31.048
Madagascar	MD	-	3	-	-18.767	46.889
Mauritius	MR	2	7	1	-20.170	57.449
Seychelles	SY	4	2	2	-4.6792	55.419
Mascarene Plateau	MP	-	1	-	-10.000	59.990
Total		36	71	51		

2.2. LABORATORY PROCEDURES

2.2.1. DNA extraction

Total genomic DNA was extracted using the protocol of the Promega DNA purification kit (Madison, New York, USA) after cutting the tissue samples into small pieces. The extraction process involved digestion by incubating samples at 55°C with Proteinase K for 1-3 hours or overnight. RNase solution was then added before incubating for another 15-30 minutes in a water bath at 37°C. Extracted DNA was visualised by loading 5 µl of the DNA template mixed with 2 µl of Bromophenol blue on a 1% agarose gel containing Ethidium bromide. The loaded gels were electrophoresed for 15-20 minutes at 90 mV while submerged in 1X TBE buffer. Gel electrophoresis is a widely used technique for the preparation and analysis of DNA extracts and (PCR) products. Each gel was visualised under ultra-violet light (UV

Transilluminator), to confirm the quality of DNA extracted. DNA extraction aliquots were labeled and stored in a -20°C freezer before PCR.

2.2.2. Polymerase Chain Reaction (PCR)

To amplify the selected gene fragments, standard PCR was performed. This PCR technique has opened up the possibility of examining the genetic changes in fish populations (Chauhan and Rajiv 2010). PCR allows for accurate production of millions of copies of a target DNA sequence in a matter of hours (Kocher *et al.* 1989). PCR was conducted for the amplifications of three genes: NADH dehydrogenase subunit 2 (ND2), large subunit of the 16S ribosomal protein (16S) and the recombination activation gene-1 (RAG1). Each PCR reaction mixture contained: 3-6 µl template DNA; 10X reaction buffer (2.5 µl), 2.5 mM MgCl₂ (2.5 µl), 0.125 mM of dNTP's (Promega) (2.5 µl); 2 units of Super-therm Taq (Southern Cross Biotechnology, Cape Town) DNA polymerase (0.2 µl) and 10 pmol (1 µl) of the specific forward and reverse primers for the region being amplified (Table 2.2) and DNA free water to top up the mixture to 25 µl. PCR amplifications were performed in either a Thermo Hybaid PX2 or Multi-Block Thermal cycler (Thermo Electron Corporation) and Mastercycler gradient (Eppendorf) under optimal conditions that were specific for each gene with the last step for all reactions being a final extension at 72°C for 10 minutes. The specific PCR cycling parameters for each gene were:

- 1) ND2: Initial denaturation at 90°C for 4 min, followed by 35 cycles of 45 sec of denaturation at 94°C, 1 min of annealing at 50°C, and 90 sec of extension at 72 °C.
- 2) RAG1: Initial denaturation at 94°C for 2 min followed by 35 cycles of 30 sec of denaturation at 94 °C, 1 min of annealing at 57°C, and 2 min of extension at 72°C.
- 3) 16S: Initial denaturation at 96°C for 3 min followed by 30 cycles of 50 sec of denaturation at 94°C, 30 sec of annealing at 56°C, and 50 sec of extension at 72°C.

Table 2.2. Primers used for the amplification of the mtDNA genes ND2 and 16S and the RAG1 nuclear gene region.

Gene	Primer	Sequence (5' - 3')	Source
ND2	ND2R	CGCGTTTAGCTGTAACTAA	Kocher <i>et al.</i> 1995
	ND2F	CTACCTGAAGAGATCAAAAC	
RAG1	RAG1R3	CTGAGCTGCAGTCAGTACCATAAGATGT	Lopez <i>et al.</i> 2004
	RAG1F1	GTCTTGTGSAGGTAGTTGGT	
16SrRNA	16SBR	CCGGTCTGAACTCAGATCACGT	Palumbi <i>et al.</i> 1991
	16SAR	CGCCTGTTTATCAAAAACAT	

2.2.3. DNA sequencing

The PCR products were purified using a QIAquick PCR purification kit (Qiagen, Valencia, CA). This protocol was designed to purify single or double-stranded DNA fragments from PCR reactions. The purified DNA was verified by loading on a 1% gel by adding one volume of loading dye to five volumes of purified DNA. PCR cycle sequencing was conducted using an ABI sequencing kit (Big Dye Terminator Cycle Sequencing v 3.1, Applied Biosystems) using 1 µl of Big dye, 1.5 µl of the sequencing buffer, 0.5 µl of either the reverse or forward (10 µM) primer, 4 µl of DNA template and 13 µl of DNA free water. This mixture was sequenced in a thermocycler using the following conditions: 25 cycles of 10 sec at 96°C, 5 sec at 50°C and 4 min at 60°C.

When the sequencing process was conducted, the PCR tubes were stored at 4°C until DNA precipitation. Precipitation involved mixing cycle sequencing products with a master mix of Sodium acetate and ethanol. This master mix contained, 1 µl of 3 M NaOAc (pH 4.6), 1 µl of 0.25M EDTA (pH 8), and 50 µl of 99% ethanol. The mixture was left at room temperature for 15 minutes to precipitate before centrifuging for 20 minutes at 13000 rpm. The supernatant was removed and the pellet rinsed with 70% ethanol. After discarding the

supernatant, the pellet was dried at 60°C for 5-10 minutes. The cycle sequencing products that were analysed on an ABI Prism 310 or 3100 DNA genetic sequencers (Applied Biosystems) operated by the Science Faculty, Rhodes University or the sequencing facilities of Macrogen Inc. (South Korea).

2.3. STATISTICAL ANALYSES

2.3.1. Sequence alignment

DNA sequences were analysed during sequence alignment using gaps that are inserted between the nucleotide bases so that positions with identical or similar DNA base characters are aligned in successive columns. Gaps (codons) were only included into the analyses and considered as either missing data or as another character state in addition to the four nucleotides (i.e., gaps as fifth state) for the RAG1 nuclear gene only (Maake 2009). All DNA sequence chromatograms were edited by eye on CHROMAS Pro version (v) 1.5 (Technelysium) and checked and aligned in LASERGENE v.9.0.5 sequence editor (DNA Star Inc., Madison, WI). For the nuclear RAG1 data set, the sequences generated were aligned and then phased using the PHASE option (and the default parameters) in the DNASP 5.10 software package (Librado and Rozas 2009) to identify the component alleles of each individual from the ambiguities/polymorphisms present in the DNA sequence. DNASP was also used to transform the sequence alignments into different file formats for further analyses using other software.

2.3.2. Genetic diversity estimates

Several genetic indices and extensive population genetics analyses were estimated as implemented in DNASP 5.10 in order to address current and recent historical levels of variation and genetic diversity within the goatfishes. Unique haplotypes were also identified for each of the three genes for each species. Genetic diversity was assessed by calculating the

nucleotide (π) and haplotype diversities (HD) of each gene for each species and within each locality. Haplotype diversity is a measure of the uniqueness of a particular haplotype in a given population (Nei and Tajima 1981), while nucleotide diversity is the average number of nucleotide differences per site between two sequences (Nei and Li 1989; Tamura and Nei 1993). Haplotype and nucleotide diversities were calculated using ARLEQUIN v3.5 (Excoffier and Lischer 2010) only for localities with more than three individuals. Relationships among haplotypes were investigated using a parsimony median-joining network in the program NETWORK v4.6 (Bandelt *et al.* 1999). In this program, the outgroup probabilities are assigned to each haplotype based on the correlation between haplotype frequency and the age of the haplotype in the lineage (Templeton *et al.* 1995).

2.3.3. Mismatch distributions and tests of neutrality

Distributions of pairwise sequence differences frequently called mismatch distributions have generally been used to estimate the demographic parameters of past populations (Schneider and Excoffier 1999). Mismatch distributions summarise information about genetic differences between pairs of subjects in a sample (Rogers *et al.* 1996; Excoffier and Heckel 2006). To test for population expansion, we used ARLEQUIN with 10000 bootstrap replicates to estimate the distribution of observed pairwise haplotype differences (mismatch distribution; Rogers and Harpending 1992) among individuals. This analysis calculates the observed and expected values for expanding and stationary populations (Slatkin and Hudson 1991; Rogers and Harpending 1992; Rogers 1995). Two different scenarios may be inferred from this analysis: a unimodal distribution characteristic of populations which have experienced a recent demographic expansion; or a multimodal distribution indicative of a stable population (Cassone and Boulding 2006; Silva *et al.* 2010; Krithika and Vasulu 2013). Within DNASP, a sum of square deviation test was used to test for the fit of the data sets to a model of sudden population expansion.

Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997) estimates were tested for significance using parametric bootstrapping and 1000 bootstrap replicates using the infinite sites model (Schneider and Excoffier 1999). Tajima's D statistic tests various predictions made by the neutral theory of molecular evolution (Kimura 1983). Apart from confirming the neutrality of the gene, significant negative values for these tests may also be interpreted as indicative of sudden population expansion (Tajima 1989). Tajima's D value can also be interpreted together with mismatch distribution results, since a model of sudden expansion is predicted by a negative D value while positive values of D indicate possible balancing selection of a population (Tajima 1989). A negative value of the statistic F_s is an indication of an excess number of alleles as would be expected from a recent population expansion or from genetic hitchhiking (Holsinger 2006). A positive value is an indication for deficiency alleles as that can be expected from a recent population bottleneck or from over dominant selection. It has been suggested that Fu's F_s is more sensitive to population expansion and genetic hitchhiking (Krithika and Vasulu 2013).

2.3.4. Genetic structure and analysis of molecular variance (AMOVA)

The best-fit model of DNA evolution was determined using MEGA v5 (Tamura *et al.* 2011). The parameters of the resulted best-fit model were used in the analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) which was conducted using ARLEQUIN to measure the apportionment of genetic variance at various hierarchical levels such as among populations and within defined groups (Roewer *et al.* 1996). This method takes into account the molecular relationship of alleles/haplotypes, rather than just their frequency. AMOVA also generates Φ_{ST} , an analogue of Wright's (1965) F -statistics based on the frequency of haplotypes and the genetic distance between haplotypes. The Φ_{ST} statistic is a way of comparing differentiation among two or more subpopulations. AMOVA was conducted, as an overall treatment of genetic diversity within localities for each species, and between

defined regions (Bowen *et al.* 2001). The grouping of the various localities into regions of the WIO for AMOVA analyses was employed according to geographic proximity or defined biogeographic provinces following Santini and Winterbottom (2002).

CHAPTER THREE

RESULTS

3.1. *PARUPENEUS BARBERINUS*

3.1.1. Molecular diversity and haplotype relationships

Analyses were conducted for seven sampling localities (Oman, the Maldives, Kenya, Tanzania, Mozambique, Seychelles and Mauritius) of *P. barberinus* using three gene regions (16S, ND2 and RAG1). The alignment of amplified sequences of all genes resulted in 515 bp of 16S, 702 bp of ND2 and 912 bp of RAG1 data alignments. Molecular diversity indices for most of the localities were characterised by moderate to high haplotype and low nucleotide diversities, generally suggesting high levels of genetic differentiation among different localities for this species (Table 3.1).

Table 3.1. The DNA sequence diversity estimates for *Parupeneus barberinus* for the mitochondrial (16S and ND2) and nuclear (RAG1) gene datasets for each of the six sampling localities. The number of individuals (N), haplotypes (H), private haplotypes (N_{PH}), haplotype diversity (HD) alleles (A), private alleles (N_{PA}), allele diversity (AD) and nucleotide diversities (π) are indicated for each locality.

Locality	16S 515 bp					ND2 702 bp					RAG1 912 bp				
	N	H	N_{PH}	HD	Π	N	H	N_{PH}	HD	π	N	A	N_{PA}	AD	π
OM						1	-	-	-	-					
ML	7	4	3	0.71	0.004	1	-	-	-	-	16	8	4	0.76	0.005
KE	5	2	-	0.60	0.005	3	3	2	1	0.006	18	7	3	0.82	0.005
TZ	2	1	-	-	-	2	2	1	1	0.003	2	1	-	-	-
MZ	7	2	1	0.29	0.001	7	6	5	0.95	0.004	16	6	2	0.82	0.005
MR	2	1	-	-	-	2	2	1	1	0.003	4	3	-	0.83	0.001
SY	4	2	1	0.50	0.001	3	2	1	0.67	0.003	8	6	2	0.93	0.002
Overall	27	7	5	0.61	0.004	17	11	12	0.84	0.004	64	16	11	0.88	0.006

The dataset of the 16S gene (515 bp) had 27 sequences representing six sampling localities from the Maldives, Kenya, Tanzania, Mozambique, Seychelles and Mauritius. This data had four singletons and eight variable nucleotide sites of which only four were parsimony informative which defined seven unique haplotypes. Five of these haplotypes were private, (Slatkin 1985) being only present in one individual and therefore restricted to a locality. The other two were shared with haplotype H1 being present in almost all of the analysed localities (except the Maldives) while H3 was only shared between individuals from Kenya and the Maldives (Figure 3.1A). The 16S data had a northern lineage of *P. barberinus* consisting of Kenya and the Maldives (H3 to H5) which was restricted from the more SWIO localities south of Kenya. These analyses also indicated that individuals from Maldives were more genetically different from other localities with a higher number of mutational steps in the network.

The 19 sequences analysed for the ND2 gene data were from seven localities (Oman, the Maldives, Kenya, Tanzania, Mozambique, Seychelles and Mauritius). These mtDNA sequences had 20 variable sites, including 13 singletons and seven parsimony informative sites and defined 13 unique haplotypes (Figure 3.1B). There was only one haplotype (N1) that was shared and widespread among five of the localities (except Oman and the Maldives). The other 12 ND2 haplotypes were private and restricted to specific localities. There were three missing haplotypes in the network which are indicated as median vectors (mV) in the network. The ND2 data also suggested that Oman was differentiated from the SWIO with four mutational steps isolating its haplotype from the main network. Unfortunately this single sample from Oman could not be used in the estimation of diversities due to sample size. Some individuals from Kenya were also isolated from the main network by a high number (>2) of mutational steps.

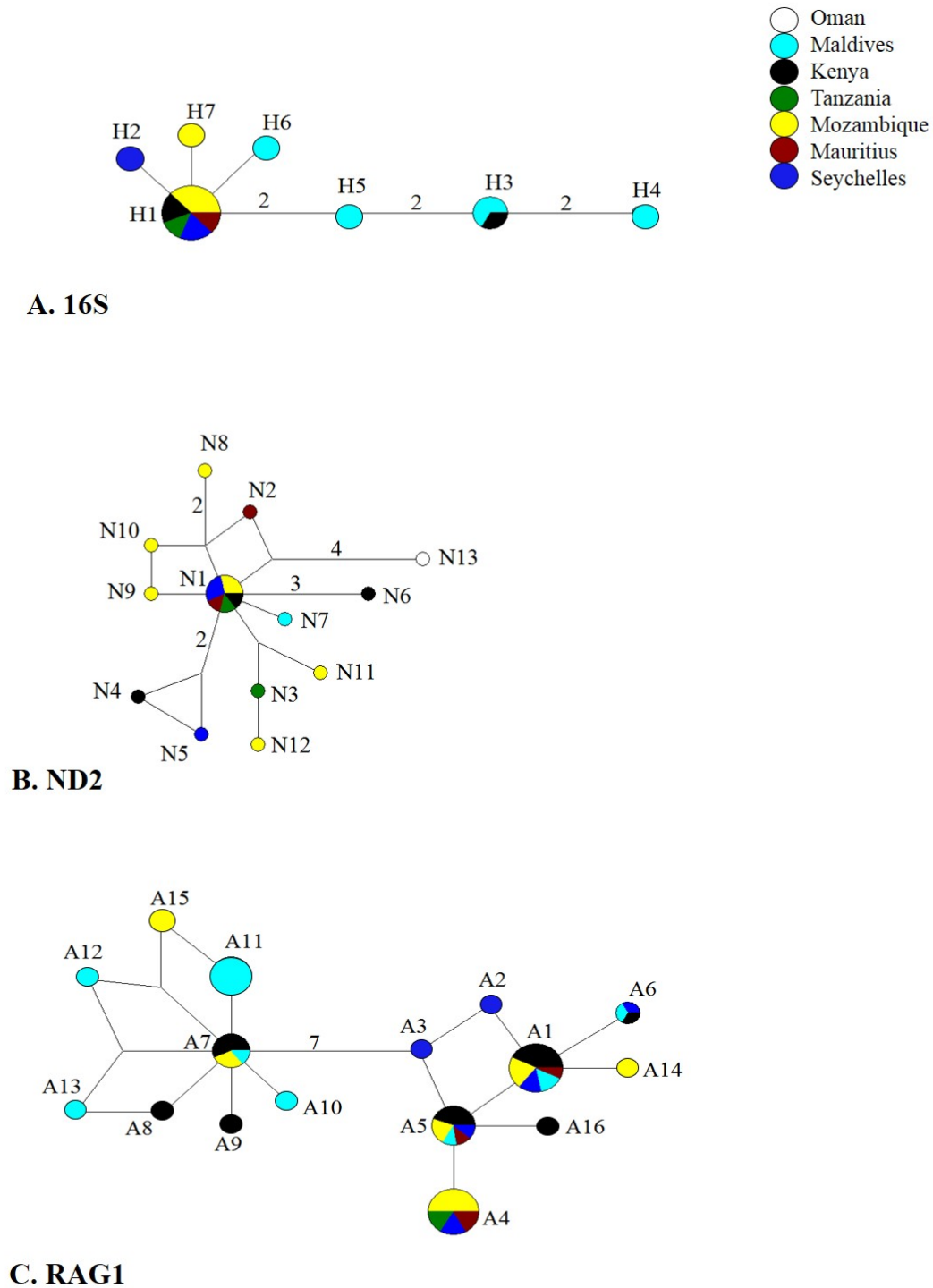


Figure 3.1. The haplotype/allele networks of *Parupeneus barberinus* for the 16S (A), ND2 (B) and RAG1(C) gene sequences. Haplotypes are represented by the circles while the colours represent the different sampling localities. The size of each circle is proportional to the frequency of each haplotype. Haplotype network connections with two or more mutations have the numbers of mutational steps also displayed. The connection where there are no coloured circles are the missing haplotypes called median vectors (mv).

The phasing of the aligned 32 RAG1 individual sequences (912 bp) of *Parupeneus barberinus* resulted in 64 sequence alleles. After phasing, these sequence alleles had 19 variable sites that included four singletons with 15 sites being parsimony informative sites. Sixteen unique alleles (Table 3.2) were identified among these 32 individuals with eight being shared among individuals but only five of these being more widespread and shared among three or more localities (A1, A4, A5, A6 and A7). Two lineages were observed in the nuclear network (Figure 3.1C), with one widespread lineage having four high frequency alleles and the other being a more range restricted lineage with the exclusion of individuals from the Mascarene Islands (Mauritius and Seychelles) and Tanzania. The Maldives also had more private alleles (4) supporting the genetic isolation that was observed in the 16S mtDNA data sets. The distinct groups separated by several mutational steps may be related to independent dispersal events.

Table 3.2. The nuclear DNA (RAG1) alleles for six sampling localities of *Parupeneus barberinus* with the numbers of individuals (N) collected per locality within the WIO.

Allele	ML	KE	TZ	MZ	MR	SY	N per allele
A1	2	6	-	3	1	2	14
A2 and A3	-	-	-	-	-	2	2
A4	-	-	2	6	2	2	12
A5	1	4	-	2	1	1	9
A6	1	1	-	-	-	1	3
A7	1	4	-	2	-	-	7
A8 and A9	-	2	-	-	-	-	2
A10 to A13	11	-	-	-	-	-	11
A14 and A15	-	-	-	3	-	-	3
A16	-	1	-	-	-	-	1
Locality (N)	16	18	2	16	4	8	64

3.1.2. Demographic history of *Parupeneus barberinus*

The mismatch distributions of the 16S, ND2 and RAG1 datasets had bimodal graphs (Figure 3.2) suggesting that the species has high genetic differentiation and may consist of more than one population unit. Tajima's D value was negative, for the 16S (-0.22) and ND2 (-1.92) mtDNA genes with the statistical test being insignificant ($P > 0.10$) for the 16S data but significant ($P < 0.05$) for the ND2 gene data set.

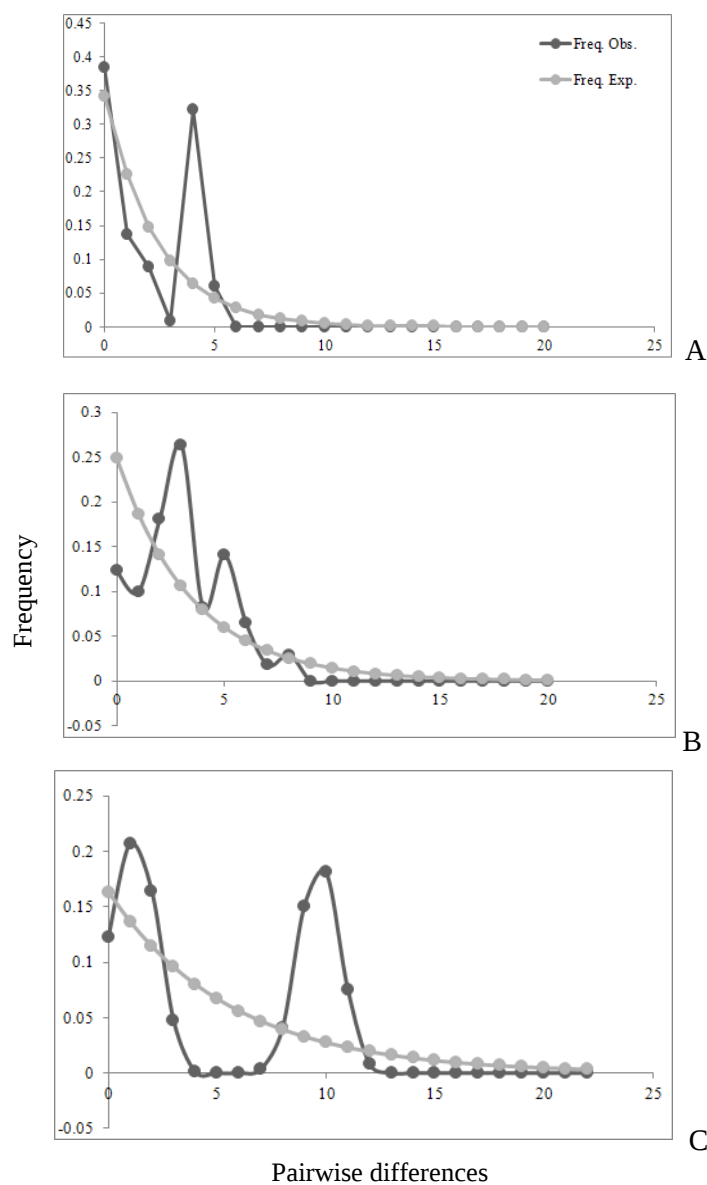


Figure 3.2. Mismatch distribution curves for *Parupeneus barberinus* for all the three genes; 16S (A), ND2 (B), and RAG1 (C).

For the nuclear DNA RAG1 data, Tajima's D was positive (0.83) and not significant ($P > 0.10$). The negative Tajima's D values and other tests of neutrality such as Fu's F_s statistic (-0.75, -7.13 and -0.98 for the 16S, ND2 and RAG1, respectively) indicate a model of sudden population expansion for this species in the WIO (Tajima 1989).

3.1.3. Genetic structure of *Parupeneus barberinus*

The substitution models for *P. barberinus* with the lowest BIC scores (Bayesian Information Criterion) under the Akaike Information Criterion (AIC) that best fits the data were: the Jukes-Cantor (JC) (Jukes and Cantor 1969) model (16S dataset), the Tamura-Nei (TN93) (Tamura and Nei 1993) (ND2 dataset), Tamura 3-parameter (T92) (Tamura 1992) (RAG1 dataset). The pairwise F_{ST} comparison values from the 27 analysed sequences of 16S data, suggested some genetic differentiation among some pairs of localities (Table 3.3). Pairwise F_{ST} values with the Maldives were substantially higher than all other comparisons suggesting that the Maldives is genetically differentiated from the Seychelles ($F_{ST} = 0.55$), Tanzania ($F_{ST} = 0.46$) and Mozambique ($F_{ST} = 0.62$) samples. These significantly different ($P < 0.05$) comparisons suggest very great differentiation as all values were greater than 0.4 (Wright 1978). The RAG1 pairwise F_{ST} (Table 3.4) values also suggested that *P. barberinus* from the Maldives were genetically different from the rest of the SWIO as comparison estimates with the Seychelles, Mauritius, Mozambique, Tanzania and Kenya were all greater than 0.05. However, no significant differentiation was detected among pairwise comparisons in the ND2 data set even though two haplotype lineages were observed in the haplotype network. This could be due to the low sample size of this data.

Table 3.3. A comparison of pairwise F_{ST} values between six localities based on mtDNA sequences (16S, below diagonal and ND2, above diagonal). Significant estimates ($P < 0.05$) are indicated in bold font. See Table 3.1 for locality abbreviations.

Locality	ML	KE	TZ	MZ	MR	SY
Maldives	-	-0.33	0.00	-0.11	0.00	0.00
Kenya	0.06	-	-0.07	0.08	-0.07	-0.23
Tanzania	0.46	-0.02	-	-0.20	0.00	0.00
Mozambique	0.62	0.29	-0.09	-	-0.13	0.02
Mauritius	0.46	-0.02	-0.31	0.00	-	0.00
Seychelles	0.55	0.18	0.03	0.00	-0.26	-

Table 3.4. A comparison of pairwise F_{ST} values between six localities based on RAG1 sequences. Significant F_{ST} values ($P < 0.05$) are indicated in bold font.

Locality	ML	KE	TZ	MZ	MR
Maldives	-				
Kenya	0.23	-			
Tanzania	0.50	0.15	-		
Mozambique	0.29	-0.00	-0.06	-	
Mauritius	0.51	0.11	-0.02	-0.02	-
Seychelles	0.53	0.12	0.23	0.05	-0.06

An analysis of molecular variance (AMOVA) with the sampling localities of *P. barberinus* being grouped according to biogeographic regions (Table 3.5) detected significant ($P < 0.05$) genetic structuring in the mtDNA 16S data set among groups (Va) and also within localities (Vc). This significant differentiation accounted for 59.9% of the genetic variation among the four defined groups: (1) the southern mainland areas (Tanzania and Mozambique), (2) the Mascarene Islands (Seychelles and Mauritius), (3) Kenya and (4) Maldives. The AMOVA however revealed no significant genetic variation among these same four regional groups in the ND2 data in agreement with the pairwise F_{ST} population comparisons. However, the

RAG1 data also revealed significant genetic differentiation ($P < 0.05$) among groups and within localities as was indicated by the 16S AMOVA analysis, but only when three groups were defined: (1) African mainland areas (Kenya, Tanzania and Mozambique), (2) Mascarene Islands (Mauritius and Seychelles) and (3) the Maldives. This significant genetic structure accounted for 30.4% of the genetic variance in the data. These 16S and RAG1 results therefore suggest that there is some significant regional genetic sub structuring in *P. barberinus* in the WIO.

Table 3.5. The source of variation, degrees of freedom (d.f.), sum of squares, variance components and percentage of genetic variation among different groups, localities within groups and within localities of *Parupeneus barberinus* that resulted from the AMOVA analyses of the 16S, ND2 and RAG1 genes. This analysis had three variance estimates: Va (Variance among groups), Vb (variance among localities within groups) and Vc (variance within localities).

Gene region	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	P. value
16S	Among groups (4)	3	11.72	0.65 Va	59.94	0.00
	Among localities within groups	2	0.11	-0.19 Vb	-18.40	0.49
	Within localities	21	13.32	0.63 Vc	58.45	0.00
ND2	Among groups (4)	3	6.84	0.28 Va	17.40	0.19
	Among localities within groups	3	3.87	-0.03 Vb	-1.93	0.57
	Within localities	12	16.66	1.39Vc	84.53	0.19
RAG1	Among groups (3)	2	36.76	0.89 Va	30.42	0.03
	Among localities within groups	3	5.58	-0.02 Vb	-0.78	0.31
	Within localities	58	118.8	2.05 Vc	70.35	0.00

3.2. *PARUPENEUS MACRONEMUS*

3.2.1. Molecular diversity and haplotype relationships

The analyses of *Parupeneus macronemus* were conducted using samples collected from ten WIO localities (Oman, Red Sea, the Maldives, Kenya, Tanzania, Mozambique, South Africa, Seychelles, Mauritius and Madagascar). Only two genes (16S and RAG1) were analysed for this species. The molecular diversity indices for these two genes among localities were characterised by zero, low (0.27) to high (0.88) haplotype and low nucleotide (0.001-0.002) diversities. These results together indicate varying levels of genetic differentiation in *P. macronemus* among different areas in the WIO (Table 3.6).

There were nine variable nucleotide sites among the aligned 523 bp of the mtDNA 16S data set that included seven singletons and two parsimony informative sites. Ten unique haplotypes were detected among the 10 localities (Table 3.6), of which eight were private with two being shared among localities. One of the shared 16S haplotypes (H1) was widespread and present in all localities while the other one (H7) was only shared in the north between the Red Sea and Oman (Figure 3.3). There were two mutational steps separating the Red Sea (H10) and South African (H5) private haplotypes from the main network. The network tree of *P. macronemus* for this mtDNA gene had a star-like pattern with a central haplotype and various different connections to private haplotypes. The central widespread haplotype probably indicates a common ancestral haplotype in the species (Templeton *et al.* 1995).

There were 29 polymorphic DNA sequences that were amplified for the RAG1 nuclear gene (912 bp) representing eight localities (Red Sea, the Maldives, Kenya, Tanzania, Mozambique, Madagascar, Mauritius, and Seychelles). These were phased into 58 allele sequences after alignment. About 22 nucleotide sites were variable among sequences with 13

of these being singletons while nine were parsimony informative. These resulted into 24 unique RAG1 sequence alleles (Table 3.7) identified among these individuals of *P. macronemus*. Six of these alleles were shared representing three widespread high frequency alleles (A1, A2, and A4), one shared allele (A11) between Mozambique and Kenya, while the other two shared alleles were found among individuals from the same locality. The rest of the 18 alleles that were also private and restricted to specific localities were rare being only represented by an individual in the dataset (Figure 3.3). The allele diversity was lowest in Mauritius (0.33) and highest in Madagascar and Maldives (1.00).

Table 3.6: Molecular diversity indices of *Parupeneus macronemus* for mitochondrial (16S) and nuclear (RAG1) genes for ten sampling localities. Indicated are the numbers of individuals (N), haplotypes (H), private haplotypes (N_{PH}), haplotype diversity (HD), alleles (A), allele diversity (AD), private alleles (N_{PA}) and nucleotide diversities (π)

Locality	16S 523 bp					RAG1 912 bp				
	N	H	N_{PH}	HD	π	N	A	N_{PA}	AD	Π
Oman	2	2	-	1.00	0.002	-	-	-	-	-
Red Sea	4	4	2	1.00	0.004	8	6	3	0.89	0.002
Maldives	2	1	-	-	-	4	4	4	1.00	0.006
Kenya	17	2	1	0.12	-	16	12	8	0.96	0.003
Tanzania	4	1	-	-	-	6	4	1	0.87	0.001
Mozambique	24	2	1	0.08	-	10	5	2	0.87	0.002
South Africa	3	3	2	1.00	0.005	-	-	-	-	-
Madagascar	3	1	-	-	-	2	2	1	1.00	0.001
Mauritius	7	2	1	0.29	0.001	6	2	-	0.33	-
Seychelles	3	2	1	0.67	0.001	6	4	1	0.80	0.001
Overall	69	10	8	0.27	0.001	58	24	20	0.88	0.002

Table 3.7. Nuclear region (RAG1) alleles for eight sampling localities with the number of individuals (N) per locality within the WIO for *Parupeneus macronemus*.

Alleles	RS	ML	KE	TZ	MZ	MD	MR	SY	N per allele
A1	3	-	3	2	2	-	5	3	18
A2	1	-	2	1	2	-	1	1	8
A3	-	-	-	1	-	-	-	-	1
A4	1	-	1	2	-	1	-	1	6
A5	-	-	-	-	-	1	-	-	1
A6	-	-	-	-	-	-	-	1	1
A7 to A10	-	-	5	-	-	-	-	-	5
A11	-	-	1	-	2	-	-	-	3
A12 to A15	-	-	4	-	-	-	-	-	4
A16 to A19	-	4	-	-	-	-	-	-	4
A20 to A21	-	-	-	-	4	-	-	-	4
A22 to A24	3	-	-	-	-	-	-	-	3
Locality (N)	8	4	16	6	10	2	6	6	58

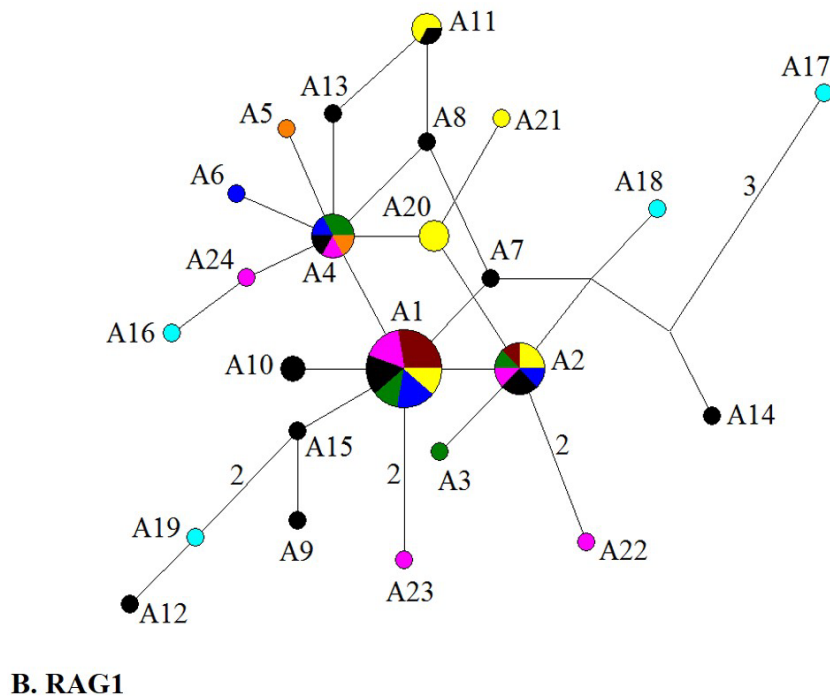
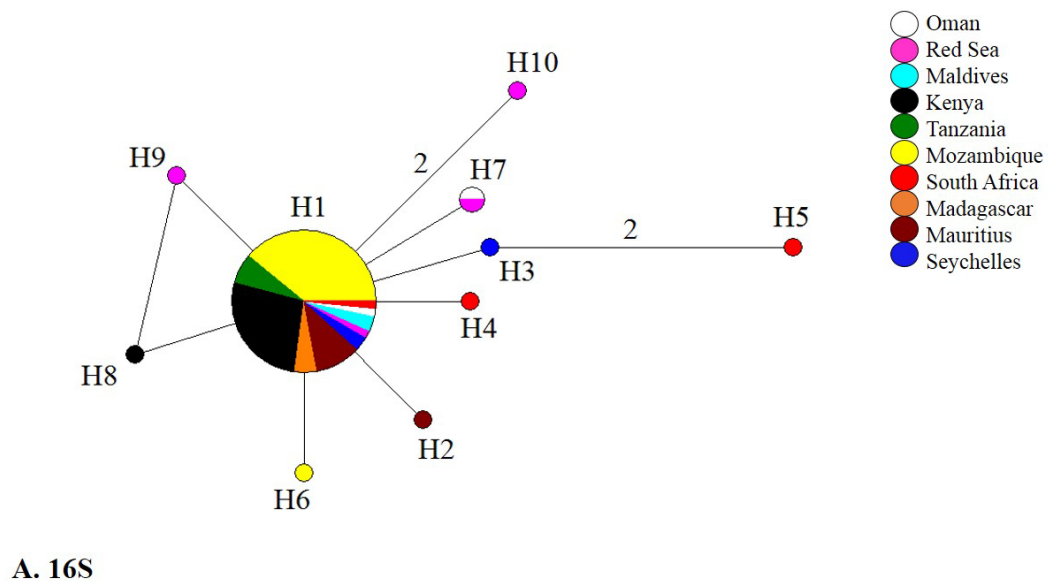


Figure 3.3. The haplotype/allele networks of *Parupeneus macronemus* for the mtDNA 16S gene (A) and nuclear RAG1 gene (B). Unique haplotypes are indicated by the circles while the colours represent the different sampling localities in which a haplotype was found. The size of each circle is proportional to the frequency of each haplotype. The number of steps for network connections with two or more mutational steps is also indicated. The connections where there are no circles are the missing haplotypes called median vectors (mv).

3.2.2. Demographic history of *Parupeneus macronemus*

The mismatch distributions for each of the two genes were unimodal and suggested a model of sudden population expansion for *Parupeneus macronemus* in the WIO (Figure 3.4). The estimated Tajima's D statistics were all negative but significant ($P < 0.01$) for the mtDNA gene and not significant ($P > 0.05$) for the nuclear gene. Fu's F_s statistic tests of neutrality were also negative -11.23 and -19.30 but significant ($P < 0.05$) for both the 16S and RAG1 genes respectively. The sudden population expansion model for this species was further supported by the observed high haplotype and low nucleotide diversities among genes (Avice 2000).

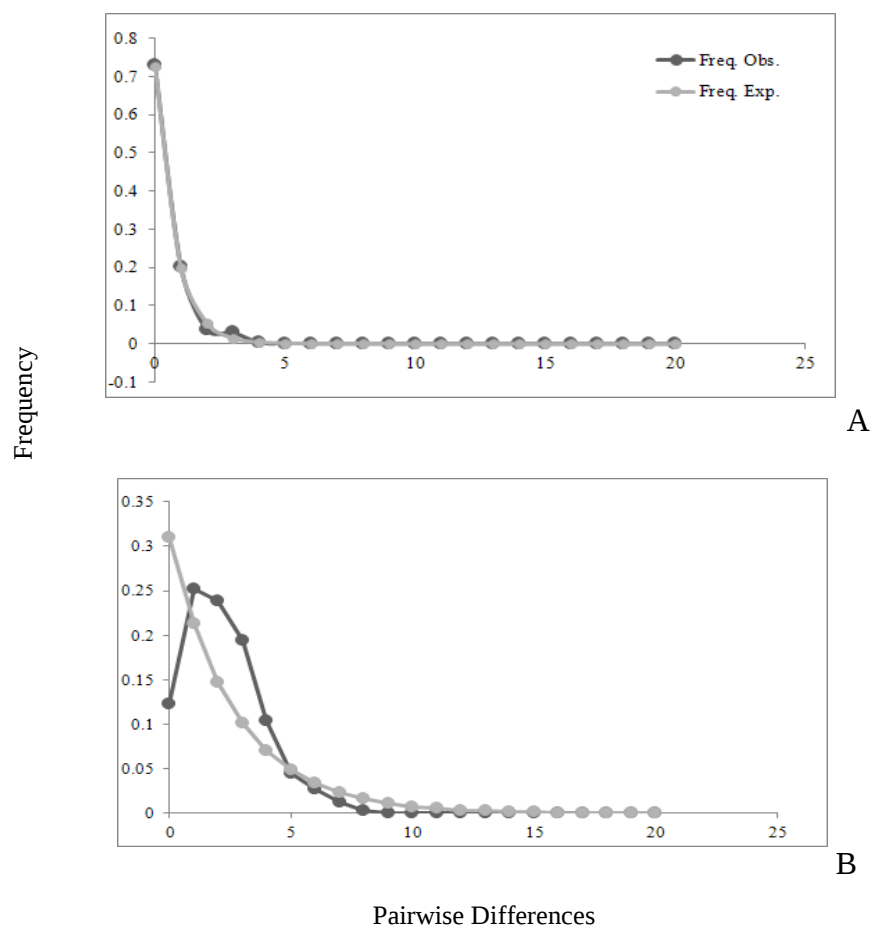


Figure 3.4. Mismatch distribution curves obtained for the 16S (A) and RAG1 (B) genes for *Parupeneus macronemus* within the WIO. The expected curves are shown by the solid line and the dashed curves are the observed data.

3.2.3. Genetic structure of *Parupeneus macronemus*

The best model of evolution for the 16S data was the same as the one selected for *P. barberinus* [Jukes-Cantor (JC), Jukes and Cantor (1969) model]. For the nuclear gene datasets the fit model was Jukes-Cantor (JC+G). The F_{ST} pairwise comparisons for the 16S mtDNA data revealed significant ($P < 0.05$) genetic differentiation between the Red Sea and two of the more southerly or SWIO localities: Kenya (0.29) and Mozambique (0.40). There was also a very great genetic differentiation ($F_{ST} = 0.48$) between individuals from Kenya and South Africa (Table 3.8). The RAG1 nuclear gene data also had pairwise F_{ST} comparison values among localities which were generally high and significantly different from each other. The RAG1 data indicated that the Maldives and Mauritius were significantly differentiated ($P < 0.05$) from some of the localities and each other ($F_{ST} = 0.26$). In general, the Maldives was genetically differentiated from the more southerly WIO localities including Seychelles while Mauritius was also different from Madagascar (0.72) without a clear pattern of differentiation among these localities.

Table 3.8. Pairwise F_{ST} comparison values between the ten localities of *Parupeneus macronemus* based on 16S mtDNA (below diagonal) and RAG1 clear gene sequences (above diagonal). Significant estimates ($P < 0.05$) are indicated in bold font.

Locality	OM	RS	ML	KE	TZ	MZ	SA	MD	MR	SY
Red Sea	-0.30	-	0.17	-0.00	-0.09	0.08	-	0.15	-0.05	-0.08
Maldives	0.00	-0.26	-	0.12	0.17	0.20	-	0.10	0.26	0.20
Kenya	0.55	0.29	-0.33	-	-0.03	0.06	-	0.12	-0.00	-0.03
Tanzania	0.38	0.00	0.00	-0.13	-	0.01	-	0.23	0.02	-0.11
Mozambique	0.64	0.40	-0.33	0.00	-0.14	-	-	0.13	0.24	0.08
South Africa	-0.09	0.01	-0.20	0.48	0.11	0.56	-	-	-	-
Madagascar	0.25	-0.09	0.00	-0.19	0.00	-0.19	0.00	-	0.72	0.24
Mauritius	0.27	0.10	-0.31	0.04	-0.09	0.08	0.22	-0.17	-	0.01
Seychelles	0.04	-0.04	-0.20	0.30	0.11	0.40	-0.15	0.00	0.09	-

The AMOVA of the 16S data of *P. macronemus* that was conducted for four biogeographic regions (Table 3.10) which were defined as African mainland areas (1: Kenya, Tanzania, Mozambique, and South Africa), the Mascarene Islands and Madagascar (2: Madagascar, Mauritius and Seychelles), the north western localities (3: Oman and Red Sea) and the Maldives (4) was not significant ($P > 0.05$). The AMOVA for the nuclear gene also revealed no significant differentiation ($P > 0.05$) among these four biogeographic groupings: Island regions (1: Madagascar, Mauritius and Seychelles), Mainland areas (2: Kenya, Tanzania and Mozambique), Maldives (3) and the Red Sea (4). Nevertheless, AMOVA analysis revealed significant ($P < 0.05$) genetic differences within localities for 16S as well as for RAG1 with the high percentage of variation of 82.4% for 16S and 91.2% for RAG1 respectively .

Table 3.9. The source of variation, degrees of freedom (d.f.), sum of squares, variance components and percentage of variation among different groups, localities within groups and within localities of *Parupeneus macronemus* that resulted from the AMOVA analyses of 16S and RAG1 genes. This analysis had three variance components: Va (Variance among groups), Vb (variance among localities within groups) and Vc (variance within localities).

Gene	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	<i>P.</i> value
16S	Among groups (4)	3	1.191	0.01Va	5.33	0.12
	Among localities within groups	6	1.944	0.02Vb	12.26	0.16
	Within localities	59	9.615	0.16Vc	82.41	0.01
RAG1	Among groups (4)	3	6.726	0.08Va	6.80	0.14
	Among localities within groups	4	5.019	0.02Vb	1.95	0.06
	Within localities	50	55.269	1.10Vc	91.25	0.02

3.3. *PARUPENEUS RUBESCENS*

3.3.1. Molecular diversity and haplotype relationships

Analyses were conducted for six southern WIO localities (Kenya, Tanzania, Mozambique, South Africa, Mauritius and Seychelles) for only the 16S and RAG1 genes. The samples from Mauritius did not amplify for ND2 gene such that only five localities were analysed in this dataset. The diversity indices for all three genes varied among the six localities with generally moderate to high haplotype and low nucleotide diversities (Table 3.10). High haplotype and low nucleotide diversities usually indicate that populations are genetically divergent and maybe geographically subdivided (Grant and Bowen 1998).

Table 3.10. Diversity indices of *Parupeneus rubescens* for the mtDNA (16S and ND2) and nuclear (RAG1) genes for six sampling localities with the number of individuals (N), haplotypes (H), private haplotypes (N_{PH}), haplotype diversity (HD), alleles (A), private alleles (N_{PA}), allele diversity (AD) and nucleotide diversities (π).

Locality	16S 551bp					ND2 790 bp					RAG1 912 bp				
	N	H	N_{PH}	HD	π	N	H	N_{PH}	HD	π	N	A	N_{PA}	AD	π
KE	1	-	1	-	-	2	2	-	1.00	0.002	6	4	2	0.80	0.003
TZ	1	-	-	-	-	2	2	1	1.00	0.004	4	2	-	0.50	0.001
MZ	32	2	1	0.06	-	23	8	5	0.80	0.002	54	20	14	0.92	0.003
SA	9	4	3	0.58	0.001	3	3	1	1.00	0.002	9	5	-	0.86	0.002
MR	1	-	-	-	-	-	-	-	-	-	2	1	1	-	-
SY	2	1	-	-	-	2	2	-	1.00	0.004	4	3	-	0.83	0.002
Overall	46	5	5	0.18	0.000	32	11	7	0.85	0.002	80	23	17	0.91	0.003

The 46 sequences of the 551 bp mtDNA 16S dataset had five variable nucleotide sites that included five singletons. There were no parsimony informative sites in the data set with only six unique haplotypes being identified among these sequences and six localities (Table for

haplotype frequencies not shown). One haplotype was in high frequency and widespread (H1; 89% of the sample) being present among all localities except Kenya (Figure 3.5), while the other five haplotypes were rare and private being restricted to specific localities. The high frequency haplotype was central in the star-like network. For the ND2 data, the 32 aligned sequences were 790 bp, and had eight variable nucleotide positions with two singletons and six parsimony informative sites. Eleven haplotypes were identified among all localities (Table 3.11), of which seven were private, while two (N2 and N4) were widespread and shared among three out of five localities. The two other haplotypes were only shared between Mozambique and two localities namely South Africa (N1) and the Seychelles (N6).

The 40 polymorphic 912 bp sequences which were amplified for the RAG1 data were phased into 80 sequence alleles. These sequences which had 20 variable nucleotide sites of which two were singletons and 18 were parsimony informative. About 23 unique RAG1 allele sequences were identified among these individuals (Table 3.12). Four widespread alleles (Figure 3.5: A2, A4, A5 and A6) shared among more than three localities were detected with two other shared alleles (A1 and A3), being shared only between Mozambique and South Africa. The only allele from Mauritius (A7) was isolated from the rest of the network with four mutational steps. However, most of the alleles were very closely related and connected by very few mutational steps in the network. Mozambique had more rare and private alleles than all other analysed localities due to a larger sample size.

Table 3.11. ND2 haplotypes of *Parupeneus rubescens* for the six WIO sampling localities indicating the number of individuals (N) per locality.

Haplotype	KE	TZ	MZ	SA	SY	N per haplotype
H1	-	-	1	-	1	2
H2	1	-	-	1	1	3
H3	-	1	-	-	-	1
H4	1	1	9	-	-	11
H5	-	-	-	1	-	1
H6	-	-	2	1	-	3
H7 to H11	-	-	11	-	-	11
Locality (N)	2	2	23	3	2	32

Table 3.12. Nuclear gene (RAG1) alleles for *Parupeneus rubescens* for the six WIO sampling localities indicating the number of individuals (N) per locality.

Allele	KE	TZ	MZ	SA	MR	SY	N per allele
A1	-	-	7	4	-	-	11
A2	-	3	4	2	-	-	9
A3	-	-	1	1	-	-	2
A4	-	1	8	-	-	2	11
A5	-	1	1	1	-	1	4
A6	2	1	9	2	-	1	15
A7	-	-	-	-	2	-	2
A8 to A19	-	-	22	-	-	-	22
A20 to A21	2	-	-	-	-	-	2
A22 to A23	-	-	2	-	-	-	2
Locality (N)	4	6	54	10	2	4	80

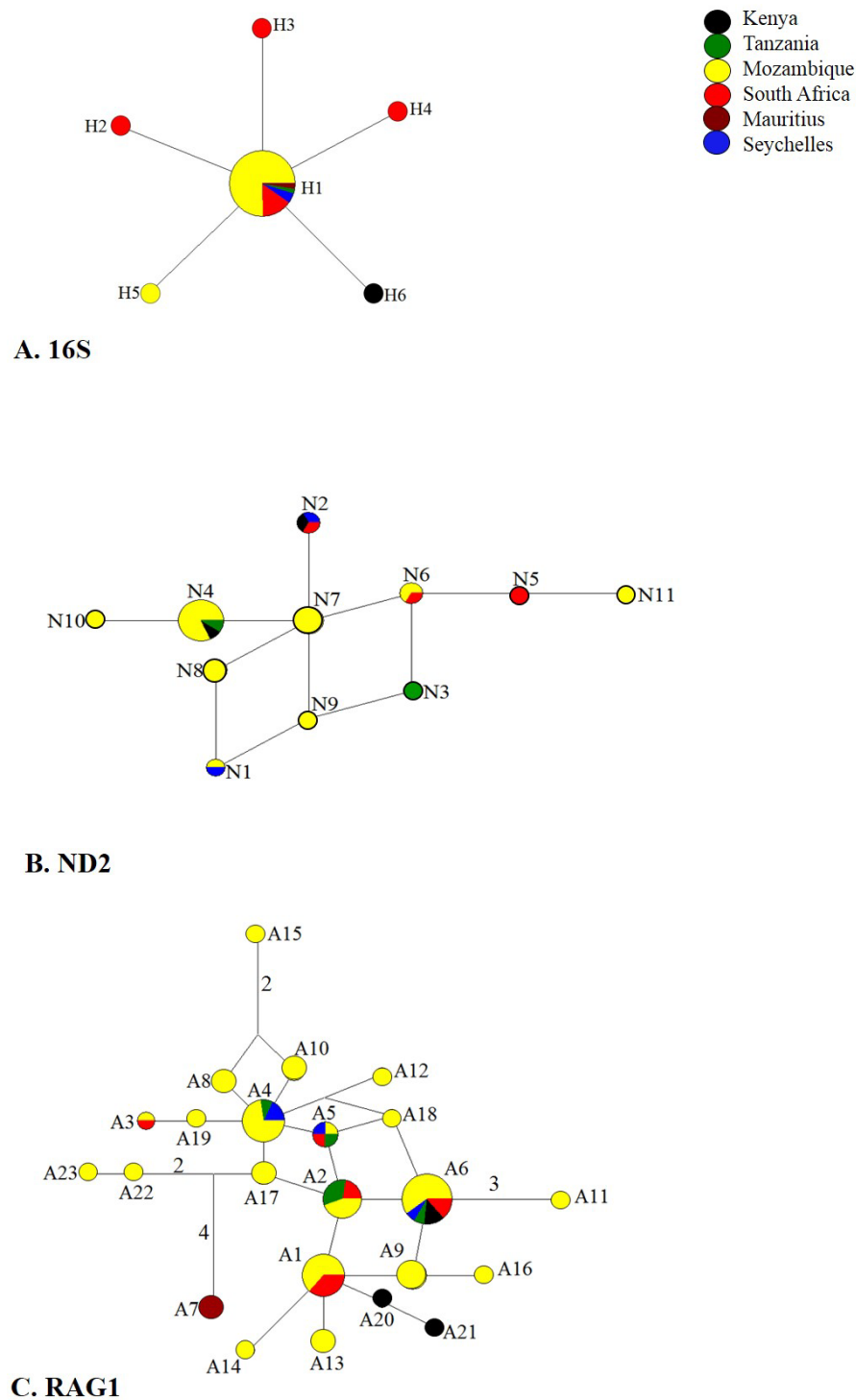


Figure 3.5. The haplotype network of *Parupeneus rubescens* for the 16S (A), ND2 (B) and RAG1 (C) genes. Haplotypes/allele are indicated by the circles while the colours represent the different sampling localities. The size of each circle is proportional to the frequency of each haplotype. The numbers of steps for network connections with two or more mutational steps are also displayed. Missing haplotypes are called median vectors (mv).

3.3.2. Demographic history of *Parupeneus rubescens*

The mismatch distributions for all the analysed genes were unimodal and suggested a model of sudden population expansion for *Parupeneus rubescens* in the WIO (Figure 3.6). The estimates of Tajima's D test of neutrality were negative (-1.99, -0.40, and -1.00) for the 16S, ND2 and RAG1 genes respectively suggesting that *P. rubescens* has undergone a recent population expansion.

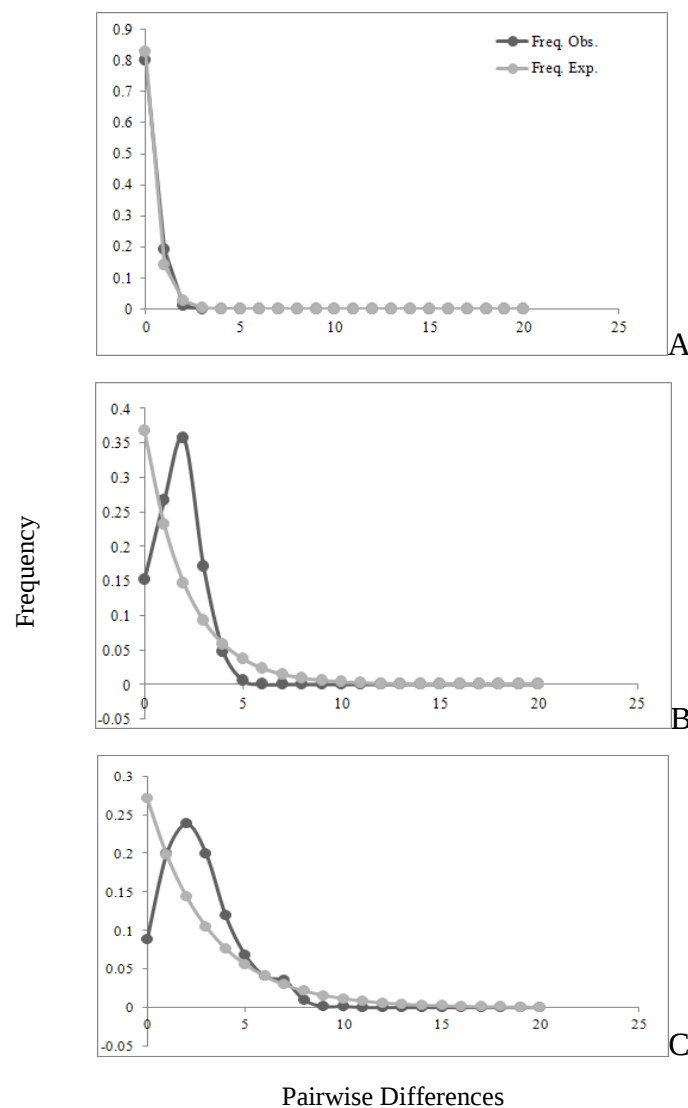


Figure 3.6. Mismatch distribution curves obtained for *Parupeneus rubescens* for all the three genes 16S (A), ND2 (B) and RAG1 (C). The expected curve is shown by the solid line and the dashed curve is the observed.

Although the estimates from the ND2 and nuclear data sets were not significant for this test ($P > 0.10$), the 16S data estimate was significant ($P < 0.05$). The Fu's F_s statistic tests were also all negative for the 16S (-6.61), ND2 (-4.88) and RAG1 (-12.02) datasets and significant ($P < 0.001$). The plots of the mismatch distributions (Figure 3.6) were unimodal for all the genes also supporting the model for a recent expansion as indicated by the negative Tajima's D values.

3.3.3. Genetic structure of *Parupeneus rubescens*

The substitution models with the lowest BIC scores (Bayesian Information Criterion) for mtDNA data of this species were the same as those selected for *P. barberinus*. The Kimura 2-parameter (Kimura 1980) was determined as the best substitution model fitting the RAG1 nuclear DNA data. The population pairwise F_{ST} comparisons were only conducted for the ND2 and RAG1 datasets. The 16S data could not be compared due to sample size as Kenya and Mauritius had less than two individuals. Only the comparisons between individuals from South Africa and Mozambique revealed significant ($P < 0.05$) genetic differentiation (0.22) for ND2 dataset (Table 3.13).

Table 3.13. Comparison of pairwise F_{ST} values between six localities of *Parupeneus rubescens* based on mtDNA sequences (ND2). Significant estimates ($P < 0.05$) are indicated in bold font.

Locality	KE	TZ	MZ	SA	SY
Kenya	-				
Tanzania	-0.25	-			
Mozambique	-0.07	-0.05	-		
South Africa	-	-0.10	0.22	-	
Seychelles	-0.25	-0.20	0.17	0.04	-

The pairwise F_{ST} comparisons (Table 3.14) for the nuclear gene data revealed significant ($P < 0.05$) and very great genetic differentiation only between Mauritius and most of the other localities, namely Kenya (0.74), Mozambique (0.67) and South Africa (0.78).

Table 3.14. Comparison of pairwise F_{ST} values between six localities of *Parupeneus rubescens* based on RAG1 sequences. Significant ($P < 0.05$) F_{ST} values are indicated in bold.

Locality	KE	TZ	MZ	SA	MR
Kenya	-				
Tanzania	0.05	-			
Mozambique	-0.01	0.04	-		
South Africa	-0.21	-0.03	0.01	-	
Mauritius	0.74	0.94	0.67	0.78	-
Seychelles	0.05	0.20	-0.01	0.15	0.82

The AMOVA partitioning of molecular variance of *P. rubescens* among all three hierarchical levels are indicated in Table 3.15. AMOVA revealed a significant genetic differentiation ($P > 0.05$) among three defined groups: East Africa (Tanzania and Kenya), southern Africa (Mozambique and South Africa) and the Mascarene Islands (Mauritius and Seychelles) for the 16S mtDNA dataset. For the ND2 data, AMOVA revealed significant genetic differentiation ($P < 0.05$) among the three groups: the tropical African mainland areas (Kenya, Tanzania and Mozambique), the Mascarene Islands (Seychelles) and South Africa (SA) the southernmost locality.

The AMOVA for the nuclear RAG1 gene also revealed significant genetic differentiation ($P < 0.05$), however this was only when localities were divided into two groups; the Mascarene Islands (Mauritius and Seychelles) and all the continental mainland areas (Kenya, Tanzania,

Mozambique and South Africa). Furthermore, the test showed revealed significant ($P < 0.05$) genetic differences among localities within localities as well as within localities sampled and this significant differentiation accounted for 73.8% of the genetic variation.

Table 3.15. The source of variation, degrees of freedom (d.f.), sum of squares, variance components and percentage of variation among different groups, localities within groups and within localities of *Parupeneus rubescens* that resulted from the AMOVA analyses of 16S, ND2 and RAG1. Significant values ($P < 0.05$) are indicated in bold. This analysis had three variance components: Va (Variance among groups), Vb (variance among localities within groups) and Vc (variance within localities).

Gene region	Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation	<i>P.</i> value
16S	Among groups (3)	2	0.49	0.01Va	11.42	0.00
	Among localities within groups	3	0.77	0.03Vb	22.06	0.00
	Within localities	40	3.64	0.09Vc	66.52	0.07
ND2	Among groups (3)	2	3.14	0.22Va	22.14	0.00
	Among localities within groups	2	1.24	-0.06Vb	-5.86	0.59
	Within localities	27	22.34	0.83Vc	83.72	0.19
RAG1	Among groups (2)	1	5.29	0.19Va	13.41	0.00
	Among localities within groups	4	13.06	0.20Vb	12.75	0.03
	Within localities	74	95.93	1.21Vc	73.84	0.00

CHAPTER FOUR

4.0. DISCUSSION

In this study, two mitochondrial DNA genes (16S and ND2) and one nuclear marker (RAG1) were used to determine regional differentiation and connectivity of three goatfishes (*Parupeneus spp.*) within the WIO. The selected goatfishes were examined to establish whether their distribution among biogeographic regions of the WIO was affected by dispersal and/or vicariant events. To date, this is the first reported study to assess differentiation in *Parupeneus barberinus*, *P. macronemus* and *P. rubescens*. Samples of all species from the analysed localities were characterised by few widespread or high frequency WIO haplotypes, while most haplotypes were rare and generally represented by an individual in one locality. Such results could indicate that gene flow is reduced or restricted among these WIO localities, even between geographically close sampling sites.

For marine species with pelagic larvae or high dispersal capabilities, several factors can be involved in determining the genetic structuring of a population (Hedgecock 1986; Palumbi 1994; Mora 2004; Kesäniemi *et al.* 2014). These can include the effects of oceanographic features such as ocean currents and physical barriers such as islands in the species distribution range and the dispersal potential based on the life history features of the species being studied. It is generally expected that a population with high levels of adult dispersal and long pelagic larval life duration (PLD) would have little genetic structure in the sea (Palumbi 1992; Crandall *et al.* 2010; Reece *et al.* 2011). For example, Gaither *et al.* (2013) indicated that marine larvae can be transported 100's or even 1000's of km before settlement, increasing the levels of connectivity among populations. However, Riginos and Victor (2001) indicated that a short larval duration is expected to exhibit strong genetic structuring,

due to a limit of the amount of dispersal among populations in widespread species. Unfortunately there is no specific information on the larval dispersal of these three goatfish species to verify this. Therefore, studies are needed to be conducted on their specific biology and life history strategies. It is also important to note that the length of the pelagic larval phase is just one factor affecting dispersal in marine organisms that can also explain the difference in genetic population structure (Sogabe and Takadi 2013). Evidence suggests the importance of other factors, such as currents and larval retention (Carreras-Carbonell *et al.* 2006), that may cause strong differentiation and should also be taken into account when considering larval dispersal and its effect on population genetic structure (Sogabe and Takadi 2013). Differences in interpretation of the results for these targeted species were required in this study. The reason for such is that these study taxa had different number of samples and not all of them were found in the eleven WIO localities which were sampled and analysed.

4.1. *Parupeneus barberinus*

The assessment of genetic connectivity and levels of differentiation of the dash and dot goatfish *P. barberinus* through the analysis of two mitochondrial DNA (16S and ND2) and one nuclear DNA (RAG1) data revealed some general diversity patterns in the WIO region. The DNA sequence diversity for these three gene datasets all similarly revealed moderate to high haplotype and low nucleotide diversities (and also among the six localities), suggesting high levels of genetic differentiation within *P. barberinus* in the WIO. The high haplotype diversities among haplotypes can be interpreted as evidence of recent population expansion (Rogers 1995; Graves 1998). The low nucleotide diversities that were observed among these genes may be a result of the relatively short existence of haplotypes followed by recent population expansion or colonisation among areas as suggested by the negative Tajima's D values (Cassone and Boulding 2006; Silva *et al.* 2010).

Analysis of nuclear DNA variation (using haplotype networks) revealed two lineages separated by seven mutational steps. The first more northern-WIO and East African lineage was dominated by private alleles from Maldives and Kenya with a few individuals from Mozambique. The second lineage was widespread and dominated by high frequency alleles that were present in all localities in the WIO. These results therefore suggested that the species is genetically divergent with a restricted north-east WIO and a more widespread WIO lineage which included the Mascarene Islands. The genetic separation between these two lineages could be due to major historical events or geographic barriers or breaks that occur in this species distribution range. Obura (2012) stated that the flow of the SEC may isolate the Island regions and banks of the Mascarene Plateau from the main flow of this current and from each other. This current therefore establishes a clear upstream-downstream gradient between these Mascarene areas and the Mozambique Channel as well as the East African mainland coast (Kenya in this study). Therefore, the SEC may be the main oceanographic barrier that has restricted gene-flow between these two lineages in this species.

The Maldives also appeared to be genetically differentiated from all other localities from the 16S and RAG1 gene analyses. The genetic isolation of the Maldives as well as the high haplotype frequency and diversity estimates could also be because this locality represented the most distant sampling site in the sample. Thus the genetically differentiation of the Maldives compared to the southerly WIO localities could mean that connectivity was inhibited by geographic distance. However, isolation by distance could not be tested due to sample sizes and sampling range. This isolation of the Maldives in this data could also be due to other factors, including the effect of the monsoon winds that are created by an upwelling that is characterised by low-salinity waters in the Maldives (Sarkar and Gupta 2009). These winds are stronger and steadier during the South West Monsoon than during the North East (Shankar *et al.* 2002). The monsoon winds are also the drivers of the water movement

(eddies) which circulates around the Maldives islands at practically all levels. These eddy-like circulatory motions of the waters keep the fish eggs and larvae within the vicinity of the islands for a considerable length of time (Sinha 1994), thereby preventing or limiting dispersal to the rest of the WIO region.

The results obtained from the nuclear data were very similar to the mtDNA 16S data, where the haplotypes from the Maldives were in a lineage isolated from the high frequency central haplotype which was widespread but absent from the Maldives. The lineage that had the private haplotypes from Maldives included a haplotype that was also shared with Kenya as was observed in the RAG1 dataset. However, the ND2 gene had slightly different diversity patterns, probably because it included fewer specimens than the nuclear DNA and 16S datasets due to unsuccessful amplification for most specimens. The ND2 mtDNA data had only one widespread shared haplotype and had fewer samples from the Maldives to verify these regional scale patterns. However, the haplotypes from the Maldives and Oman were private and restricted, suggesting that similar patterns of isolation in the northern-WIO might be detected in this dataset with increased sample sizes. The haplotype from Oman was separated from the widespread haplotype by four mutational steps, with Kenya also being separated by three mutational steps within the network.

The bimodal mismatch distribution observed for the RAG1 gene data set also provided further strong evidence for the presence of two genetically distinct lineages or populations in this the species. Two peaks were observed which might suggest that isolating mechanisms in the WIO have played a role in shaping the genetic differentiation found in this species. In addition, F_{ST} population pairwise comparison values were considerably high for the 16S and RAG1 analyses and revealed significant differentiation among localities. The AMOVA also revealed high levels of genetic structuring among defined regional groups for these two

datasets with almost 60% of the total 16S genetic variance being found among four areas; (1) African mainland localities, (2) Mascarene Islands, (3) Kenya and (4) the Maldives. This observed genetic structure among *P. barberinus* populations could also be because of the larval dispersal processes and pathways formed by the South Equatorial Current, the counter-currents and eddy systems in the Mozambique Channel and upper Agulhas Current region major circulation systems that operate between these defined regions. These water current movements can either disperse larvae over long distances (von der Heyden *et al.* 2008; Groeneveld *et al.* 2012), promoting connectivity or return/retain them near their origin and lead to genetic isolation (Chiswell and Booth 1999).

However, no significant population structuring was found among localities for ND2, as the F_{ST} values were low and not significant. Furthermore, AMOVA of the ND2 data revealed low and insignificant levels of genetic structuring among four defined groups which accounted for 17.37% of the total genetic variance in the sample. Several factors such as a complex interaction of biology and geography, and low rates of mitochondrial evolution can influence genetic diversity and variability (Grant *et al.* 2006; Xiao *et al.* 2009; Shih *et al.* 2011; Xu *et al.* 2012). However, ND2 is one of the faster evolving protein-coding gene (Meyer 1993), such that these low levels of genetic structure could be due to low sample size. A study by Dorenbosch *et al.* (2006) investigating the genetic structure of the Dory snapper, *Lutjanus fulviflamma*, from the sites along the East African coastline (Kenya and Tanzania) and the Comoros Archipelago had similar results that also revealed low genetic differentiation among populations. The authors suggested that the Dory snapper may be genetically connected over large distances in the WIO.

The analyses of all the genes revealed haplotypes that were widespread and shared among localities within *Parupeneus barberinus*. This suggests that there is ongoing gene flow for

these widespread lineages and haplotypes suggesting that connectivity was not inhibited by the geographic distance for localities that were far from each other. However, the observed star-shaped networks could also possibly indicate that these common and widespread haplotypes are ancestral (Templeton *et al.* 1995), when they were central in the network, as they support various different connections of all other haplotypes from all the sampled localities.

4.2. *Parupeneus macronemus*

Analyses of *P. macronemus* revealed low to high haplotype (0.27 - 0.88) and low nucleotide (0.001-0.002) diversities based on 16S and RAG1 gene sequences from 10 localities. Therefore, *P. macronemus* appears to have higher nuclear (RAG1) than mtDNA genetic diversity. The 16S data had one widespread high frequency haplotype that was present in all localities and only one other shared haplotype between Oman and Red Sea in the north. The rest of the haplotypes were private suggesting a recent population expansion from the common ancestral haplotype in the star-like network (Avice 2000). This demographic change in *P. macronemus* was also supported by the unimodal mismatch distribution and the negative Tajima's D estimate. A unimodal mismatch distribution, statistically significant and negative values of Tajima's D and Fu's Fs neutrality tests were also observed in the RAG1 data and were also consistent with the hypothesis of a recent population expansion subsequent to a genetic bottleneck.

Recent population expansions have also observed in other studies of WIO marine species, such as the study by Silva *et al.* (2010) on the mangrove crab *Perisesarma guttatum* using mtDNA COI gene. This study was conducted along the East African coast with the aim of estimating the levels of population diversity and differentiation along a latitudinal cline. Their study also observed unimodal mismatch distributions for southern Mozambique

populations, a star-like network pattern within each clade and statistically significant negative values of Tajima's *D* and Fu's *F* are also consistent with these demographic hypotheses. Benzie *et al.* (2002) obtained similar results in a study on mtDNA variation in Indo-Pacific populations of the giant tiger prawn *Penaeus monodon*, with the population of southeast Africa and Western Australia revealing patterns of a relatively recent population bottleneck. According to these authors, the fall in sea level in the Pleistocene was likely to have removed shelf habitat suitable for *P. monodon* in southern Africa, and these would have been reinvaded by populations further north after the latest major sea level rise (Forbes *et al.* 1999).

Similar results were also obtained by Kochzius and Nuryanto (2008) for the giant clam *Tridacna crocea*, where the mismatch distribution test also indicated a recent population expansion using mtDNA COI gene data. These authors suggested that the changes in population size, could be explained by the reduction of habitat and subsequent population bottlenecks during periods of low sea level stands, whereas the availability of new habitats after the rise of the sea level at the end of the last glacial maxima (16000 years ago) enabled recolonisation and growth of the reduced populations. Many marine species were affected directly in their population distribution, diversity and demographic expansions as a consequence of the sea level and temperature fluctuations during the Pleistocene-era (Peters *et al.* 2005; Li *et al.* 2009; Huang and Lin 2011; Van de Putte *et al.* 2012). The effect of the sea level fluctuations during Pleistocene events appears to have completely interrupted gene flow of several marine taxa between the African mainland and WIO (Daniels *et al.* 2002; Ragionieri *et al.* 2009; Daniels 2011).

These results of demographic expansion are similar to the results observed for *P. barberinus* and this may also have been the case, as these events might also have similarly occurred with

this species. The significant moderate to great differentiation observed in the mtDNA 16S dataset for pairwise comparisons between the Red Sea versus three SWIO localities, Mauritius, Kenya and Mozambique could be isolation due to the cold upwelling in the Red Sea in the north as well as the eddies and gyres in the Mozambique Channel in the south. These features could act as barriers limiting the dispersal of fish and may thus have an impact on connectivity among these areas and the Red Sea. In fact, the northern entrance of the Mozambique Channel is dominated by a large seasonal anti-cyclonic cell (Donguy and Piton 1991), whereas the central part is characterised by a series of mesoscale anticyclonic and cyclonic eddies along the Mozambique coast that play a major role in fish distribution (Schouten *et al.* 2003; Swart *et al.* 2010).

A similar study by Gopurenko *et al.* (1999) that used mtDNA control region sequences also demonstrated genetic differentiation in this same region among populations of the mangrove crab *Scylla serrata* from the Red Sea, Mauritius and South Africa due to these same ocean features. There were three widespread alleles in the nuclear data (RAG1), with the alleles from the Maldives being absent from the main part of the network indicating some patterns of genetic isolation in this dataset. A similar pattern was observed for *P. barberinus* where the Maldives (the distant locality in the north-east) was restricted from the more southerly WIO localities which could be due to the effect of the monsoon winds and isolation by distance. The pairwise F_{ST} comparisons also revealed that Mauritius was significantly differentiated from the Maldives, Mozambique and Madagascar. The separation of Mauritius was also associated with the influence of the SEC and other features mentioned above, which could act as a barrier to fish dispersal promoting isolation of Mauritius.

The apportioning of genetic variance among samples of *P. macronemus* was measured with AMOVA to explain the differentiation among groups in the structure observed. Very little

genetic variation was observed among four groups (mainland areas, Mascarene Islands and northern localities) for both of these genes (16S = 5.36% and RAG1 = 6.71%) with only the within group variation having high significant percentages. Although these groups were the logical grouping based on regional biogeography, these AMOVA results suggest that there is weak geographic sub-structuring in this species. The low AMOVA levels of genetic differentiation among defined groups were in contrast to the statistically significant and high F_{ST} values that were found in pairwise comparisons between some localities for example the Red Sea and the Maldives. In conclusion, a high level of population genetic diversity and low differentiation were found in this species from 11 locations of the WIO region. More genetic studies should be carried out with high sample numbers to verify the regional structuring in this species.

4.3 *Parupeneus rubescens*

Although the analyses on this species were limited because of the small sample sizes for most localities and also influenced by a higher number of samples from Mozambique and South Africa, some trends in genetic diversity were observed and will be discussed here. The DNA sequence diversity for the 16S, ND2 and RAG1 datasets revealed varying levels of diversity ranging from low to high haplotype and generally low nucleotide diversities. There were therefore high levels of genetic differentiation among localities and within the species as was observed in the other two goatfish species (*P. barberinus* and *P. macronemus*). Analysis of 16S mtDNA sequence data revealed low to high haplotype diversities in Mozambique and South Africa, whereas the ND2 data were characterised by high haplotype diversities in all localities. Analysis of nuclear DNA sequence data revealed some populations that were characterised by moderate to high allelic diversities. The highest mtDNA haplotype diversities (> 0.80) were recorded from Kenya, Mozambique, South Africa and Seychelles, whilst the lowest (< 0.50) were from Tanzania and Mauritius. These

high haplotype diversities among localities can also be interpreted as evidence of a recent population expansion (Rogers 1995; Graves 1998). Although Mozambique had the largest number of samples (> 60 % of sample), it only had a higher number of private or restricted haplotypes than the other localities, in the ND2 and RAG1 datasets. The 16S data had low haplotype diversity and its network revealed a star-shaped tree with one high frequency ancestral haplotype (Templeton *et al.* 1995) that was found in all the sampled localities. The star-like shape of the network is often considered as a signature of a recent population expansion. The low haplotype and low nucleotide diversities also suggest that there may be little genetic differentiation within the 16S gene for this species. The ND2 data was also a star-shaped tree but slightly different from the 16S data, as there were more shared haplotypes among localities. However, some individuals and the central haplotype (restricted to Mozambique) were private; indicating that patterns of isolation might be detected in this dataset with increased sample sizes. The network tree for the nuclear gene (RAG1) revealed that Mauritius in the Mascarene Plateau was genetically separated (four mutational steps in the network) from other localities. Individuals from Mauritius were not included in the widespread and shared haplotypes.

The results clearly indicated that the species is genetically divergent with a restriction of Mauritius Island and a more widespread group which included the Seychelles. The pairwise F_{ST} comparisons also supported that Mauritius is significantly restricted from other localities. The isolation of Mauritius from the mainland areas and Seychelles could be because of the effect of the zonal flow of the SEC when it approaches Madagascar. This flow isolates the northern (Seychelles) and southern Mascarene Islands and its banks from the main current flow and from each other. The current establishes a clear upstream-downstream gradient between these and the Mozambique Channel and East African coast (Obura 2012), as previously discussed. The observed pairwise F_{ST} comparison differences between

Mozambique and South Africa in both mtDNA datasets could be because of the flow along the Mozambique coastline and the anti-cyclonic circulation of the channel. Although the circulation in this channel is not yet fully understood (Lutjerhams 2006), the anti-cyclonic circulation pattern should create the major part of the flow through the Mozambique Channel and may cause isolation among areas in the region. A study of green turtles by Bourjea *et al.* (2007) also revealed a pattern of connectivity and differentiation among Mozambique Channel localities. However, these results must be interpreted carefully and need to be reevaluated due to the low number of samples of *P. rubescens* from other localities in this study.

The significantly negative values of the Fu's F_S as well as Tajima's D indicated that *P. rubescens* in the WIO has also experienced a rapid population expansion (Grant and Bowen 1998), which is generally associated with little or no population structure (von der Heyden *et al.* 2007). In recent years statistical tests, such as Tajima's D statistic, which were originally developed to test for selective neutrality of mutations, have been implemented to detect such population growth (Ramos-Onsins and Rozas 2002; Zhao *et al.* 2008) and these tests are generally based on the distribution of pair-wise differences between sequences within populations (de Jong *et al.* 2011). In addition, the unimodal distributions of pairwise distances between haplotypes of *P. rubescens* that were obtained for all three genes were also a possible consequence of recent demographic changes. The associated high haplotype diversity together with low nucleotide diversity can also be a signature of a rapid demographic expansion from a small effective population size (Grant and Bowen 1998; Avise 2000). Therefore, these results suggest that *P. rubescens* has also experienced a recent demographic change in its recent history. The detection of significant structuring among defined groups of localities in the AMOVA would be expected because of this recent range expansion as well as the high haplotype and low nucleotide diversities that were observed.

Nonetheless, this was not the case in the 16S gene region in this species but this could be due to a much smaller sample size.

4.4. CONCLUSIONS

In conclusion, this study revealed diversity patterns and varying levels of connectivity with some geographic differentiation among these three goatfishes in the WIO. These results were dependant on the markers that were used, with some differences in results that could be associated with rates of evolution of the genes. These patterns of differentiation were detected because of the major ocean current circulation and biogeographic barriers among areas in the region. The Maldives were found to be genetically differentiated when compared to the southerly WIO localities for these goatfishes. Kenya was also genetically different from most of the other mainland and SWIO localities and this was mainly associated with the South Equatorial Current that limits dispersal.

A similar study on the parrot fish *Scarus ghobban* by Visram *et al.* (2010) that included samples from coastal East Africa and the Mascarene Islands, also found similar patterns but also identified this difference as isolation by distance among areas. Most of the differentiation that they observed was due to the separation of Kenya and Seychelles from Tanzania and Mauritius. Their result was also attributed to the influence of the equatorial current system, specifically the SECC. These Ocean currents may promote the transport of larvae from the Mascarene Islands in the WIO and the East African Mainland (Visram *et al.* 2010). The region also has a complex system of eddies which are suspected to have an impact on the genetic structure of marine fishes in the area (Bourjea *et al.* 2007). These currents may not only act as a barrier, even when the distributions appear to be constant, but may also allow gene flow between populations (Silva *et al.* 2010). This could be the reason that contracting patterns of connectivity and differentiation were observed in this study. It is

clear from comparative analyses of these three goatfishes that oceanographic barriers and geological features can limit gene flow among marine species, and that in some cases ocean currents can isolate populations, rather than facilitate wide dispersal of *Parupeneus* species within WIO area.

Although the results of this study explain aspects of the genetic structure of *Parupeneus* species within the WIO area, future studies are needed to confirm and expand these observations. It is important to address the biological factors (e.g. life history strategies such as pelagic larval duration) that affect the distribution of these *Parupeneus* species and their effects on genetic structure. This study found evidence for regional differentiation supporting the presence of genetic breaks among known biogeographic areas that could either be due to the historical isolation or due to geographic and oceanic barriers such as the Mascarene Plateau and the Agulhas Current eddies in the Mozambique Channel. The findings of this study support the hypotheses that the physical factors (geographic barriers and oceanographic characteristics) do have an impact on the distribution of fishes in the WIO and that the life history processes need to be studied to understand the genetic differentiation of these *Parupeneus* reef fishes. Although the molecular markers could not identify the complete structure of these widely distributed goatfishes due to sample size, the data revealed some of the mechanisms responsible for the observed regional structures. Verifying these biogeographic analyses for *Parupeneus* requires a larger population genetic study that should provide a more concise conclusion on the biogeography of the WIO and this genus.

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