REGIONAL CONNECTIVITY, DIFFERENTIATION AND BIOGEOGRAPHY OF THREE SPECIES OF THE GENUS *LUTJANUS* IN THE WESTERN INDIAN OCEAN

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By

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DECLARATION

I declare that the thesis hereby submitted to Rhodes University, Grahamstown, South Africa, for the degree of Master of Science in Ichthyology, has not previously been submitted for a degree at this or any other university. The work contained herein is my own. I am aware of the University's policy on plagiarism, and the material (ideas, phrases and illustrations) presented from other authors have been duly acknowledged.

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ABSTRACT

Snappers of the genus *Lutjanus* are small to large predatory fishes occurring in inshore circumtropical and subtropical waters throughout the world. These fishes support fisheries across their distribution range. Within the Western Indian Ocean (WIO), previous studies on *Lutjanus kasmira* revealed limited spatial genetic differentiation, whereas *Lutjanus fulviflamma* showed high genetic connectivity. The phylogenetic relationships among WIO snappers are unknown. Previous studies in the Indo-Pacific (IP) did not include any WIO representatives. This study examined (1) the phylogeographic patterns in *Lutjanus bohar*, *L. fulviflamma* and *L. lutjanus* to understand the origins and factors influencing the distribution of diversity in the region, (2) how the physical environment, biological, and ecological factors influence genetic diversity, (3) the placement of WIO snappers in context to those from the IP, as well as the placement of taxa not included previously, (4) extent of differentiation among conspecifics from the two regions, and (5) the relationship of the Caesionidae to the Lutjanidae.

Samples were sourced from across the WIO and from peripheral localities, where possible. DNA sequence data were generated from two mitochondrial gene regions (cyt-*b* and NADH-2) and a nuclear gene region (S7 intron 1). Data were analysed under a phylogeographic framework to examine genetic structure, diversity and differentiation among identified regions for each of the three species. Other sequence data were generated from two mitochondrial gene regions (COII and 16S rDNA) to examine the phylogenetic placement of WIO snappers in context of the IP snappers and the relationship of the Caesionidae to the Lutjanidae.

Lutjanus bohar and *L. fulviflamma* displayed high genetic diversity, but lower diversities were observed for *L. lutjanus*. Genetic differentiation was observed between Mozambique and Maldives in *L. bohar*. *Lutjanus fulviflamma* was differentiated in South Africa, Mozambique, Mauritius and Thailand, while differentiation was observed between Kenya and Tanzania in *Lutjanus lutjanus*. Overall, low genetic differentiation and high connectivity were observed for each of the three species. This differentiation may result from intrinsic features of the species and extrinsic features of the environment, whereas the connectivity is mainly influenced by the pelagic larval duration. These patterns of differentiation are in accordance

with a proposed vicariant biogeographic hypothesis for the origins of regional faunas of the IP. Phylogenies were similar to those published, with additional taxa not altering the previous groupings found. Conspecifics from the two regions clustered together, with varying degrees of differentiation among the WIO and IP, depending on the species. Members of the Caesionidae were nested within Lutjanidae, suggesting that morphological characters separating the two families are taxonomically insignificant. This affirms previous notions that the Caesionidae should be a subfamily within the Lutjanidae. This is the first multi-gene study, examining differentiation in multiple species of snapper over a wide geographic area in the WIO, and the results of this study could have potential implications for fisheries management and conservation.

Keywords: marine, genetics, phylogeography, biogeography, mitochondrial DNA, nuclear DNA,

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16S rDNA	16S ribosomal DNA	KEN	Kenya
AC	Agulhas Current	LGR	Lower gill rakers
A_D	Allelic diversity	LLS	Lateral line scales
AFLP	Amplified Fragment Length Polymorphism	MAD	Madagascar
AL	Anal fin length	MAL	Maldives
AMOVA	Analysis of Molecular Variance	MAU	Mauritius
AUS	Australia	MC	Mozambique Current
BD	Body depth	MCL	Middle caudal lobe
BI	Bayesian inference	MCMC	Markov Chain Monte Carlo
BW	Body width	ML	Likelihood
CHI	China	MOZ	Mozambique
COI	Cytochrome *c* oxidase I	MP	Parsimony
COII	Cytochrome *c* oxidase II	MPA(s)	Marine Protected Area(s)
COM	Comoros	mtDNA	mitochondrial DNA
CPD	Caudal peduncle depth	*N*	Number of individuals
CPL	Caudal peduncle length	N_A	Number of alleles
CVA	Canonical Variate Analysis	NADH-2	Nicotinamide adenine dinucleotide dehydrogenase-2
Cyt *b*	Cytochrome *b*	N_h	Number of haplotypes
DFA	Discriminant Function Analysis	NJ	Neighbor-joining
D-III-L	3rd Dorsal spine length	NNA	Neural Network Analysis
D-II-L	2nd Dorsal spine length	N_{PA}	Number of private alleles
D-I-L	1st Dorsal spine length	N_{PH}:	Number of private haplotypes
D-IV-L	4th Dorsal spine length	OD	Orbit diameter
DL	Dorsal fin length	PAL	Pre-anal length
DNA	Deoxyribonucleic acid	PAUP	Phylogenetic Analysis Using Parsimony, software
DnaSP	Population genetics and molecular analysis software	PCA	Principal Component Analysis
DR	Dorsal rays	PCR	Polymerase Chain Reaction
DS	Dorsal spines	PDL	Pre-dorsal length
EACC	East African Coastal Current	PL	Pectoral fin length
FAO	Food and Agriculture Organisation of the United Nations	PLD(s)	Pelagic larval duration(s)
FL	Fork length	PR	Pectoral rays
GoO	Gulf of Oman	PVL	Pre-pelvic length
H_D	Haplotype diversity	*r*	Raggedness index statistic
HL	Head length	REU	Reunion
IBD	Isolation by distance	RS/R-Sea	Red Sea
IO	Inter-orbital width	S7 intron 1	First intron of the S7 gene
IP	Indo-Pacific	SA	South Africa
IWP	Indo-West Pacific	SAIAB	South African Institute for Aquatic Biodiversity

SC	Somali Current	TL	Total length
SCC	Somali Counter Current	tRNA	transfer Ribonucleic acid
SCS	South China Sea	UCL	Upper caudal lobe
SEC	South Equatorial Current	UGR	Upper gill rakers
SECC	South Equatorial Counter Current	UNEP	United Nations Environmental Programme
SEMC	Southeast Madagascar Current	WIO	Western Indian Ocean
SEY	Seychelles	WP-Is	West-Pacific Island
SL	Standard length	YEM	Yemen
SNL	Snout length	π	Nucleotide diversity
SOM	Somalia	Φ_{CT}	Random permutation of all localities across regions
TAN	Tanzania	Φ_{SC}	Random permutation of individuals across localities within the same region
THA	Thailand	Φ_{ST}	Random permutation of individuals across localities without regard to their original locality or region

CHAPTER ONE

GENERAL INTRODUCTION

1.1. Population structure and phylogeography

Several studies on marine species (Warwick & Clarke, 2001; Borsa, 2003; Dawson & Hamner, 2005; Teske *et al*., 2008; Chlaida *et al*., 2009) emphasise that the marine environment lacks obvious physical barriers to dispersal, which means that species can potentially disperse over large distances. The main mode of species dispersal is thought to be by oceanic currents, mainly during the pelagic larval stage (Dawson & Hamner, 2005). However, some species are also highly mobile as adults (Árnason, 2004; Domeier & Speare, 2012), leading to high rates of gene flow between distant localities (Palumbi, 1994; Chlaida *et al*., 2009). D'Amato and Carvalho (2005) suggested that gene flow, population history and environmental conditions influence genetic diversity. Over time, separated populations accumulate mutations, resulting in population divergence; the opposite occurs when individuals from other populations migrate into local populations and populations converge through successful reproduction (Bernardi *et al*., 2001). Schizas *et al*. (1999) suggest that population structure can occur in species independent of dispersal rates. Population history, habitat preference, local adaptation and oceanographic conditions are some of the mechanisms influencing marine population structure (Palumbi, 1994; González-Wangüemert *et al*., 2010). For example, Vollmer and Palumbi (2007) found great differentiation among the staghorn coral *Acropora cervicornis* populations separated by greater than 500 km, and even among reefs that were 2 km apart. Palumbi (1995) detected great genetic differentiation between populations of tide-pool copepods separated by a few kilometres, despite a larval stage of more than six weeks and the ability of adults to drift along rocky outcrops on which they live.

Isolation by distance (IBD), i.e., genetic differences induced by geographically-restricted gene flow (Wright, 1943), is another way that marine populations diverge and become genetically differentiated (Hardy & Vekemans, 1999; Bohonak, 2002). This has been shown by the isolation of Marquesas from other West Pacific localities in surgeonfish *Acanthurus triostegus*, influenced by ocean currents (Planes & Fauvelot, 2002). Palumbi *et al*. (1997)

detected strong genetic structure among populations of sea urchins (genus *Echinometra*) separated by 5000 – 10 000 km, but low structure among populations separated by 2500 – 5000 km, despite the high dispersal potential of these species.

1.2. Larval dispersal and connectivity

Dispersal refers to the movement of individuals among populations (Chenoweth *et al*., 1998; Shulman, 1998), leading to connectivity (Mora & Sale, 2002). Dispersal of larvae is important in maintaining gene flow over great distances, particularly in organisms with sedentary adults (Kinlan & Gaines, 2003). Individuals of some species are highly mobile as adults (e.g. Atlantic cod, *Gadus morhua*) and are able to travel as individuals of reproductive age over large distances, thus adding to the potential for short- and long-distance gene flow over large areas (Árnason, 2004; Leis *et al*., 2007; Wu *et al*., 2009). The pelagic larval duration (PLD) is generally assumed to promote species distribution and connectivity in the marine environment (Roberts, 1997; Riginos & Victor, 2001; Leis, 2002; Palumbi, 2003), and this has implications for the understanding of biogeography and population dynamics (Wellington & Victor, 1989; Cowen *et al.*, 2000). Larval transport is crucial for maintaining the integrity of many marine species and for inhibiting speciation that could result from the isolation of adult individuals in local patches of habitat (Planes, 2002). Pelagic larval duration can last for a few days to several months, depending on the species (Wellington & Victor, 1989; Almany *et al*., 2007). For example, the PLD of the pomacentrid fish *Amblyglyphidodon curacao* is 15 – 22 days (Leis *et al*., 2007), whereas it can last for two to three months in the crab *Cancer pagurus* (Ungfors *et al*., 2009). Variability in larval transport will be determined by the interactions of water masses, winds, tides, currents and water temperature (Cowen, 2002; Leis & McCormick, 2002). These processes could either result in reduced gene flow and resultant population differentiation or facilitate genetic connectivity (Endler, 1973). Furthermore, oceanographic features like eddies and fronts can prevent mixing and diffusion of pelagic larvae, and two adjacent sites on different sides of an oceanographic front may rarely exchange migrants (White *et al*., 2010). Some studies have shown that larvae often recruit to their natal reefs, resulting in reduced dispersal (Taylor & Hellberg, 2003; Almany *et al*., 2007). Local larval retention and larval behaviour, i.e., the swimming abilities of many coral reef fishes, are some of the factors assumed to reduce dispersal of marine fish larvae (Meekan *et al*., 1993; Cowen *et al*., 2000; Leis, 2002; Taylor & Hellberg, 2003; Leis *et al*., 2007).

1.3. Molecular data

Molecular data can be categorised by function (protein-coding vs. non-coding vs. structural RNA) and by genome (mitochondrial vs. nuclear) and different categories have distinct properties (Springer *et al*., 2001). Molecular methods offer a wealth of characters for systematic studies (Avise, 1994). Hackett (1996) regarded some of the advantages of molecular data, particularly DNA sequences, as being the known genetic base and mode of inheritance of variation, and the large number of variable characters that can be reviewed. These markers are very useful in evolutionary studies of animals and plants as they can be interpreted more objectively than earlier methods, which involved morphological characters (Hillis & Moritz, 1990). Earlier methods employed to determine genetic variation were based on the indirect expression of DNA loci in immuno-assays, protein profiles and allozymes (Kocher *et al*., 1989; Begg & Waldman, 1999). The major impact on systematics and determining genetic variation occurred with the advent of DNA sequencing (Sanger *et al*., 1977) and polymerase chain reaction (PCR: Saiki *et al*., 1988) technologies. These methods were useful in unravelling the systematics of species that were morphologically similar. Consequently, molecular systematics and phylogeographic approaches grant the opportunity to make inferences about species biogeography, phylogeographic structure and relationships, and the processes that have led to present-day distribution of genetic diversity (Hillis & Moritz, 1990; Avise, 2009).

Molecular markers have provided the means to assess the extent to which reef fish species are genetically structured, the spatial scale of partitioning and the temporal pattern of population connectivity between isolated populations (Horne *et al*., 2008). Molecular data have also been used to resolve species relationships within different groups/taxa such as lutjanids (Guo *et al*., 2007; Miller & Cribb, 2007), gobies (Wang *et al*., 2001), sunfishes and black basses (Near *et al*., 2004). In determining population differentiation, Waples (1987) found a negative correlation between estimated dispersal potential and genetic differentiation among various species of shore fishes. Veilleux *et al*. (2011) found differentiation between western and eastern Australian populations of *Lutjanus carponotatus* due to local adaptation. Viñas *et al*. (2010) detected genetic differentiation in between eastern and western populations of the swordfish *Xiphias gladius* in the Mediterranean Ocean, influenced by oceanographic currents.

Marine fishes often have high fecundity, large population sizes and large geographic ranges, leading to the expectation of high gene flow among populations (Borsa, 2003). Although many studies confirm this expected pattern for species with high dispersal capabilities, there are, however, exceptions to this paradigm in the marine environment (Avise *et al*., 1987; Palumbi, 1994). For example, the cleaner goby *Elacatinus evelynae* shows strong genetic differentiation, influenced by larval retention, despite a pelagic larval duration of 21 days potentially allowing for great dispersal (Taylor & Hellberg, 2003). Therefore, high dispersal abilities do not always result in genetic homogeneity, and population differentiation can be due to factors such as geographic isolation, marine barriers (ocean circulation patterns, water temperature, eddies and jets), behavioural limits to dispersal and natural selection leading to reduced gene flow (Schizas *et al*., 1999).

1.3.1. Mitochondrial DNA (mtDNA)

Animal mitochondrial DNA (mtDNA) is the most well studied piece of eukaryotic DNA. It is a small duplex, circular molecule (Figure 1.1), that is easy to purify relative to other organellar genomes, due to its buoyant density and high copy number (Wilson *et al*., 1985). The mtDNA of multicellular organisms has two ribosomal RNA genes (12S and 16S), 22 transfer RNA (tRNA) genes, and 13 proteins that are tightly packed in about 15 kilobases of a double-stranded DNA molecule (Wilson *et al*., 1985). Although, mtDNA is a small component of the macromolecules in a mitochondrion, it plays an important role in energy-yielding metabolism and protein synthesis within the mitochondrion and possibly contributes to the structure of the cell surface (Wilson *et al*., 1985; Saccone, 1994). Mitochondrial DNA has proved powerful in understanding the evolutionary relationships among individuals, populations and species (Irwin *et al*., 1991). Mitochondrial DNA sequence analysis has become one of the most widely used tools in studies of molecular phylogeny and phylogeography among vertebrates, including fish, because it is easy to handle and can be easily purified and sequenced (Avise, 2000). However, the applications largely depend on the gene of choice and its mutation rate. Several studies have used mitochondrial DNA to examine phylogeography (Rocha *et al*., 2008; Winters *et al*., 2010), population genetics (Ovenden & Street, 2003; Ovenden *et al*., 2004), and phylogenetics and biogeography (Orrel *et al*., 2002; Bernardi *et al*., 2004) of marine fishes.

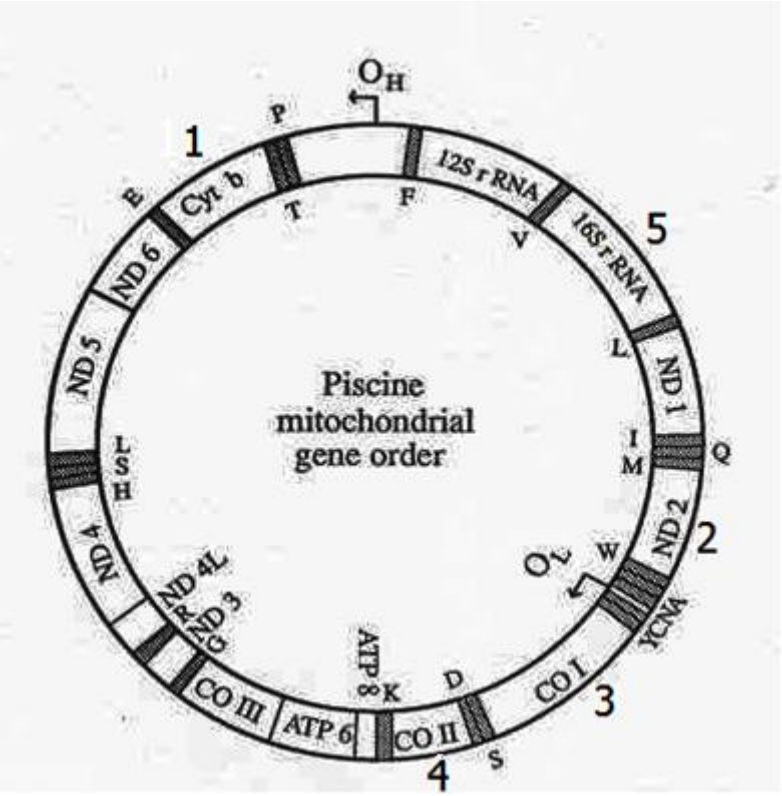


Figure 1.1. The animal mitochondrial gene order, illustrating the different genes, including those used in the current study: cyt *b* (1), ND2 (2; NADH-2), COI (3), COII (4) and 16S r RNA (5; 16S). Figure taken from Meyer (1993).

Mitochondrial DNA is the marker of choice because of some of its characteristics, which include maternal transmission, higher mutation rates compared to nuclear DNA, a lack of recombination, its haploid nature and smaller effective population size (Wilson *et al*., 1985; Saccone, 1994; Neigel, 1997; Avise, 2000; Sunnucks, 2000; Rokas *et al*., 2003). It also accumulates mutations faster than nuclear DNA, making it suitable to infer genealogical relationships among recently diverged species and for estimating divergence times (Kocher *et al*., 1989; Wilson *et al*., 1985; Avise, 1994; Neigel, 1997). To examine regional genetic structure in the three species, cytochrome *b* (cyt *b*) and NADH dehydrogenase 2 (NADH-2) were used in the current study. Cytochrome *b* has been successfully used in resolving population structure in the soldierfish *Myripristis berndti* (Craig *et al*., 2007; Muths *et al*., 2011), the snapper *Lutjanus purpureus* (De Salles *et al*., 2006) and the nine-spined stickleback *Pungutius pungutius* (Teacher *et al*., 2011). Bradman and Appleton (2011) successfully used NADH-2 to detect differentiation among populations of the swordfish

Xiphias gladius. For the study of phylogenetic relationships among WIO snappers, 16S rDNA, cytochrome *c* oxidase I and II (COI and COII, respectively) were selected. Hanel *et al*. (2002) successfully used 16S rDNA to resolve the phylogenetic relationships and brood-care behaviour in the wrasse tribe Labrini (Labridae).

For the study of phylogenetic relationships, Elliot *et al*. (1999) used cytochrome *b* and 16S rDNA to determine evolution in anemonefishes (family Pomacentridae). The phylogenetic relationships of angelfishes (Pomacanthidae) were resolved using 12S rDNA and 16S rDNA (Bellwood *et al*., 2004). Fessler and Westneat (2007) used combination of 12S rDNA, 16S rDNA, NADH-3, Tmo-4C4 and RAG-2 to resolve phylogenetic relationships of butterflyfishes (Chaetodontidae). Chakrabarty (2006) successfully applied a combination of mitochondrial (including 16S rDNA and COI) and nuclear (S7 intron 1 and Tmo-4C4) markers to resolve relationships within the Cichlidae. Guo *et al*. (2007) resolved the phylogenetic relationships of the South China Sea (SCS) snappers by applying cytochrome *b* and COII. Based on these studies, it is clear that these genes can successfully be used independently or in combination with other genes to either determine connectivity among populations or the phylogenetic relationships among fish species.

As with most applications, there are drawbacks associated with the use of mtDNA (Meyer, 1993; Saccone, 1994). These are (1) the lack of recombination, which greatly diminishes the power to detect significant spatial patterning (Palumbi & Barker, 1994; Sunnucks, 2000), and (2) the exclusive reliance on mtDNA data alone will allow only the re-construction of maternal lineages (Avise *et al*., 1987; Slatkin & Maddison, 1990). Heteroplasmy, when two or more genotypes co-exist within the same individual, is another limitation. Nevertheless, heteroplasmy poses few problems since it is rare (Avise *et al*., 1987). There is also the possibility of paternal transmission, which is estimated to be one paternal mtDNA per thousand maternal mtDNAs transferred to the zygote in insects and mice (Lansman *et al*., 1983; Gyllensten *et al*., 1985).

1.3.2. Nuclear DNA (nDNA)

The nuclear genome provides an array of markers for evolutionary studies. Among others, it is characterised by both the coding (exon) and non-coding (intron) regions that evolve at different rates, allowing for inferences of intra-population dynamics based on markers from independent linkage groups (Sunnucks, 2000; Fujita *et al*., 2004). For most organisms, exons were widely used for systematic studies. Introns are now becoming the focus of systematic studies (Zhang & Hewitt, 2003), as they appear to harbour a greater degree of genetic polymorphism within and between species and an increased rate of evolution when compared to exons, with exons presenting ideal places to place PCR primers (Palumbi & Barker, 1994; Chow & Hazama, 1998; Zhang & Hewitt, 2003). Furthermore, introns are relatively free from the functional (coding) constraints imposed on exons, resulting in molecular markers that show little base compositional bias, low transition-transversion ratios and minimal among-site rate heterogeneity (Guo & Chen, 2010). Von der Heyden *et al*. (2008) applied a combination of mitochondrial and nuclear genes to detect population structure among populations of the endemic South African bluntnose klipfish *Clinus cottoides*. They were able to detect significant geographic differentiation with mitochondrial genes and shallow but significant population structure with the nuclear gene. Schinske *et al*. (2010) used a combination of mitochondrial and nuclear genes, and morphological data to study the phylogeography of the flatfish *Hypsopetta guttulata*. Therefore, the inclusion of nDNA markers in evolutionary and population-genetic studies is indispensable for a better understanding of evolutionary processes that have occurred, due to differing mutation rates and inheritance properties (Zhang & Hewitt, 2003). Parallel analysis of nuclear DNA and mtDNA is necessary as it offers insight into different patterns of evolution and reflects different aspects of population biology and history (Palumbi & Barker, 1994; Fujita *et al*., 2004).

1.4. Lutjanidae (snappers)

1.4.1. Phylogenetic relationships

Fishes of family Lutjanidae (snappers) are mainly confined to tropical and subtropical marine waters distributed throughout the eastern Pacific, Indo-West Pacific, and eastern and western Atlantic (Allen, 1985; Allen & Talbot, 1985). Snappers are perch-like, reef-dwelling marine

fishes that are slender- to deep-bodied with a truncate to deeply forked caudal fin. They have scaly sheaths at the bases of the dorsal and anal fins, teeth present on the vomer, and are without filamentous soft dorsal or anal fin rays (Allen, 1985). The family is composed of 17 genera and over 120 species. The Lutjanidae has been split into four subfamilies (Allen 1985, 1987): (1) Etelinae, containing five genera: *Aphareus*, *Aprion*, *Etelis*, *Pristipomoides*, and *Randallichthys*; (2) Apsilinae, containing four genera: *Apsilus*, *Lipocheilus*, *Paracaesio* and *Parapristipomoides*; (3) Paradicichthyinae, containing two monotypic genera: *Symphorus* and *Symphorichthys*; and (4) Lutjaninae, containing six genera: *Hoplopagrus*, *Macolor*, *Ocyurus*, *Pinjalo*, *Rhomboplites* and *Lutjanus*. The Etelinae (jobfishes) are the most primitive group, with Apsilinae intermediate to the Etelinae and the Lutjaninae + Paradicichthyinae (the most advanced groups). The Paradicichthyinae is the sister-taxon to the Lutjaninae and Caesionidae, which are often included in the Lutjanidae (see below) (Johnson, 1980). The genus *Lutjanus*, which is the focus of this study, is the largest in the subfamily with 65 known species (Allen, 1985). Three species from this genus inhabit freshwater streams, while the majority are found in the marine environment, from shallow to intermediate depths (Allen, 1985).

The Caesionidae (fusiliers) are related to the Lutjanidae and together these comprise the superfamily Lutjanoidea (Johnson, 1980). There is an ongoing debate about the relationship between the Lutjanidae and Caesionidae; whether they should be treated as separate families or whether the Caesionidae be placed as a subfamily within the Lutjanidae (Johnson, 1980; Leis, 1987; Carpenter, 1990; Johnson, 1993; Miller & Cribb, 2007). The Caesionidae is divisible in two subfamilies (Allen, 1985; Carpenter, 1987, 1990): (1) Caesioninae, containing two genera: *Caesio* and *Pterocaesio*, and (2) Gymnocaesioninae, containing two genera: *Gymnocaesio* and *Dipterygonotus*. These fishes are planktivorous, thus, differing widely in feeding habits from the Lutjanidae, which are mostly benthic carnivores (Carpenter, 1987).

1.4.2. Study taxa

Three species from the genus *Lutjanus*, the twin-spot red snapper *Lutjanus bohar* (Forsskål, 1775), the dory snapper *Lutjanus fulviflamma* (Forsskål, 1775) and the bigeye snapper *Lutjanus lutjanus* Bloch, 1790, were selected for the study of genetic differentiation and connectivity across the Western Indian Ocean (WIO). Various other species from the genus

were also included to determine the phylogenetic relationships and placement of the WIO snappers in context to the Indo-Pacific (IP) snappers. Literature has shown that lutjanids have a wide distribution across the Eastern Pacific, Indo-Pacific, Eastern Atlantic and Western Atlantic (Allen, 1985).

Specific studies done on representatives of the family Lutjanidae have looked at population genetic structure of *Lutjanus campechanus* (Garber *et al*., 2004; Pruett *et al*., 2005) and *Lutjanus fulviflamma* (Dorenbosch *et al*., 2006), larval development in the genus *Macolor* (Leis, 2007), and reproductive biology of *Lutjanus argentiventris* (Muhlia-Melo *et al*., 2003). Only scant information is known about other species in the genus. Two papers examining phylogenetic relationships within the genus *Lutjanus* were published; one covered the phylogenetic relationships of the South China Sea (SCS) snappers (Guo *et al*., 2007) and the other IP snappers (Miller & Cribb, 2007). However, none of them included the WIO representatives. Therefore, a combined data set from the current study and these studies was used to examine differentiation among conspecific representatives from the two regions and to determine the position of the WIO snappers in the wider IP.

Members of the genus *Lutjanus* have variable external colouration, often consisting of yellow, reddish, grey or brown colours, with a pattern of stripes or bars on the sides (Allen, 1985). The external colouration of most species appears the same and often species are misidentified in the field. For instance *L. bengalensis* and *L. quinquelineatus* are both "blue-lined" species but the former has four stripes while the latter has five stripes (Allen, 1985). *Lutjanus fulviflamma* and *L. ehrenbergii* both have a prominent black-spot, with six to seven stripes for *L. fulviflamma*, while *L. ehrenbergii* has four to five stripes (see Allen, 1985). Morphometrics are included in the present study to determine if the patterns of intraspecific differentiation seen in genetics are also seen in the morphology (or vice versa). This will be done strictly for *Lutjanus fulviflamma* (Chapter 3) because of better sample size.

1.4.2.1. *Lutjanus fulviflamma* (Forsskål, 1775) – dory snapper

The dory snapper (Figure 1.2) is associated with coral reefs across the Indo-Pacific (IP) from Samoa to East Africa (with their distribution extending southwards to East London in South Africa), and from Australia northwards to the Ryukyu Islands (Allen, 1985). The dory

snapper is often found in occurrence with big-eye and blue-stripped snappers (*Lutjanus lutjanus* and *L. kasmira*, respectively). Juveniles of this species are found in the mangrove habitats or in the lower reaches of freshwater streams (Allen & Talbot, 1985).

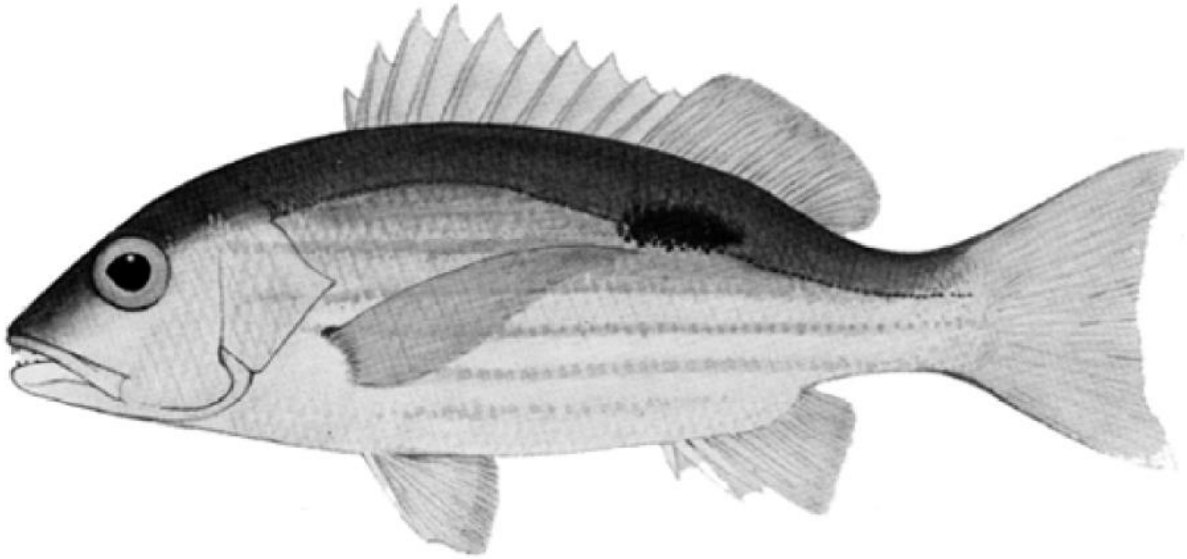


Figure 1.2. Illustration of *Lutjanus fulviflamma* (Forsskål, 1775). Illustration taken from Allen (1985).

These environments are often nutrient rich and offer safe refuge from predators. *Lutjanus fulviflamma* can grow to a total length (TL) of 35 cm but more commonly reaches 25 cm (Allen, 1985; Branch *et al*., 1994; Froese & Pauly, 2006). For a description of the species, consult Allen (1985).

1.4.2.2. *Lutjanus bohar* (Forsskål, 1775) - twin-spot red snapper

The twin-spot red snapper (Figure 1.3) is associated with coral reefs, including sheltered lagoons and outer reefs across the Indo-West Pacific (IWP); occurring from the Marquesas and Line Islands to East Africa (with the distribution extending as far south as Durban), and from Australia northwards to the Ryukyu Islands (Allen, 1985). *Lutjanus bohar* can grow to a maximum 75 cm (TL) but more commonly attains 50 cm (Allen, 1985). For a species description, consult Allen (1985). Twin-spot red snappers are large, slow growing, solitary

species, often found adjacent to steep outer reef slopes and occasionally found in groups (Allen & Talbot, 1985; Branch *et al*., 1994; Marriot *et al*., 2007).

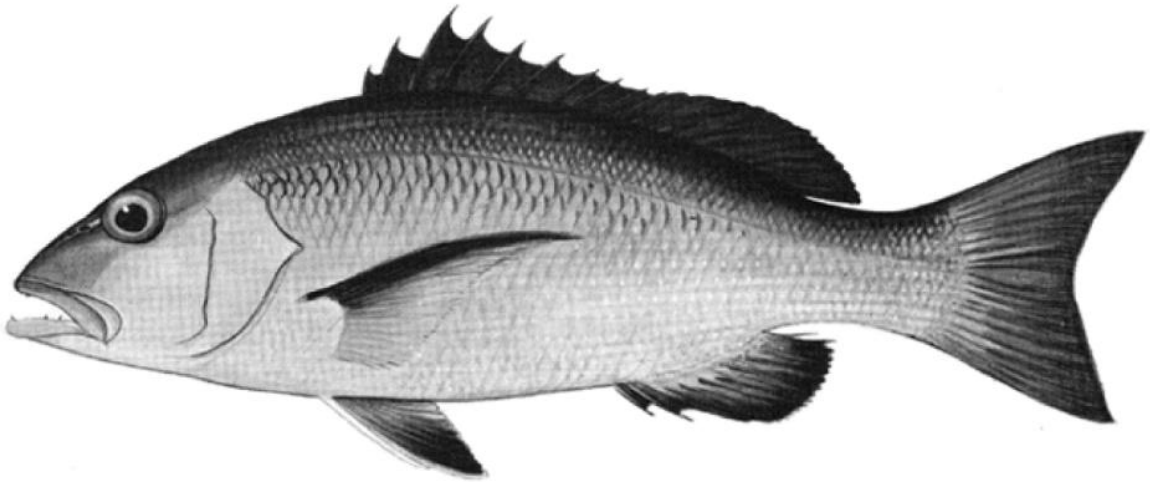


Figure 1.3. Illustration of *Lutjanus bohar* **(**Forsskål, 1775). Illustration taken from Allen (1985).

1.4.2.3. *Lutjanus lutjanus* Bloch, 1790 – bigeye snapper

The bigeye snapper (Figure 1.4) is associated with rocky and coral reefs across the Indo-West Pacific; from the Solomon Islands to East Africa, and from Australia to southern Japan (Allen, 1985). They are found in large schools of more than 100 individuals with other *Lutjanus* species (Allen, 1985; Smith & Heemstra, 2003). *Lutjanus lutjanus* can reach a maximum of 30 cm (TL) but common commonly grows to 19 cm (Allen, 1985; Froese & Pauly, 2006). For the description of the species, consult Allen (1985).

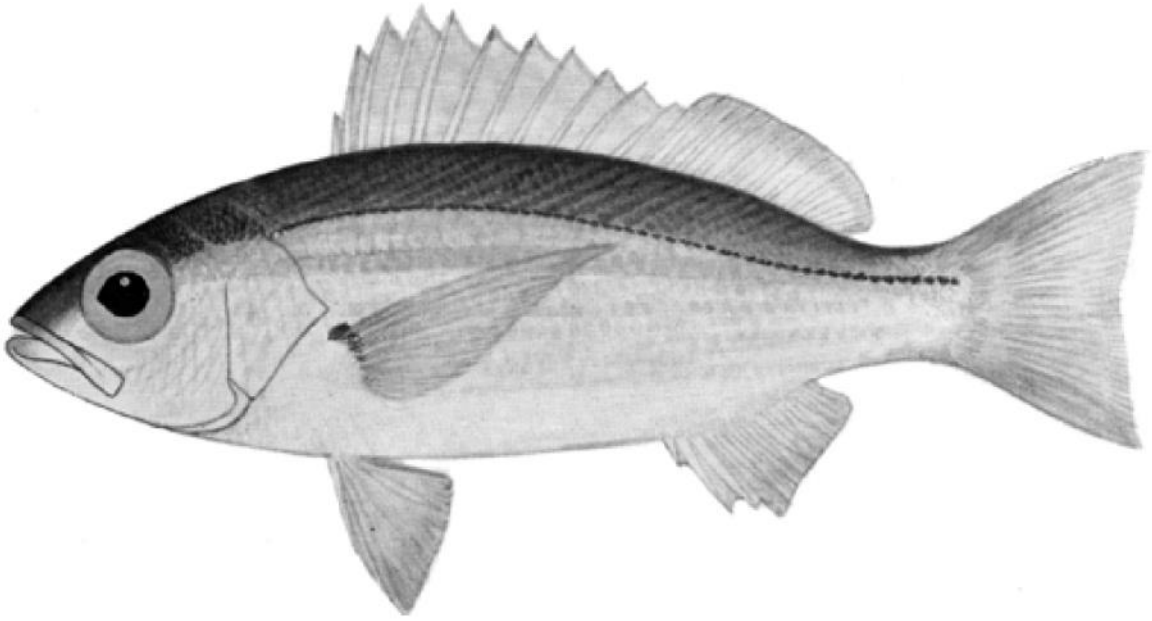


Figure 1.4. Illustration of *Lutjanus lutjanus* Bloch, 1790. Illustration taken from Allen (1985).

1.4.3. Habitat and Biology

Snappers are active predators, feeding mostly at night on a variety of foods, including fishes (the dominant diet of most species), crustaceans, gastropods, cephalopods and urochordates (Allen, 1985; Smith & Heemstra, 2003). Lutjanids spawn small, spherical, pelagic eggs, with incubation times generally ranging from 17 to 36 hours, depending on the species and temperature (Allen, 1985; Grimes, 1987; Leis, 1987). To some degree, spawning occurs year-round with maximum reproductive activity during the northern hemisphere spring and summer, mostly between April and July (Leis, 1987; Shimose & Tachihara, 2005; Grandcourt *et al*., 2006). The larvae develop elongate fin spines and extensive spination on the head. Lutjanids, like other tropical marine reef fishes, have a biphasic life cycle that includes sedentary or reef-associated adults and a pelagic larval stage, allowing for great dispersal potential (Fisher *et al*., 2005). Zapata and Herron (2002) studied the PLD of five species of snappers, *Hoplopagrus guntheri*, *Lutjanus argentiventris*, *L. guttatus*, *L. novemfasciatus* and *L. viridis*. Their results showed *L. viridis* to have a higher PLD (37.9 days) with *L. novemfasciatus* having the lowest (20.6 days). It is assumed that longer PLD will allow for

the colonisation of distant localities. Compared to other lutjanids, these PLDs are short. For instance, the PLD of the jobfish *Pristipomoides filamentosus* can last up to 180 days (Gaither *et al*., 2011a).

1.5. Study area

The Food and Agriculture Organization of the United Nations (FAO, 1993, 2011a) divided the world's marine environment into 19 major fishing areas. The present study region, the Western Indian Ocean (WIO, Area 51), accounts for 8% of total marine waters (Van der Elst *et al*., 2005). The WIO (Figure 1.5) is considered a biogeographic sub-region of the Indian Ocean (Gullström *et al*., 2002). It stretches along the East African coast from Somalia to South Africa, and includes the Red Sea, Gulf of Oman and island states (Comoros, Seychelles,

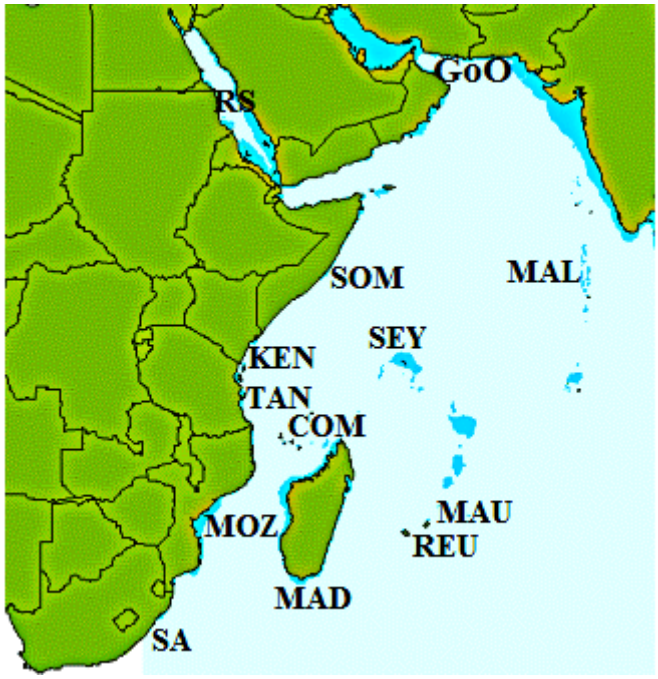


Figure 1.5. The delimitation of the Western Indian Ocean. Countries and island states indicated include South Africa (SA), Mozambique (MOZ), Tanzania (TAN), Kenya (KEN), Somalia (SOM), Maldives (MAL), Seychelles (SEY), Comoros (COM), Madagascar (MAD), Reunion (REU) and Mauritius (MAU). The Red Sea (RS) and Gulf of Oman (GoO) are included within the WIO.

Reunion, Mauritius and Madagascar), and extending towards the central Indian Ocean (Gullström *et al*., 2002; Wafar *et al*., 2011). This area comprises over 12 200 km of coastline with coastal ecosystems including major estuaries, coastal lagoons, mangrove forests, coral reefs, sea-grass beds, mud flats, algal beds, barrier islands, and sandy and rocky beaches (Smith & Heemstra, 1986; Van der Elst, 1990; Ngoile & Linden, 1997). These environments sustain diverse marine life and are critical as a source of subsistence for the coastal communities (Ngoile & Linden, 1997).

The WIO is one of the most dynamic and variable large marine ecosystems in the world (Vousden *et al*., 2008; Van der Elst *et al*., 2009). The physical complexity of the WIO (Gullström *et al*., 2002; Francis & Torell, 2004; Lutjeharms, 2006) makes it suitable to formulate testable hypotheses regarding species diversity, population dynamics (with reference to population connectivity and differentiation) and biogeography.

1.5.1. Physical oceanography of the study area

The WIO is characterised by complex climate and current systems (Figure 1.6), strongly influenced by monsoonal winds (Schott & McCreary, 2001; Benny, 2002). The South Equatorial Current (SEC) is the major oceanic current in the WIO. It flows from the eastern Indian Ocean to northern Madagascar and proceeds towards the continent to northern Mozambique and southern Tanzania (Warren *et al*., 1966; Kemp, 1998). On contact with the continent, it splits into two components, which flow north and south. One component continues northward forming the East African Coastal Current (EACC) during the Southeast Monsoon (April – October) season (Figure 1.6: A) and leaves the continent. During the Northeast Monsoon (November – March) season (Figure 1.6: B), it forms the Somali Counter Current (SCC) (McClanahan, 1988). According to McClanahan (1988), these currents cause a major downwelling along Tanzania and southern Kenya during the Northeast Monsoon, whereas an upwelling occurs along northern Somalia during the Southwest Monsoon. The southward-flowing SEC continues down as the Mozambique Current (MC) into the Mozambique Channel. The flow of water within the channel shows a strong poleward

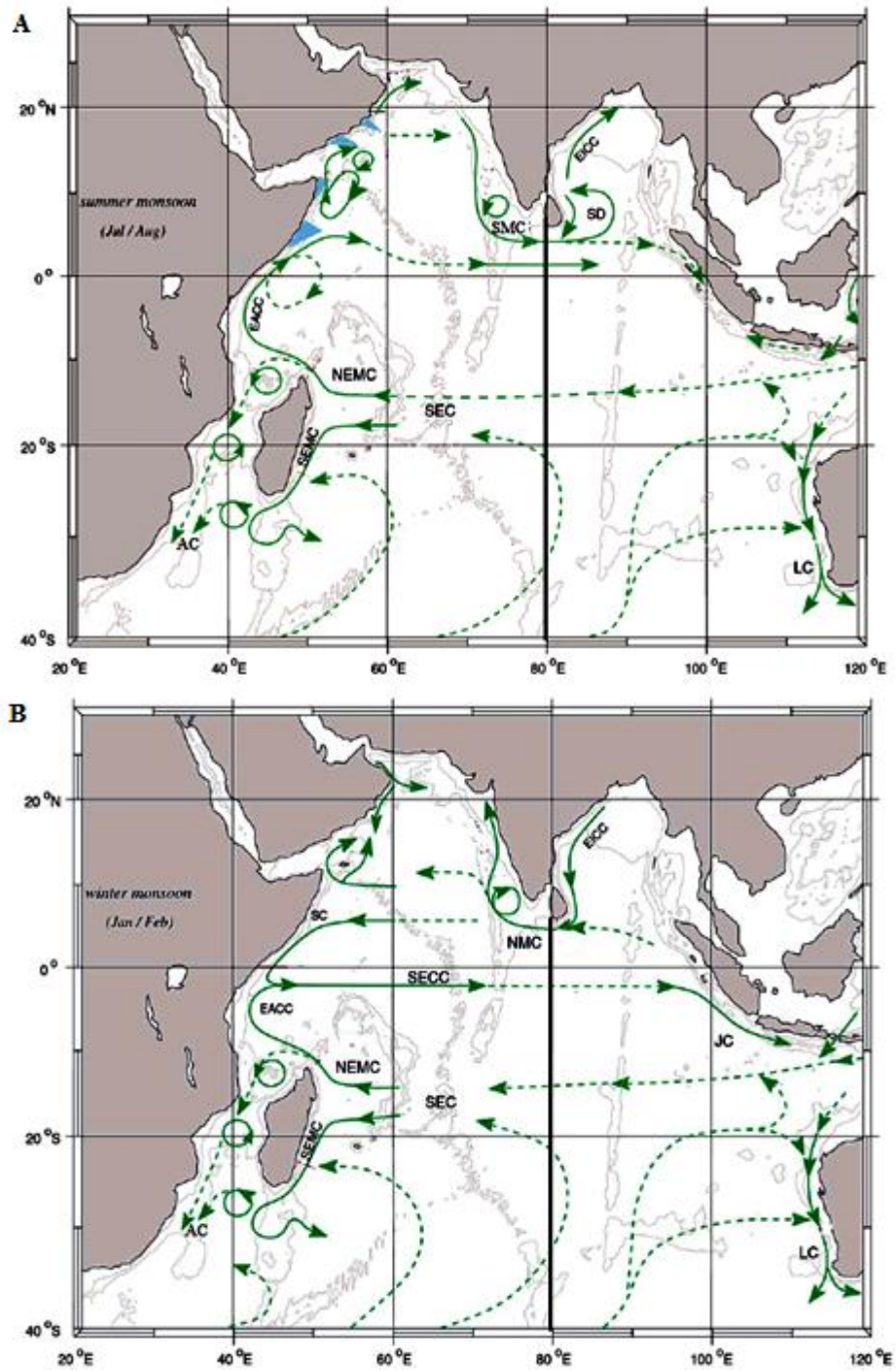


Figure 1.6. Schematic presentation of the Western Indian Ocean currents during the Southwest Monsoon (A) and Northeast Monsoon (B). The solid lines indicate the eastern boundary of the Western Indian Ocean region. Current branches include the South Equatorial Current (SEC), East African Coastal Current (EACC), Somali Current (SC), Agulhas Current (AC) and Southeast Madagascar Current (SEMC), as discussed in the text. Figure from Schott & McCreary (2001).

movement along the eastern shelf of Mozambique (Donohue & Toole, 2003). Quartly and Srokosz (2004) used satellite observations and indicated the presence of cyclonic eddies off the shelf edge along the southwestern coast of Madagascar, which draw coastal water into the channel. To the south, towards the end of the channel, the Mozambique shelf is wide and constitutes the Delagoa Bight (Lutjeharms, 2006). Some water entering this bight comes from the passing anti-cyclonic Mozambique eddies, resulting in the Delagoa Bight eddy (Lutjeharms, 2006). The Agulhas Current (AC) is composed of water from the SEC and from recirculation in the southwest Indian Ocean subgyre (Stramma & Lutjeharms, 1997; De Ruijter *et al*., 2005; Lutjeharms, 2006, 2007). How the SEC acts as a source for the AC has not been determined, but most water comes from the subgyre (Lutjeharms, 2006). Nonetheless, it flows past the southern tip of Africa and, upon reaching the Agulhas Bank, it swings back eastwards in the south Indian Ocean as the Agulhas Return Current (Lutjeharms, 1998). Along KwaZulu-Natal, just north of Durban, the AC flows closer to shore forming the Natal Bight circulation (Lutjeharms *et al*., 2000; Lutjeharms, 2007). An upwelling upstream of the bight is an important hydrodynamic feature in the region of the bight (Lutjeharms & De Ruijter, 1996; Lutjeharms *et al*., 2000; Donohue & Toole, 2003; Lutjeharms, 2007). This upwelling and the presence of the AC causes the circulation off the KwaZulu-Natal shelf to undergo frequent current reversals, restricting the distribution of the coastal biota, mostly from the Indo Pacific (IP), with the open ocean (Schumann, 1988; Lutjeharms *et al*., 2000; Van der Elst *et al*., 2005). Little is known about the oceanography east of Madagascar. However, Lutjeharms (2006) noted the presence of the Southeast Madagascar Current (SEMC) and a likely upwelling on the southeast of the island – the interaction between the two remains unexplored. Other currents indicated in Figure 1.2 are not discussed since they are outside the study area.

The currents discussed above may have played a role in influencing connectivity or differentiation in the WIO. For example, the EACC together with the SEC and the SECC resulted in larval exchange on large geographic scales in the blue-barred parrotfish *Scarus ghobban* (Visram *et al*., 2010a). Complex current systems and eddies along the Mozambique Channel have also been shown to play a role in influencing dispersal and establishing biogeographic patterns (Procheş & Marshall, 2002; Samyn & Tallon, 2005; Bourjea *et al*., 2007). While the inshore waters along the KwaZulu-Natal coast retain eggs and fish larvae in

this region, offshore spawned eggs and larvae are transported southward in the AC (Whitfield, 1990).

1.5.2. WIO fish diversity

The WIO is characterised by high levels of regional endemism (Branch *et al*., 1994). Areas of high endemism, however, are not uniformly distributed and are found around island states, such as Mauritius and Reunion, along South Africa (the KwaZulu-Natal province) and southern Mozambique, and in the Red Sea (Van der Elst, 1988; Cox & Moore, 2005; Van der Elst *et al*., 2005). Smith and Heemstra (1986) recorded 2 200 species occurring in southern African waters, which comprise about 15% of the total marine fishes in the world. Van der Elst (1985) regarded the South African fauna as originating from the Indo-Pacific and later divided it into five categories based on its origin (Van der Elst, 1988), where he described 15.9% as endemic, 73.8% from the Indo-Pacific, 1.8% from the Atlantic and 0.3% from the Southern Ocean, while 8.4% was circumglobal. This diversity and composition led to a conclusion that the South Western Indian Ocean, particularly off KwaZulu-Natal, possesses a fauna that is distinctive from elsewhere in the Indian Ocean (Van der Elst, 1988).

The diverse fish fauna of the WIO is hypothesised to have originated from the IP (Briggs 1999). The Indo-Malayan region has been described as a centre of speciation or origin, with species distributions maintained by dispersal or migration from this centre (Randall, 1998; Briggs, 1999; Bellwood & Wainwright, 2002; Heads, 2005). There is a reduction in species diversity away from this centre and the older taxa are found to the periphery of this region (Briggs, 1999). Apart from dispersal, vicariance has been proposed for the origin of regional faunas of the WIO. Vicariance is described as the separation of continuously-distributed taxa due to a geographic or physical barrier. This leads to the separation of allopatric populations, resulting in the discrete distributions of taxa and a pattern of sequentially-related groups being geographically adjacent to each other (Hocutt, 1987; Pandolfi, 1992; Santini & Winterbottom, 2002). Santini and Winterbottom (2002) proposed biogeographic regions for the Indo-West Pacific, which included the Red Sea, Somali Basin, Mozambique Basin, Arabian Basin, Maldives/Chagos Laccadive Ridge and Mascarene Plateau within the WIO. In order to support vicariant hypotheses, historical events separating species will have to be identified. This would require the use of the fossil record, which is poor in this region (Bellwood &

Wainwright, 2002). These processes (Indo-Malayan connections, vicariance and regional current-mediated dispersal) could act individually or in combination to produce regional faunas (Procheş & Marshall, 2002; Samyn & Tallon, 2005), but their contributions have not been established (Bellwood & Wainwright, 2002).

1.5.3. Western Indian Ocean biogeographic regions

Several biogeographic hypotheses have been proposed for the WIO (Hocutt, 1987; Pandolfi, 1992; Santini & Winterbottom, 2002). However, Santini & Winterbottom's (2002) hypothesis is more congruent with the features of the region (Figure 1.7). These regions are discussed below:

1.5.3.1. Red Sea

The Red Sea lies between about 13 °N and 30 °N (Sheppard 2000). It is narrow and deep (Figure 1.7: 1), forming part of the Great East African Rift Valley system, and is connected to the Gulf of Aden and Indian Ocean by a shallow channel in the south (Roberts *et al*., 1992). The Red Sea is unique because it is partially isolated from the open ocean, with no river inflow and scant rainfall (Shaikh *et al*., 1986). The Red Sea is known for its steep, clear-water, coral reefs in the north and vast areas of sedimentary shallows suitable for mangroves and sea grass growth in the southern half (Sheppard, 2000). Regions of the Red Sea are physically distinguishable in terms of temperature, salinity and endemic species (Van der Elst, 1988). Roberts *et al*. (1992) described the thermal (increasing from north to south) and salinity (decreasing from north to south) gradients in the Red Sea. Similarly, a seasonal upwelling in the Gulf of Aden also forms a barrier to dispersal between Red Sea and the Indian Ocean (Sheppard *et al*., 1992). Within the Red Sea, most endemics are confined to the cooler and deeper northern part (Roberts *et al.*, 1992), while the warm waters in the middle and southern parts could be a barrier to fishes adapted to these cooler waters (Randall, 1998).

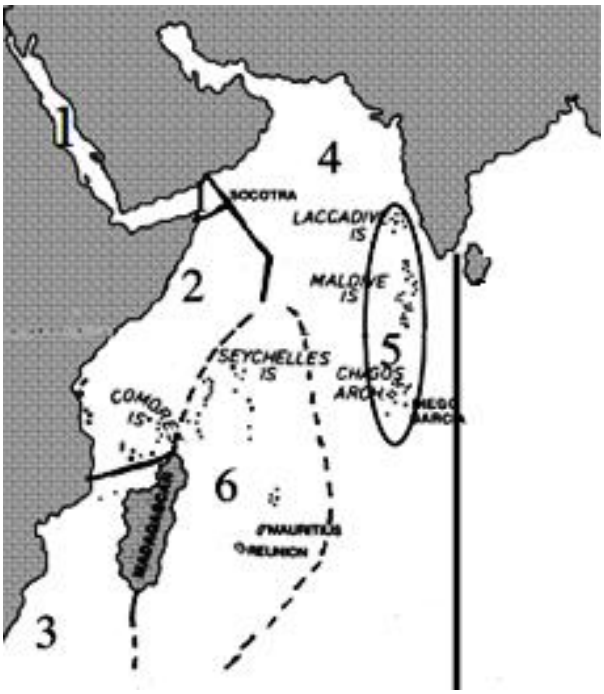


Figure 1.7. Western Indian Ocean biogeographic regions as proposed by Santini and Winterbottom (2002): Red Sea (1), Somali Basin (2), Mozambique Basin (3), Arabian Basin (4), Maldives/Chagos Laccadive Ridge (5) and Mascarene Plateau (6).

1.5.3.2. Somali Basin

The Somali Basin (Figure 1.7: 2) is confined in the west by continental Africa, in the east by the Carlsberg Ridge and in the north by the continental shelf surrounding the island of Socotra (Beal *et al*., 2000; Santini & Winterbottom, 2002; Pushcharovsky, 2007). The basin is fed by the water from the Somali Current, driven by the Southwest Monsoon from May through to September (Smith & Codispoti, 1980; UNEP, 2004). In the northern winter, the current flows from north of the equator to about 2 ºS and in winter it reverses direction and flows north (Duing *et al*., 1980; Carbone & Accordi, 2000; UNEP, 2004). The surface waters of the Somali Basin are characterised by waters derived from the Arabian Sea and the Gulf of Aden (Warren *et al*., 1966). The Southwest Monsoon upwelling off the Somali coast (Warren *et al*., 1966; UNEP, 2004) complicates interaction of these waters. This upwelling creates a physico-chemical and thermal barrier (Kemp, 1998).

1.5.3.3. Mozambique Basin

Simpson *et al*. (1979) described the Mozambique Basin (Figure 1.7: 3) as approximately 483 km wide, bounded by the Mozambique Ridge and the Madagascar Ridge in the north, and in the south it connects to the South-West Indian Ridge east of the Prince Edward Fracture Zone system. The basin covers the central and southern, and on- and offshore parts of Mozambique (Salman & Abdula, 1995), and the northern parts of the KwaZulu-Natal province of South Africa (Samyn & Tallon, 2005). Along the coast, it is characterised by coral outcrops around Inhaca (Mozambique) and the northern parts of KwaZulu-Natal (Van der Elst, 1985; Samyn & Tallon, 2005). The east and south coasts of South African have been divided into biogeographic provinces characterised by different species assemblages and hydrological conditions; the warm temperate south coast and the subtropical east coast (Turpie *et al*., 2000; Harrison, 2002). However, the boundaries separating these provinces are not agreed upon (see Turpie *et al*., 2000; Von der Heyden *et al*., 2008). Griffiths *et al*. (2010) recognised additional regions along the South African coast (see Figure 1.8).

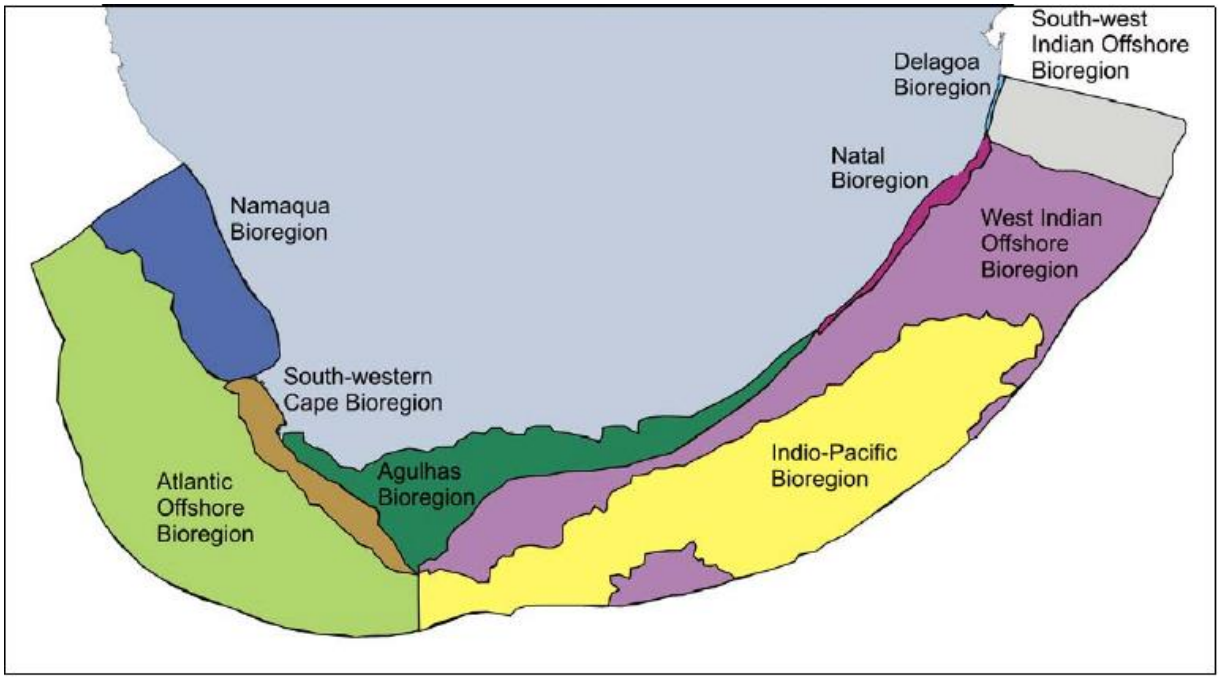


Figure 1.8. The nine South African marine biogeographic regions as taken from Griffiths *et al*. (2010).

1.5.3.4. Maldives/Chagos Laccadive Ridge

The Chagos are coral islands rising from the long, broad and slightly-curved plateau known as the Maldives/Laccadive Ridge (Figure 1.7: 5) (Heezen & Tharp, 1966), stretching over 2000 km from the Indian subcontinent southwards (Parson & Evans, 2005). The Maldives Archipelago is at the centre of this ridge. There are 1 190 coral islands with numerous sand cays and faroes within the 23 atolls (Rajasuriya *et al*., 2002). Maldives functions as an important stepping-stone in transoceanic species dispersal (Sheppard, 1999, 2000; Rajasuriya *et al*., 2002). Winterbottom and Emery (1986) and Winterbottom *et al*. (1989) found that recruitment of pelagic larvae to the Maldives could come from other WIO localities or from the West Pacific, but the distances involved could be expected to filter out a proportion of those reef fishes with short pelagic stages. In addition, the distances involved could result in genetic differentiation within species between Maldives and other WIO localities.

1.5.3.5. Mascarene Plateau

The crescent-shaped Mascarene Plateau (Figure 1.7: 6) consists of shallow banks, ridges and channels (UNEP, 2004; Parson & Evans, 2005; New *et al*., 2005, 2007). It has no significant land mass and partially lies in the path of the South Equatorial Current (Turner *et al*., 2000; Gallienne *et al*., 2004; Conway, 2005; Gallienne & Smyth-Wright, 2005). This plateau has both continental (resulting from the breakup of Gondwana) and oceanic, volcanic components, which contribute substantially to its diversity (Payet, 2005). The northern part of the plateau is affected by the Northeast Monsoon and South Equatorial trade winds (from December to February), and by the South Equatorial Current during the remainder of the year (Gallienne *et al*., 2004; New *et al*., 2005). During the Northeast Monsoon, the Mascarene Ridge affects the westward flow of the South Equatorial Current, causing it to diverge and resulting in an increased mass of water downstream of the ridge (Gallienne *et al*., 2004; New *et al*., 2005).

The Arabian Basin (Figure 1.7: 4) is not discussed here, because samples from this region were not included in the present study.

1.6. Rationale

Studies of connectivity and differentiation on a historical (i.e. biogeographic) timescale are confounded by the fact that biogeographic accounts of the WIO have not considered the region in its entirety or have considered the region incidentally to the larger IP (Pandolfi, 1992; Santini & Winterbottom, 2002). This knowledge gap exists despite the considerable diversity and endemism of some regions (see above). This study will contribute to this knowledge gap by sampling various localities in the region to examine patterns of connectivity and differentiation in lutjanid species. A molecular genetic approach will be followed to provide insights into the origin and relationship of regional faunas using three species (*Lutjanus bohar*, *L. fulviflamma* and *L. lutjanus*) as representatives or typical models, as they are widespread in the region (WIO). Snappers are sought after by recreational, commercial and artisanal fishers in the region (Allen, 1985), which is bordered by people living in developing countries and whose livelihoods depend on marine resources (Walmsley & Ninnes, 2006). By considering intraspecific relationships, phylogeographic breaks (genetic discontinuities separating geographic regions) can be detected. Intraspecific variation gives insights into contemporary and historical patterns of migration, connectivity, and the isolation of regions. The inclusion of species with different biology, habitat preferences and dispersal potentials (see Chapter 2) allows one to determine whether these species react similarly to the same environmental processes. These biogeographic breaks, patterns of genetic structure and species distributions can then be considered against life history and dispersal potential (Cowen, 2002; Planes, 2002). The interaction between environmental features and species biology could influence genetic differentiation (Todd *et al*., 1988). This has implications for understanding biodiversity and interactions among regional faunas (Santini & Winterbottom, 2002).

1.6.1. Research aims

Studies focusing on population genetic structure of lutjanids were carried out in the Gulf of Mexico (Pruett *et al*., 2005; Saillant & Gold, 2006; Karlsoon *et al*., 2009), around Australia (Ovenden & Street, 2003; Salini *et al*., 2006; Van Herwerden *et al*., 2009), and East Asia (Zhang *et al*., 2006). Within the WIO, Dorenbosch *et al*. (2006) and Muths *et al*. (2012) conducted genetic studies on a single species each, with the former study (on *Lutjanus*

fulviflamma) not covering the entire region. Therefore, this study will include multiple species to examine population structure/connectivity in the three species (*Lutjanus bohar*, *L. fulviflamma* and *L. lutjanus*) in Chapters three and four. These chapters aim to:

- Determine the relationships and interaction among, and the evolutionary history of different geographic regions in the WIO for each of the target species.
- Identify the processes that have led to these relationships.
- Consider the taxonomic status of the target species in WIO by considering whether they are widespread across the area or whether they harbour cryptic diversity. Cryptic species are common in marine taxa (Bickford *et al*., 2006) and widespread taxa often show regional differentiation (Gaither *et al*., 2010a).

Studies focusing on phylogenetic relationships among the snappers have been conducted in the West Atlantic (Sarver *et al*., 1996; Gold *et al*., 2011), the Indo-Pacific (Miller & Cribb, 2007) and the South China Sea (Guo *et al*., 2007), but none included WIO representatives. Therefore, chapter five aims to:

- Examine the phylogenetic relationships of snappers found in the WIO in the context of the wider IP snappers.
- Examine the extent of differentiation among the conspecifics from the WIO and the IP.
- Determine the phylogenetic placement of taxa not included previously.
- Consider the relationship between the Caesionidae and Lutjanidae, to determine the position of the Caesionidae.

1.6.2. Key questions

The key questions relating to the overall aims of the study and posed in each of the respective chapters are:

- Is there regional differentiation or connectivity within each of the three species in the WIO? (Chapters 3 and 4).
- Is there genetic differentiation among conspecifics between the WIO and IP/SCS? (Chapter 5).

- Are widespread species conspecific? (Chapters 3 to 5).
- What are the processes or life-history characteristics that cause the observed patterns of differentiation? (Chapters 3 to 5).
- Should the Caesionidae be considered a subfamily within Lutjanidae or as a separate family? (Chapter 5)

1.7. Conservation and management

Given the substantial threats to the marine environment and the importance of marine resources to communities and economies of the WIO region (Berg *et al*., 2002; Francis & Torell, 2004; Walmsley & Ninnes, 2006), effective measures are needed for conservation and sustainable management of the resources. Artisanal fishing provides an important source of food, employment and income for most coastal communities in the region (Ngoile & Linden, 1997; Walmsley & Ninnes, 2006). A relatively limited number of specific groups (e.g. such as barracuda, rabbit-fish, sardines and mackerel) dominate this activity (UNEP, 2000). However, trends in the declared landings of marine resources from the WIO suggest that this ocean may be approaching its maximum harvest potential (FAO, 1993). Understanding intra-specific variation will aid in identification of unique stocks of exploited or exploitable species and management can then be initiated, such that exploitation in one region does not affect another negatively. For non-exploited resources, the assessment of genetic variation and stock structuring would be valuable as baselines for long-term monitoring and future sustainable management. Sustainable, well-managed fisheries are less likely to suffer over-fishing or stock collapse, thus reducing the vulnerability of fishing communities that depend on these resources (Walmsley & Ninnes, 2006). The knowledge of genetic structure of marine organisms can be used to inform conservation management. Reviews by Avise (1992) and Graves (1998) illustrated that a fuller understanding of population structure is necessary to make informed management and conservation decisions. Where this is not available, assumptions could be made based on information gained from taxonomically-related or from ecologically-similar species. For example, Bowen *et al*. (2005) suggested different management strategies at each life stage for the loggerhead turtle *Caretta caretta*. Carson *et al*. (2011) suggested marine protected areas (MPAs) for the spawning aggregates of mutton snapper, *Lutjanus analis*, for the preservation of adaptation and genetic variability. McCook *et al*. (2009) recommended a network of MPAs for coral reefs and associated habitats

throughout the Coral Triangle. Froukh and Kochzius (2007) proposed that the fourline wrasse *Larabicus quadrilineatus* be managed separately as two different stocks in the northern and the southern Red Sea. These studies present an array of cases where MPAs have been suggested in the hope that they will preserve all biotypes, prevent over-exploitation and ensure the sustainable use of marine resources (McClanahan *et al*., 2007; McCook *et al*., 2009). The results of this study can therefore have direct implications for the conservation and management of marine resources in the WIO.

CHAPTER 2

MATERIAL AND METHODS

2.1. Sampling and processing

Snappers were collected using different methods, including spearfishing, beach seine-netting (with a 10 x 2m, 1.2 cm mesh seine net), hook and line, and by applying rotenone in isolated rocky pools. However, the majority of the samples came from local fishermen and fish markets from various localities across the WIO. For molecular studies, fin or muscle tissue was cut and stored directly in 90-100% ethanol in a labelled tube in the field. Specimens were identified, photographed, measured, using standard length (SL) or total length (TL), and, where possible, voucher specimens were retained, labelled with the corresponding DNA tube number and fixed in 10% formalin. Genetic samples were later stored at -20°C until further analysis. All samples collected were accessioned in the National Fish Collection at the South African Institute for Aquatic Biodiversity (SAIAB) in Grahamstown. Specimens and tissue from other snappers were available at SAIAB and these were included in the study, while some of the samples were borrowed from other institutions or obtained from project collaborators.

2.2. Genetic data generation

2.2.1. DNA extraction

Total DNA was extracted from fin clips or tissue following the protocol of the Promega DNA Purification Kit (Madison, New York). Extracted DNA was visualised on 1% agarose gel stained with ethidium bromide under ultraviolet light (UVP Transilluminator) to verify the quality and quantity of extracted DNA against a molecular weight marker. DNA extraction aliquots were labelled and stored in a -20°C freezer until further analysis.

2.2.2. Polymerase Chain Reaction (PCR)

The PCR technique involves generating molecules of DNA from a single molecule in just a few hours, creating billions of amplicons (Mullis, 1990). For the study of regional genetic differentiation (Chapters 3 & 4) in *L. fulviflamma*, *L. bohar* and *L. lutjanus*, PCR amplification of three gene fragments (Cytochrome *b*, NADH-2 and S7 intron 1) was accomplished using universal primers (Table 2.1). Data for examining the phylogenetic relationships of these WIO snappers (Chapter 5) was gathered by amplification of two genes (16S and COII: Table 2.1). Each PCR reaction was made up to the total volume of 25 µL, consisting of 1X buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 mM of each forward and reverse primer, 1 U Taq polymerase (Southern Cross Biotechnology, South Africa), 6 µL of DNA template (*ca*. 100 ng) and DNA-free water to fill the tube to the total volume. Amplification was performed in either a Master-Cycler Gradient (Eppendorf) or a MBS Satellite 0.2G (Thermo-Hybaid) thermal-cycler. A negative control was included with every PCR run and all PCR products were visualized on 1% agarose gel stained with ethidium bromide to verify the successful amplification of the target region by comparing these to a molecular weight marker of known size.

Table 2.1. Primers used for PCR and cycle sequencing. Only the forward primers (indicated with an asterix) were used for cycle sequencing.

Gene fragment	Primer	Primer sequence (5' – 3')	Source
Mitochondrial genes			
Cytochrome *b*	CB12F*	TGG CAA GCC TAC GCA AAA A	Marko *et al*., 2004
	CB13R	TAT TCC GCC GAT TCA GGT AA	
NADH dehydrogenase II	ND2F*	CTA CCT GAA GAG ATC AAA AC	Kocher *et al*., 1995
	ND2R	CGC GTT TAG CTG TTA ACT AA	
16S ribosomal DNA	16SAR*	CGC CTG TTT ATC AAA AAC AT	Kocher *et al*., 1989
	16SBR	CCG GTC TGA ACT CAG ATC ACG T	
Cytochrome *c* Oxidase II	COIIF*	CAA GCC AAC CAC ATA ACC	Guo *et al*. 2007
	COIIR	TCG GGA GTC ACC AGT CTT TA	
Nuclear gene			
S7 intron 1	S7RPEX1F*	TGG CCT CTT CCT TGG CCG TC	Chow & Hazama, 1998
	S7RPEX3R	GCC TTC AGG TCA GAG TTC AT	

2.2.3. DNA sequencing

Prior to sequencing, all PCR products were purified using the QIAquick PCR purification kit (Qiagen), following the manufacturers' protocol. After purification, the products were eluted to a total volume of 25 µL and visualised on 1% agarose gel. Fluoro-labelled terminator cycle sequencing was conducted using the protocol and products of the BigDye® Terminator v. 3.1 Cycle Sequencing kit (Applied Biosystems). The reaction was made up of 1.5 µL Terminator Ready Reaction mix, 1.5 µL Sequencing buffer (5X), 0.5 µL primer (forward primers used in the PCR amplification diluted to 5 µM, see Table 2.1), 2 µL template DNA and DNA free water to fill the reaction to a total volume of 20 µL. After cycle sequencing the products were stored at -20 ºC. Cycle-sequencing products were then precipitated using an EDTA/NaOAc/EtOH protocol (Sambrook & Russell, 2001). The dried pellets were sent to the sequencing unit at Rhodes University (Grahamstown, South Africa) for analysis on an ABI 3100® Prism (Applied Biosystems) automated sequencer. Alternatively, some PCR products for the study of regional differentiation and all PCR products for the phylogenetic study were sent to Macrogen (Korea) for purification, cycle sequencing and analysis.

2.2.4. Statistical analyses

2.2.4.1. Sequence alignment

Prior to analysis, chromatograms were visualised with Chromas Lite 2.01 (Technelysium, available at http://www.technelysium.com.au/chromas_lite.html) and checked for errors and misreads. Manually-corrected sequences were examined in the multiple sequence editor Lasergene 9.0.5 (DNA Star Inc., Madison, WI) and finally aligned in ClustalX 2.0.12 (Larkin *et al*., 2007). For the nuclear S7 intron 1 data set, the sequences generated were aligned as above, with gaps retained and heterozygous positions coded using standard ambiguity coding, and then phased using Phase option (and the default parameters, using the MCMC algorithm) in DnaSP 5.10.1 (Librado & Rozas, 2009) to identify the component alleles of each individual from the ambiguities present in the sequence.

2.2.4.2. Nucleotide and haplotype diversity estimates

To address the current levels of variation and genetic diversity of snapper populations, several methods were used, as implemented in DnaSP 5.10.1. Genetic diversity was examined by calculating haplotype diversity (H_D) and nucleotide diversity (π) in each of the three species for cytochrome *b*, NADH-2 and S7 intron 1 for each locality and for the overall WIO sample. Nucleotide diversity is determined as the average weighted sequence divergence between haplotypes (Nei & Li, 1979). Haplotype diversity varies from zero to one (as a measure of frequency) and is the probability that two randomly drawn sequences will be different from one another (Nei & Tajima, 1980).

Genealogical relationships among haplotypes/alleles were reconstructed using median-joining networks (Bandelt *et al*., 1999) to examine relationships and the geographic distributions of haplotypes/alleles. These networks were constructed in Network 4.6 (Fluxus Technologies, http://www.fluxus-engineering.com/sharenet.htm). Networks accommodate non-bifurcating relationships, multiple equally-parsimonious connections and regard some haplotypes as being genealogically ancestral to others.

2.2.4.3. Population structure and Analyses of Molecular Variance (AMOVA)

Genetic differentiation among localities was examined using pairwise Φ_{ST}, an analogue to Wright's (1965) *F*-statistic (F_{ST}), using Arlequin (Schneider *et al*., 2000; Excoffier & Lischer 2010), for *Lutjanus bohar*, *L. fulviflamma* and *L. lutjanus*. F_{ST} –values are based on the frequency of haplotypes occurring in the different sampling localities, while Φ_{ST} –values are calculated based on the divergences among these haplotypes (Excoffier *et al*., 1992). These divergences can be corrected based on an appropriate model of nucleotide substitution. The model selected would be (or would be most similar to) that determined to be the most appropriate model for the data by the Akaike Information Criterion (AIC: Akaike, 1974), which evaluates competing models within ModelTest 3.7 (Posada & Crandall, 1998). The fixation index ranges from zero (no genetic differentiation) to one (complete differentiation between localities). These tests were used in determining genetic differentiation among localities for each of the three species for each gene region (Chapters 3 & 4).

Genetic variation was examined using Analysis of Molecular Variance (AMOVA: Excoffier *et al*., 1992) in Arlequin 3.5.1.2 (Excoffier & Lischer, 2010). AMOVA determines the partitioning of variation across three hierarchical levels based on the geographical distribution of haplotypes/alleles [in case of this study; it can also be based on ecology, phenotype, age (juvenile vs adults), etc.] and considering the pairwise distances between them (Milot *et al*., 2000). This was measured in terms of the *F*-statistic (Φ), which determines the correlation of haplotypic/allelic diversities at different levels of the hierarchical subdivision (Excoffier *et al*., 1992). In this study, Φ_{CT} is defined as the random permutation of all localities across regions, Φ_{SC} as permutation of individuals across localities within the same region, and Φ_{ST} as permutation of individuals across localities without regard to their original locality or region. This approach, however, requires *a priori* definition of group structure. This involves grouping localities at different hierarchical levels to obtain the arrangement that maximises the differentiation among regional groupings. Different biogeographic hypotheses (e.g. Pandolfi, 1992; Santini &Winterbottom, 2002) and the partitioning of genetic differentiation based on geography or current systems were considered to explain geographic-genetic structure and to determine which arrangement best explains the data sets. The details of the various arrangements examined are presented in the respective chapters.

2.2.4.4. Isolation by distance

Isolation by distance (Wright, 1943) was examined to determine whether geographic distance has influence on genetic differentiation among sampled localities. The statistical correlation between genetic differentiation and geographical distances among sampled localities was examined by a Mantel (1967) test. This test was performed using Mantel for Windows 1.19 (Cavalcanti, 2008), using 10 000 permutations to test for significance. Geographic distances between sampled localities were measured according to the minimum coastline distances using Google Earth™ (available at http://earth.google.com), and plotted against $\Phi_{ST}/(1-\Phi_{ST})$ as a measure of differentiation, following Rousset & Raymond's (1997) recommendations.

2.2.4.5 Historical demographic analyses

Changes in population sizes tend to leave traces in patterns of nucleotide diversity (Pereira *et al*., 2001), such that the distribution of pairwise sequence differences in a sample (mismatch

distribution) contains information on the population history (Rogers & Harpending, 1992). The mismatch distribution is a frequency histogram of all pairwise differences among all DNA sequences in a sample (Harpending, 1994). In a population that has been stationary for a long time, these mismatch distributions become ragged, while a population that has been expanding generates a smooth mismatch distribution and has a peak (Harpending, 1994). Thus, the position of a peak reflects the time of population growth, favouring a unimodal mismatch distribution (Harpending *et al*., 1993; Harpending, 1994). Population demographic history was examined by calculating mismatch distributions using pairwise differences under a constant population growth model in DnaSP 5.10.1. Harpending's (1994) raggedness index (*r*) was calculated in DnaSP to determine the smoothness of mismatch curve and the fit to the model curve expected under population growth model. A lower value for *r* is an indication of a smooth mismatch distribution, which could suggest population expansion.

Neutrality indices, such as Tajima's (1989) *D* and Fu's (1997) F_S statistics, were calculated in DnaSP 5.10.1. These tests are indicative of historical or demographic changes brought about by population declines or bottlenecks, founder events or population expansions (Tajima, 1989). A negative *D* is interpreted as a signal of purifying selection or, alternatively, as demographic expansion/population growth, while a positive *D* indicates possible balancing selection. Alternatively, a negative *D* could result from a population bottleneck. Similarly, Fu's *F* values are interpreted as signal of purifying selection or, alternatively, as demographic expansion/population growth (Tajima, 1989; Fu, 1997).

2.2.4.6. Phylogenetic analyses

Traditionally, phylogenetic trees were used to represent the historical relationships among groups of organisms or species (Hall, 2001). In this study, the phylogenetic approach was used to establish relationships among WIO localities for *L. fulviflamma* (Chapter 3), and for *L. bohar* and *L. lutjanus* (Chapter 4), and among species for the overall phylogenetic reconstruction to determine the position of WIO snappers in relation to the wider Indo-Pacific snappers (Chapter 5). These relationships were explored using unweighted parsimony and model-based likelihood approaches, such as Maximum Likelihood and Bayesian inference. Unweighted parsimony analyses were conducted in PAUP 4b10* (Swofford, 2002). Heuristic tree searches were employed to identify the most parsimonious or likely topologies. Nodal

support for relationships was determined by bootstrapping (Felsenstein, 1985). Bayesian inference analyses were performed using MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Detailed approaches followed for these analyses are outlined in the individual chapters.

2.3. Meristic and morphological variation (*Lutjanus fulviflamma*)

Traditional morphometrics or multivariate morphometrics has been described as the application of multivariate statistical analyses to sets of quantitative variables (Rohlf & Marcus, 1993; Adams *et al*., 2004). The results are expressed numerically and graphically in terms of linear combinations of the measured variables (Rohlf & Marcus, 1993). These statistical analyses typically include Discriminant Function Analysis (DFA) and Principal Component Analyses, PCA (Adams *et al*., 2004).

Morphological variation within the dory snapper in the WIO was quantified using analysis of morphometric and meristic characters by applying DFA. Although PCA was used in initial analyses, it was unable to reliably differentiate among localities and was, therefore, not presented in that chapter (Chapter 3). Bell *et al*. (1982) found that combination of these techniques provide useful perspectives, with one technique providing information lacking in the other. Successful applications of these or a similar approach in studies of fishes have been documented. Moran *et al*. (1998) used Canonical Variate Analysis (CVA) to detect geographical variation in the pink snapper *Pagrus auratus* (Sparidae) in the Shark Bay region of Western Australia. Pollar *et al*. (2007) used DFA and neural network analyses (NNA) to discriminate between different populations of *Tor tambroides* (Cyprinidae) in Southern Thailand. Furthermore, Vasconcellos *et al*. (2008) applied CVA, supplemented by genetics, to determine differences between yellow snapper populations in Brazil; genetic data did not reveal any differences, whereas morphometrics revealed north-south differentiation within this species. Based on these studies, it is clear that these procedures are reliable and can reveal morphological differences between and within species. In the current study, the results from the morphometric analyses were compared to those from the genetic data to check for concordance between the two procedures. Concordance between the two data sets would strengthen the conclusions regarding patterns of differentiation, whereas the discordance between the two data sets could mean that the species is responding to local processes and the influence that these have despite possible gene flow (Bell *et al*., 1982).

CHAPTER THREE

REGIONAL DIFFERENTIATION IN THE DORY SNAPPER *LUTJANUS FULVIFLAMMA* (Forsskål, 1775) IN THE WESTERN INDIAN OCEAN

3.1. Introduction

The dory snapper, *Lutjanus fulviflamma* (Forsskål, 1775), is widely distributed throughout the Indo-Pacific (IP) from the Arabian Gulf and Red Sea to South Africa (south to East London), east to Samoa and from Australia to the Ryukyu Islands in the West Pacific (Allen, 1985; Smith & Heemstra, 1986). *Lutjanus fulviflamma* mainly occurs inshore on coral reefs or rocky substrata to depths between 3 and 35 m (Allen, 1985), and in mangrove estuaries and over muddy bottoms (Smith & Heemstra, 1986). It is often found in large aggregations with *Lutjanus kasmira* and *Lutjanus lutjanus* (King, 1996; Grandcourt *et al*., 2006). Compared to other lutjanids, *L. fulviflamma* has a short life span (e.g., up to 55 years for *L. bohar*). Heupel *et al.* (2010) estimated the maximum age of *L. fulviflamma* to be 17 years. This is in accordance with 14 years found by Grandcourt *et al*. (2006), while Shimose and Tachihara (2005) estimated a maximum age of 24 years. This relatively short life span, rapid initial growth, early attainment of sexual maturity and high natural mortality suggest that the species may be resilient to exploitation (Grandcourt *et al*., 2006). Kaunda-Arara & Ntiba (1997) established that *L. fulviflamma* has a prolonged spawning period from November/December to April/May, releasing several batches of eggs in a single spawning season in Kenyan inshore marine waters. This spawning season coincides with the North-eastern Monsoon period when East African coastal waters are calm, favouring the survival of ichthyoplankton (Kaunda-Arara & Ntiba 1997). Fecundity ranged from 51 000 to 460 000 oocytes in fish of 17 cm to 30 cm TL, respectively (Grimes, 1987). However, the biology of the other spawning stocks in the region has not yet been established.

Lutjanids are high-value fish sought after by artisanal, recreational and commercial fisheries for consumption (Kaunda-Arara & Ntiba, 1997; Marriot & Mapstone, 2006). In Kenya, snappers are the third most abundant group of fishes caught and their aggressive nature makes them vulnerable to most fishing gears (Nzioka, 1984; Grandcourt *et al*., 2006). The juveniles

are found in shallow inshore waters and constitute 60% of the total catch in mangrove-lined creeks, which serve as nursery areas (Kaunda-Arara & Ntiba, 1997).

Little information is available on the larval dispersal, recruitment behaviour and stock structure of this species (e.g. Kaunda-Arara & Ntiba, 1997; Dorenbosch *et al*., 2006). This information is critical to understanding connectivity and the factors that influence this, for conservation and management strategies (Möller *et al*., 2011). Life-history traits (such as reproductive behaviour, dispersal potential, local recruitment and effective population sizes), oceanographic and historical features all play a vital role in shaping genetic structure in marine populations (Palumbi, 1994; Bohonak, 1999; Gonzalez *et al*., 2008). Given this complexity, spatial sampling covering a large area, as well as the analyses of multiple-loci (both mitochondrial and nuclear makers) are required to improve the chances of recovering evidence of population differentiation; this is often not possible with a single marker (Palumbi & Barker, 1994; Fujita *et al*., 2004).

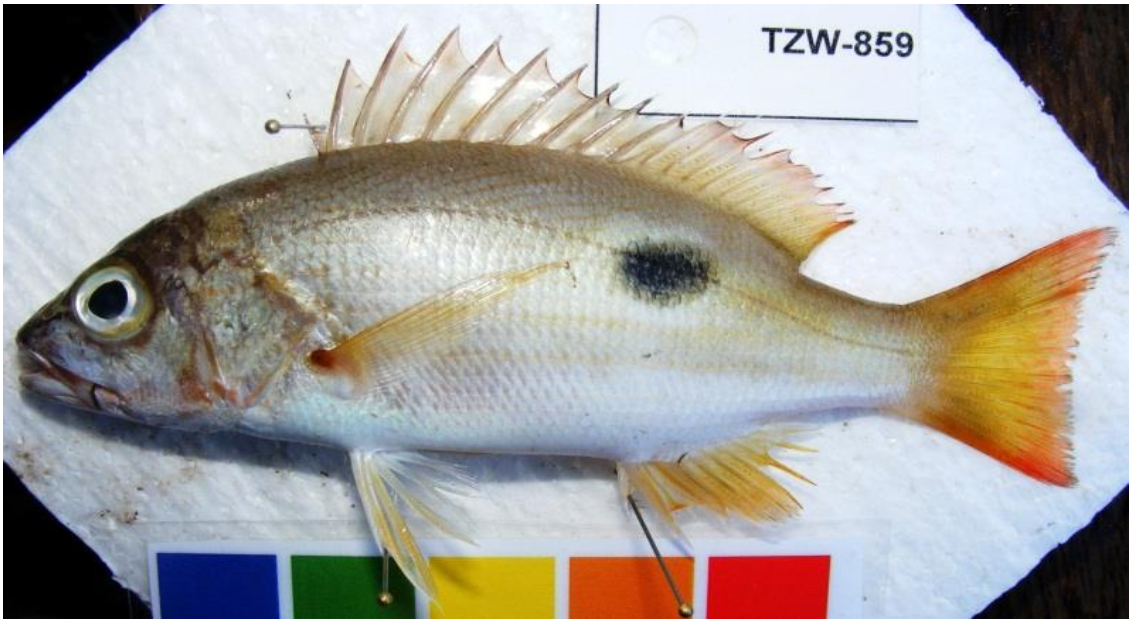
The consideration of genetic connectivity and differentiation on a historical (i.e. evolutionary or biogeographic) timescale is confounded by the fact that the biogeographic accounts of the Western Indian Ocean (WIO) have considered the WIO incidentally to the larger IP (Pandolfi, 1992; Santini & Winterbottom, 2002). This lack of knowledge exists despite the considerable diversity and endemism of various regions of the WIO (Longhurst & Pauly, 1987; Van der Elst, 1990; Branch *et al*., 1994; Randall, 1998; Turpie *et al*., 2000; Cox & Moore, 2005). The lower diversity of the WIO relative to the Indo-Malayan region has underpinned the notion that it, like other regional faunas of the IP, originated and is maintained by dispersal or migration from the Indo-Malayan region, a centre of speciation (Briggs, 1999). Evidence for dispersal is provided by some studies (Briggs, 1999; Mora *et al*., 2003). Alternatively, regional diversity could have originated by the establishment of barriers and subsequent vicariance (Pandolfi, 1992; Randall, 1998), with specific hypotheses proposed for the WIO (Pandolfi, 1992; Santini & Winterbottom, 2002). The features of the region (discussed in Chapter 1) may play an important role in species connectivity (Beldade *et al*., 2009) and the establishment of biogeographic patterns (Samyn & Tallon, 2005), and influence this diversity.

Few studies have looked at population genetic structure, genetic differentiation and/or connectivity in the WIO. Among the studies on fish, Visram *et al*. (2010a) examined genetic

connectivity of the blue barred parrotfish *Scarus ghobban*. These authors detected high gene flow, with some isolation by distance influenced by the South Equatorial Current and the Equatorial Counter Current systems. Of greatest relevance to the current study is the study by Dorenbosch *et al*. (2006). They investigated the population genetic structure of *Lutjanus fulviflamma* (77 individuals) from Tanzania, Kenya and the Comoros. They found no population structure, with low levels of genetic differentiation among populations, and concluded that the species has high genetic connectivity across the region. The shortcomings of their study were the limited spatial scale of sampling with only a few localities included, the consideration of a single marker AFLP (Amplified Fragment Length Polymorphism) and the fact that morphological differentiation was not considered. The main disadvantage of AFLPs is the difficulty in identifying homologous alleles; thus, genealogical relationships cannot be deduced with certainty (Zhang & Hewitt, 2003). In the current study, multiple DNA markers were included to test whether these reveal comparable patterns of differentiation to those found by Dorenbosch *et al*. (2006), and sampling was conducted over a broader geographic scale to determine whether the patterns these authors found (i.e., a lack of differentiation) are applicable to a much larger area.

Since *L. fulviflamma* is widespread in the region (WIO), a morphological component was included in this study to examine if patterns of genetic differentiation are reflected in morphology. The expectation is to find morphological differentiation corresponding to field observations. For example, individuals collected at Mida Creek along the Kenyan north coast (Figure 3.1: A) displayed distinctive red markings on the edges of the caudal fin, dorsal fin and the base of the pectoral fin and were paler than the characteristic bright yellow colour known from specimens collected in Tanzania (Figure 3.1: B). The snout length of the former appeared shorter and they appeared to possess a longer third dorsal spine.

A.



B.

Figure 3.1. *Lutjanus fulviflamma* (Forsskål, 1775) specimens from (A) Mida Creek, Kenya and (B) Nyama-Reef, Tanga, Tanzania (B). Note the red markings in A on the base of pectoral fin, edges of the caudal fin and at the tips of spines and rays of the dorsal fin. (Photos © SAIAB).

The aim of this study was to investigate regional differentiation in *Lutjanus fulviflamma* among the WIO localities, including representatives from Thailand and the West Pacific Islands. Genetics and morphometrics were used to examine the relationships among regions and identify the processes influencing genetic diversity. Morphological analyses can reveal the degree of adaptive differentiation that may have occurred between disjunct populations (Hermida *et al*., 2009). Although morphological differentiation may be reflected genetically, and may imply limited gene flow between localities, it is imperative to investigate the amount of genetic differentiation through more direct methods (Patterson, 1987). A combination of genetic and morphological data allows for the interpretation of patterns of variability, enabling the investigation of the source of possible inter-population variation, and contributes to the understanding of ecology and biogeography (Silva *et al*., 2010). A key underlying question, in terms of the management and conservation of this important fisheries species, is whether there is genetic connectivity or differentiation among the WIO localities.

3.2. Material and Methods

For an overview of the general methods refer to Chapter 2.

3.2.1. Sampling

Lutjanus fulviflamma specimens were collected from various localities in the WIO and peripheral localities outside the WIO (Figure 3.2). Samples for the genetic component were included from the following localities: South Africa (SA), Mozambique (MOZ), Tanzania (TAN), Kenya (KEN), Madagascar (MAD), Mauritius (MAU), Seychelles (SEY), Thailand (THA), Australia (AUS) and a West-Pacific Island (WP-Is: Tonga). Specimens for morphometric analyses (Table 3.1.) were obtained from the National Fish Collection at the South African Institute for Aquatic Biodiversity (SAIAB). These included individuals from SA, MOZ, TAN, KEN, MAR, SEY, MAD, Yemen (YEM), and China (CHI). These individuals were not necessarily those used in the genetic component. Fifty-six individuals were used for the genetic component and 89 individuals were used for the morphometric

Figure 3.2. Sampling localities for *Lutjanus fulviflamma* (Forsskål, 1775).

component. See Appendix I for the number of sampled individuals per locality and GPS co-ordinates.

3.2.2. Genetic data

Total genomic DNA was extracted and each of the three gene regions of interest, the cytochrome *b* and NADH-2 mitochondrial gene fragments and the S7 intron 1 nuclear gene fragment, were amplified by PCR, purified and sequenced. PCR conditions for the amplification of the three gene regions are detailed below (Table 3.1). Refer to Chapter 2 for details on sequence clean-up and alignment.

Table 3.1. Thermocycling regimes for the amplification of the respective mitochondrial and nuclear gene regions from *Lutjanus fulviflamma* (Forsskål, 1775).

Gene region	**PCR thermocycling profile**					
	Stage1		**Stage 2**			**Stage 3**
	Initial denaturation	**Denaturing**	**Annealing**	**Extension**	**Cycles**	**Final extension**
Cytochrome *b*	94 °C, 1 min	94 °C, 30 sec	50 °C, 1 min	72 °C, 1 min	35	72 °C, 10 min
NADH-2	94 °C, 4 min	94 °C, 45 sec	50 °C, 1 min	72 °C, 1.5 min	35	72 °C, 10 min
S7 intron 1	95 °C, 2 min	95 °C, 45 sec	53 °C, 1 min	72 °C, 1.5 min	35	72 °C, 10 min

To examine current levels of variation and genetic diversity of the dory snapper populations, several methods were used, as implemented in DnaSP 5.10.1 (Librado & Rozas, 2009). Genetic diversity was measured by calculating haplotype/allelic diversity (H_D/A_D) and nucleotide diversity (π) for each locality and for the overall sample for the cytochrome *b*, NADH-2 and S7 intron 1 gene fragments. Private haplotypes/alleles in this study are described as those restricted to a single locality.

Median-joining networks (Bandelt *et al*., 1999) were constructed using Network 4.6 (Fluxus Technologies) to examine the genealogical relationships among haplotypes/alleles and to visualise their geographic distributions.

Population differentiation among localities was examined in Arlequin 3.5.1.2 (Excoffier & Lischer, 2010). Pairwise estimates of differentiation (Φ_{ST}) were obtained for comparisons among localities. The significance of Φ_{ST} values was determined by a permutation contingency procedure using 1 000 replicates (Roff & Bentzen, 1989). These comparisons were restricted to localities with sample sizes of five or more individuals. These localities included South Africa (15 individuals), Mozambique (nine individuals), Kenya (six individuals), Red Sea (five individuals) and Thailand (five individuals). Genetic structuring among localities was examined by Analysis of Molecular Variance (AMOVA) using Arlequin. This procedure involved grouping localities based on different biogeographic hypotheses to obtain the arrangement that maximised the differentiation among regional groupings. These were based on Pandolfi's (1992) and Santini and Winterbottom's (2002) vicariant biogeographic hypotheses. Following Pandolfi's (1992) hypothesis, these regions are the Red Sea, Western-Central Indian Ocean (South Africa, Mozambique, Tanzania, Kenya, Madagascar, Seychelles and Mauritius), East Indian Ocean (Thailand), Western-Central Pacific (Australia and Tonga). Under Santini and Winterbottom's (2002) hypothesis, these six-regions were the Natal Basin (South Africa and Mozambique), Somali Basin (Tanzania and Kenya), Red Sea, Mascarene Plateau (Seychelles, Mauritius and Madagascar), Andaman Basin (Thailand) and Coral Sea (Tonga).

To test for correlation between geographic and genetic distances, isolation by distance (IBD: Wright, 1943) was examined using Mantel for Windows 1.19 (Cavalcanti, 2008) and 10 000 permutations to test for significance.

Phylogenetic relationships among individuals were determined and the relationships among regions inferred through the construction of phylogenetic trees. The out-groups used for the individual trees were different due to difficulty in amplifying some of the genes in certain species. *Lutjanus carponotatus* and *L. monostigma* were used for the cytochrome *b*, *L. kasmira* for the NADH-2 and *L. bohar* for the S7 intron 1 data sets. Each data set was analysed independently. Combined data analysis was not considered because not all individuals amplified for each of the regions. Neighbour-joining (NJ), parsimony (MP) and likelihood (ML) trees were generated in PAUP*4b10 (Swofford, 2002). Statistical support for these relationships were determined by bootstrapping (Felsenstein, 1985), using 1000, 10 000 and 100 replicates for NJ, MP and ML analysis, respectively. Bayesian inference (BI)

analyses involved four independent Markov Chain Monte Carlo (MCMC) chains running simultaneously for each data set. These chains were run over 5 000 000 generations for cytochrome *b*, 1 000 000 generations for NADH-2 and 3 500 000 generations for the S7 intron 1, and sampled every 1000th generation from the posterior probability distribution. Each analysis was run three times simultaneously to ensure convergence. To determine that the process converged, the average standard deviation of split frequecies between simultaneous analyses was monitored to ensure that it fell below the 0.05 threshold and the analyses were stopped. The first 25% of the trees were discarded as burn-in. A 50% majority-rule consensus tree was generated from the post burn-in trees of each analysis to determine the relationships and the posterior probability (support) of each node.

The cytochrome *b* data set was sufficient for examination of demographic parameters. The historical demography of *L. fulviflamma* was examined with mismatch distributions using DnaSP 5.10.1 (Librado & Rozas, 2009). Harpending's (1994) raggedness index was used to test for fit to a mismatch distribution under a population expansion model. Tajima's *D* (Tajima, 1989) and Fu's *F* (Fu, 1997) statistics were also determined in DnaSP to detect the various historical signatures of demographic change (population expansion or collapse) for the overall sample.

3.2.3. Morphometric analyses

Morphological variation within the dory snapper *Lutjanus fulviflamma* was quantified using morphometric measurements, following the procedures of Allen (1985, 1987), Allen and Talbot (1985), and Anderson (1987). Twenty-four morphological characters were measured to the nearest 0.1 mm using Vernier-callipers and recorded. Standard length (SL), fork length (FL) and total length (TL) of larger specimens were measured using a measuring tape. Six meristic counts were taken under a dissecting microscope. For most specimens, gill rakers were not well developed; therefore, rudiments were also counted. The morphometric measurements and meristic characters examined are given in Table 3.2 and illustrated in Figure 3.3.

Separate statistical analyses were conducted on morphometric and meristic data sets because morphometric data are continuous and more susceptible to environmentally-induced

variability, while meristic data are discrete and fixed early in development (Hermida *et al*., 2005; Simon *et al*., 2010). Refer to Chapter 2 for an overview of the statistical analyses.

Meristic data were not log-transformed. Counts for damaged specimens were corrected prior to analyses by replacing them with means for the respective localities. Stepwise Discriminate Function Analyses (DFA) were used to calculate the linear combinations of the morphometric and meristic variables, which maximally discriminated among the localities. To examine the extent of differentiation among localities, individuals were re-assigned to a population based on the linear combination of variables of each discriminant function. Canonical variables were then extracted from morphometric and meristic data sets, independent of the defined groups. Standard coefficients for canonical variables were extracted, and individuals were plotted according to their canonical scores along successive roots to examine differentiation among individuals and localities.

Table 3.2. Morphological measurements and meristic counts included in the morphometric analyses of dory snapper *Lutjanus fulviflamma* (Forsskål, 1775).

Character	Acronym	Description
Morphometrics		
Standard length	SL	Measurement from tip of snout to the base of the caudal fin
Fork length	FL	Measurement from tip of snout to the shortest/central portion of the tail
Total length	TL	Measurement from tip of snout to the end of the longest caudal fin lobe
Body depth	BD	Greatest depth, not including fleshy or scaly structures at fin base
Body width	BW	Width of the body at widest point
Snout length	SNL	Distance from tip of snout to anterior bony orbit of the eye
Head length	HL	Distance from tip of snout to end of opercular membrane
Orbit diameter	OD	Greatest distance between eye orbit rims
Inter-orbital width	IO	The distance across the top of the head between the eyes
Caudal peduncle length	CPL	Distance between the posterior end of the anal fin base and the base of the caudal fin rays
Caudal peduncle depth	CPD	Least depth of caudal peduncle
Upper-caudal lobe	UCL	Distance from shortest portion of forked tail to end of upper caudal lobe
Middle-caudal lobe	MCL	Distance from origin of caudal rays to middle of the shortest/central portion of the caudal fin
Dorsal fin length	DL	Length of the dorsal fin base from anterior to posterior end
Anal fin length	AL	Length of the anal fin base from anterior to posterior end
Pectoral fin length	PL	Length of the pectoral fin from the anterior to the posterior end
Pre-dorsal length	PDL	Distance between the dorsal fin origin and tip of snout
Pre-anal length	PAL	Distance between the anal fin origin and the tip of snout
Pre-pelvic length	PVL	Distance between the pelvic fin origin and the tip of snout
1st Dorsal spine length	D-I-L	Length of the first dorsal spine
2nd Dorsal spine length	D-II-L	Length of the second dorsal spine
3rd Dorsal spine length	D-III-L	Length of the third dorsal spine
4th Dorsal spine length	D-IV-L	Length of the fourth dorsal spine
Meristics		
Lateral line scales	LLS	Number of pored or tubed scales forming a sensory canal between the upper end of the gill opening and base of the caudal fin
Gill rakers, upper	UGR	Number of short protuberances of the upper gill-arch on the opposite side from the gill filaments
Gill rakers, lower	LGR	Number of protuberances of the lower gill-arch on the opposite side from the gill filaments
Dorsal spines	DS	Number of spines in the dorsal fin
Dorsal rays	DR	Number of soft rays in the dorsal fin
Pectoral rays	PR	Number of pectoral fin rays

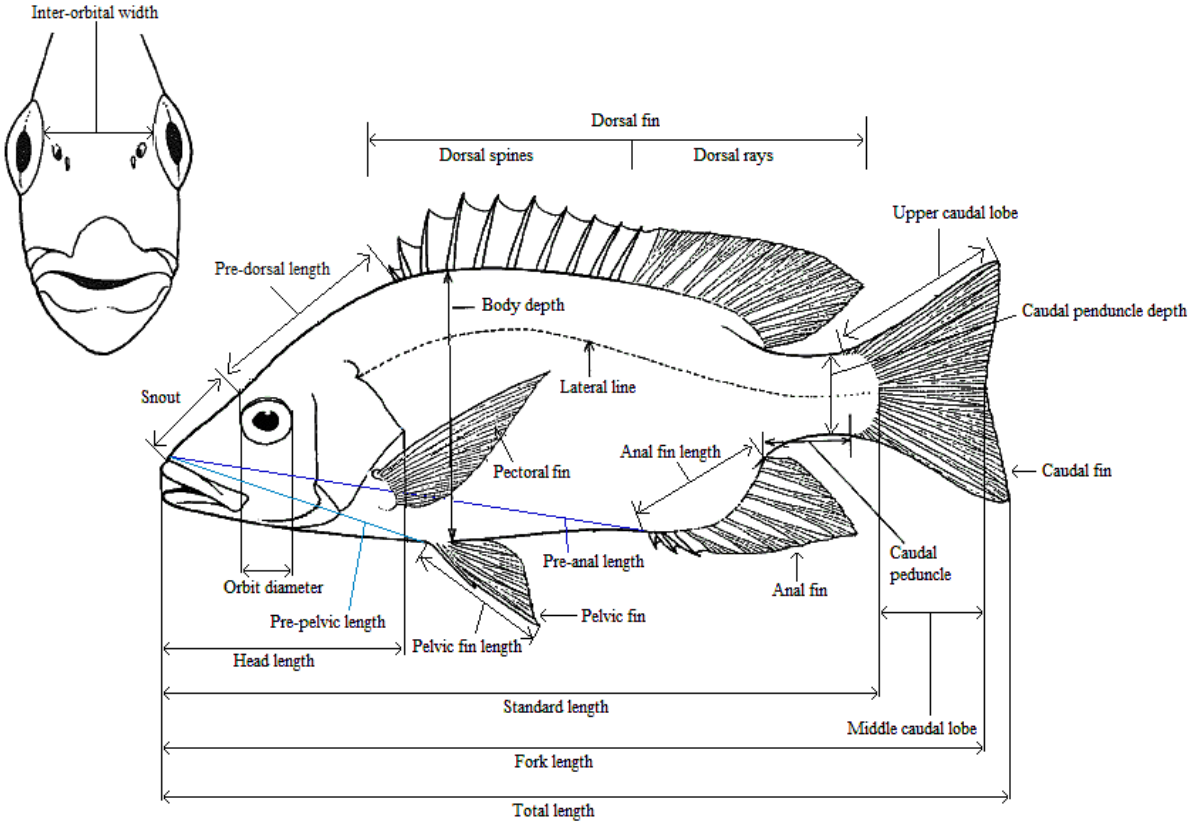


Figure 3.3. External morphology and measurements taken from *Lutjanus fulviflamma* (Forsskål, 1775) specimens. Diagram taken from Allen (1985).

3.3. Results

3.3.1. Genetic data

3.3.1.1. Cytochrome *b* analyses

The cytochrome *b* data set (636 nucleotides) included 540 sites that were conserved, 96 sites were polymorphic of which 79 were parsimony informative and 17 were autapomorphies. This data set contained 32 haplotypes from 56 individuals (Tables 3.3 & 3.4). The haplotype network (Figure 3.4) clearly showed the differentiation of individuals from the West-Pacific Island and Thailand from those of the rest of the WIO localities. Within the WIO, haplotypes H2 and H3 were present in four localities each; together these haplotypes were present in six localities. Haplotypes H13, H17 and H20 were present in two localities each. Although they

were not found in all localities, together these encompassed almost the full extent of spatial sampling in the WIO from South Africa to the Red Sea. The haplotype network was generally star-shaped with high frequency or common haplotypes (H2 and H3) having less frequent haplotypes radiating from them. Two haplotypes also appeared divergent from the main WIO network and were divergent from other haplotypes from the same locality: H18 (six mutational steps from the main network and eight mutational steps from the closest haplotype from the same locality) and H30 (four and seven mutational steps, respectively). The remaining 27 haplotypes were private. The highest proportion of private haplotypes within the WIO was found in Mauritius (4, 80%) with the lowest found in the Red Sea (1, 20%) (Table 3.3). Furthermore, many private haplotypes were found in the Mascarenes (Madagascar, Mauritius and Seychelles) and Kenya.

Haplotype (H_D) and nucleotide diversities (π) for the individual localities are presented in Table 3.3. Overall, the haplotype diversity was high, with low nucleotide diversity. Diversities for the individual localities were mostly comparable.

Table 3.3. Sample sizes (*N*) and genetic diversities at each sampling locality for the three gene regions (Cytochrome *b*, NADH-2 and S7 intron 1) examined for *Lutjanus fulviflamma* (Forsskål, 1775). Indices include number of haplotypes/alleles (N_h/N_a), the number of private haplotypes/alleles (N_{PH}/N_{PA}), haplotype diversity (H_D) or allelic diversity (A_D), and nucleotide diversity (π). Standard deviations are presented for haplotype/allelic and nucleotide diversities.

Locality	**Cytochrome *b***					**NADH-2**					**S7 intron 1**				
	N	***N_h***	***N_{PH}***	***H_D***	***π***	***N***	***N_h***	***N_{PH}***	***H_D***	***π***	***N***	***N_a***	***N_{PA}***	***A_D***	***π***
SA	15	9	7	0.924±0.044	0.004±0.003	9	6	2	0.833±0.127	0.003±0.002	14	12	8	0.978±0.035	0.017±0.009
MOZ	9	6	3	0.833±0.127	0.003±0.002	12	6	3	0.758±0.122	0.004±0.002	12	6	1	0.758±0.122	0.005±0.033
TAN	4	3	1	0.833±0.222	0.003±0.003	7	4	2	0.714±0.181	0.003±0.002	16	12	5	0.942±0.048	0.028±0.017
KEN	6	5	3	0.933±0.122	0.006±0.004	5	3	0	0.700±0.218	0.004±0.003	10	8	4	0.956±0.059	0.010±0.006
R-SEA	5	3	1	0.700±0.218	0.004±0.003	5	4	3	0.900±0.161	0.006±0.004	4	4	4	1.000±0.177	0.016±0.012
SEY	4	3	2	0.833±0.222	0.006±0.005	4	4	2	1.000±0.177	0.006±0.004	8	6	2	0.929±0.084	0.005±0.004
MAD	2	2	1	1.000±0.500	0.002±0.002						4	2	1	0.500±0.265	0.003±0.003
MAU	5	5	4	1.000±0.127	0.009±0.006	1	1	1	---	---	2	2	1	--	--
THA	5	4	4	0.900±0.161	0.002±0.002	5	3	3	0.700±0.218	0.001±0.001	2	2	1	--	--
WP-Is	1	1	1	---	---	1	1	1	---	---	2	1	1	--	--
Overall	56	32	27	0.942±0.022	0.008±0.001	49	22	17	0.906±0.002	0.009±0.002	37	38	28	0.946±0.016	0.015±0.003

Table 3.4. The distribution of cytochrome *b* haplotypes of *Lutjanus fulviflamma* (Forsskål, 1775) among South Africa (SA), Mozambique (MOZ), Tanzania (TAN), Kenya (KEN), Red Sea (R-SEA), Seychelles (SEY), Mauritius (MAU), Madagascar (MAD), Thailand (THA) and the West Pacific Island (WP-Is).

	SA	MOZ	TAN	KEN	R-SEA	SEY	MAU	MAD	THA	WP-Is	Total
H1								1			1
H2	2	1		2				1			6
H3	3	4	2		3						12
H4	1										1
H5	1										1
H6	1										1
H7			1								1
H8										1	1
H9									1		1
H10									2		2
H11									1		1
H12									1		1
H13					1		1				2
H14					1						1
H15		1									1
H16		1									1
H17		1	1								2
H18				1							1
H19				1							1
H20				1		1					2
H21	2										2
H22	3										3
H23							1				1
H24						2					2
H25	1										1
H26						1					1
H27		1									1
H28	1										1
H29				1							1
H30							1				1
H31							1				1
H32							1				1
Total	15	9	4	6	5	4	5	2	5	1	56

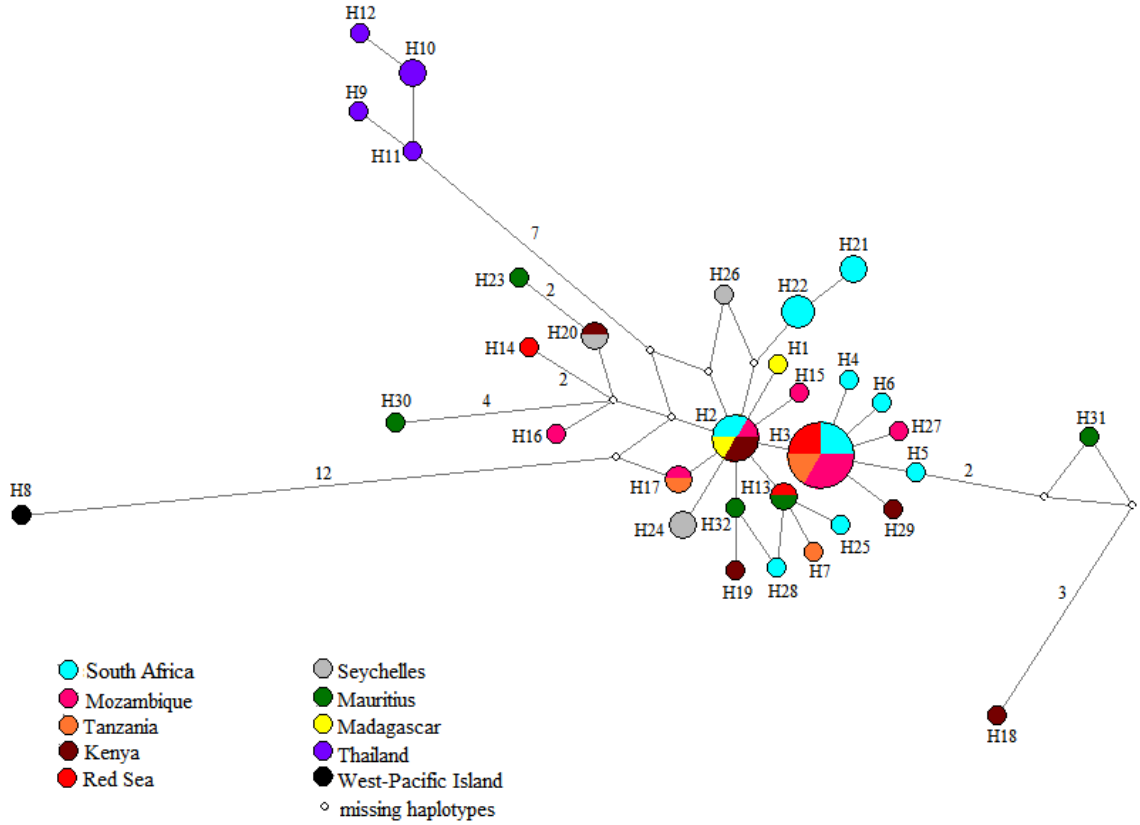


Figure 3.4. Median-joining haplotype network derived from the cytochrome *b* data for *Lutjanus fulviflamma* (Forsskål, 1775). The size of the node (haplotype) corresponds to the frequency of that haplotype and the colours represent the occurrence of that haplotype at different localities. Numbers on the branches indicate mutational differences if more than one-step.

Among-population comparisons for the cytochrome *b* were limited to localities with five or more individuals (Table 3.5). Thailand was significantly differentiated from all other WIO localities, yielding Φ_{ST} values ranging from 0.719 to 0.832 ($P < 0.05$). Mauritius was significantly differentiated from South Africa ($\Phi_{ST} = 0.223$, $P < 0.01$) and Mozambique ($\Phi_{ST} = 0175$, $P < 0.001$). Although not significant, there is evidence that South Africa shows moderate differentiation from the remaining WIO localities (Φ_{ST} values ranging from 0.033 to 0.077, $P > 0.05$). Although there was a relationship between genetic and geographic distances as determined by Mantel-test, this correlation was not significant, regardless of the distances separating the chosen localities ($r = 0.120$, $P = 0.602$: Figure 3.5).

Table 3.5. Φ_{ST} values of among-population differentiation obtained in the comparison of *Lutjanus fulviflamma* (Forsskål, 1775) representatives from South Africa (SA), Mozambique (MOZ), Kenya (KEN), Red Sea (R-SEA), Mauritius (MAU) and Thailand (THA). Significant comparisons ($P < 0.05$), as determined from permutation tests, are indicated in bold font.

	SA	MOZ	KEN	R-SEA	MAU	THA
SA	-					
MOZ	0.066	-				
KEN	0.077	0.009	-			
R-SEA	0.033	-0.073	-0.039	-		
MAU	**0.223**	**0.175**	-0.043	0.079	-	
THA	**0.802**	**0.832**	**0.759**	**0.820**	**0.719**	-

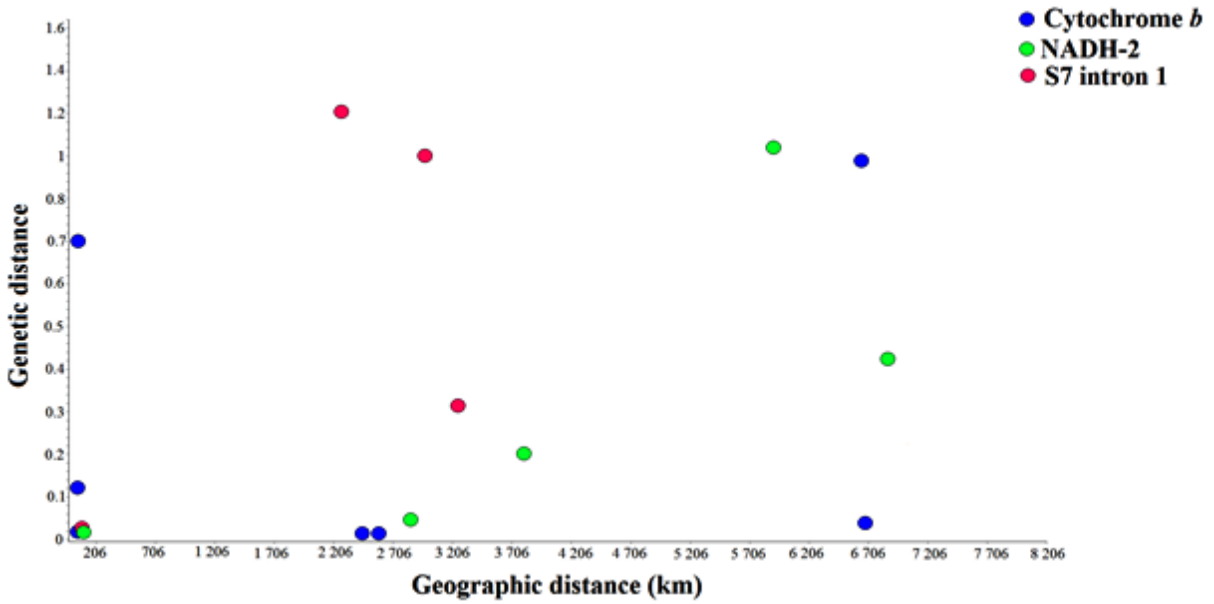


Figure 3.5. Isolation by distance graph showing pairwise genetic distance, $\Phi_{ST}/(1 - \Phi_{ST})$ for the cytochrome *b*, NADH-2 and S7 intron 1 data sets, plotted as a function of geographic distance among localities for *Lutjanus fulviflamma* (Forsskål, 1775) from South Africa, Mozambique, Tanzania, Kenya, Red Sea, Mauritius and Thailand. Mantel tests: cytochrome *b*: $r = 0.120$, $P = 0.602$; NADH-2: $r = 0.717$, $P = 0.914$ and S7 intron: 1 $r = 1.000$, $P = 0.987$.

The division of molecular variance into three components in an AMOVA allows for the estimation of the relative levels of genetic divergence that can be attributed to each level of a hierarchy. For AMOVA analyses (Table 3.6), the proportion of variation attibuted to differentiation among regions was maximised when localities werer grouped according to the biogeographic regions identified by Santini & Winterbottom (2002; see Chapter 1 and above). According to this grouping, a significant and high proportion of variation (44.76%, $P < 0.05$) observed was due to differences among regions. This was however, lower than the among-individual component (50.79%, $P < 0.001$). This suggests that most of variation for this marker is found among individuals.

Table 3.6. Results of AMOVAs examining the partitioning of differentiation at various hierarchical levels based on sequence data of the cytochrome *b*, NADH-2 and S7 intron 1 genes for *Lutjanus fulviflamma* (Forsskål, 1775). The geographical groupings were in accordance with Santini and Winterbottom's (2002) biogeographic regions. These are the Natal Basin (South African and Mozambique), Somali Basin (Tanzania and Kenya), Red Sea, Mascarene Plateau (Seychelles, Mauritius and Madagascar), Andaman Basin (Thailand) and Coral Sea (Tonga). Significant values ($P < 0.05$) from permutation tests are indicated in bold font.

Gene region	Source of variation	d.f.	Sum of squares	% variation	*P*-value
Cytochrome *b*	Among regions	5	62.071	44.76	< **0.05**
	Among localities within regions	4	8.640	4.45	0.206
	Within localities	46	66.111	50.79	< **0.001**
NADH-2	Among regions	5	91.984	68.71	< **0.001**
	Among localities within regions	3	3.329	0.00	0.781
	Within localities	40	44.340	31.29	< **0.001**
S7 intron 1	Among regions	5	24.730	3.87	0.438
	Among localities within regions	4	17.053	7.29	< **0.01**
	Within localities	64	162.285	88.84	< **0.01**

The Akaike Information Criterion (AIC) identified the most appropriate model of evolution for the dataset as HKY + G, with a gamma distribution (α) of 0.23. A transition:transversion ratio (Ti:Tv) of 4.85 and nucleotide composition (A = 0.23, C = 0.33, G = 0.15, and T = 0.29) were specified for the data set. The ML tree (-lnL = 1314.84; Figure 3.6) was constructed to examine the relationships among individuals and localities. The topologies of the strict consensus of 14 equally parsimonious trees, and the NJ and BI trees were similar to the ML tree presented. The monophyly of *L. fulviflamma* with respect to the outgroups was resolved with good statistical support (bootstraps of 100 % and posterior probability of 1.00). The West-Pacific Island individual (MA9329 WP Is) was a sister-taxon to two main clades, one including all the WIO individuals (clade A; 73/83/1.00) and the other containing the Thailand individuals (clade B; 97/99/1.00) . No regionally differentiated groups were observed within the WIO, with the exception of sub-clade I. This sub-clade was weakly supported, confined to South Africa, but did not include all the individuals from this locality.

The mismatch analysis for the cytochrome *b* data revealed a bi-modal distribution (Figure 3.7) for the entire data set. Multimodal distributions can represent populations that underwent demographic expansion, but are currently relatively stable. Harpending's (1994) raggedness index (r = 0.013) was low, indicating a good fit to the model of population expansion. Tajima's (1989) D-value was negative (D = -1.812, $P < 0.05$). The negative value of this statistic is often regarded as evidence of a historically expanding population. Similarly, Fu's (1997) F-statistic was negative and significant (F = -2.487, $P < 0.05$), indicating departure from neutrality (an excess of low frequency haplotypes).

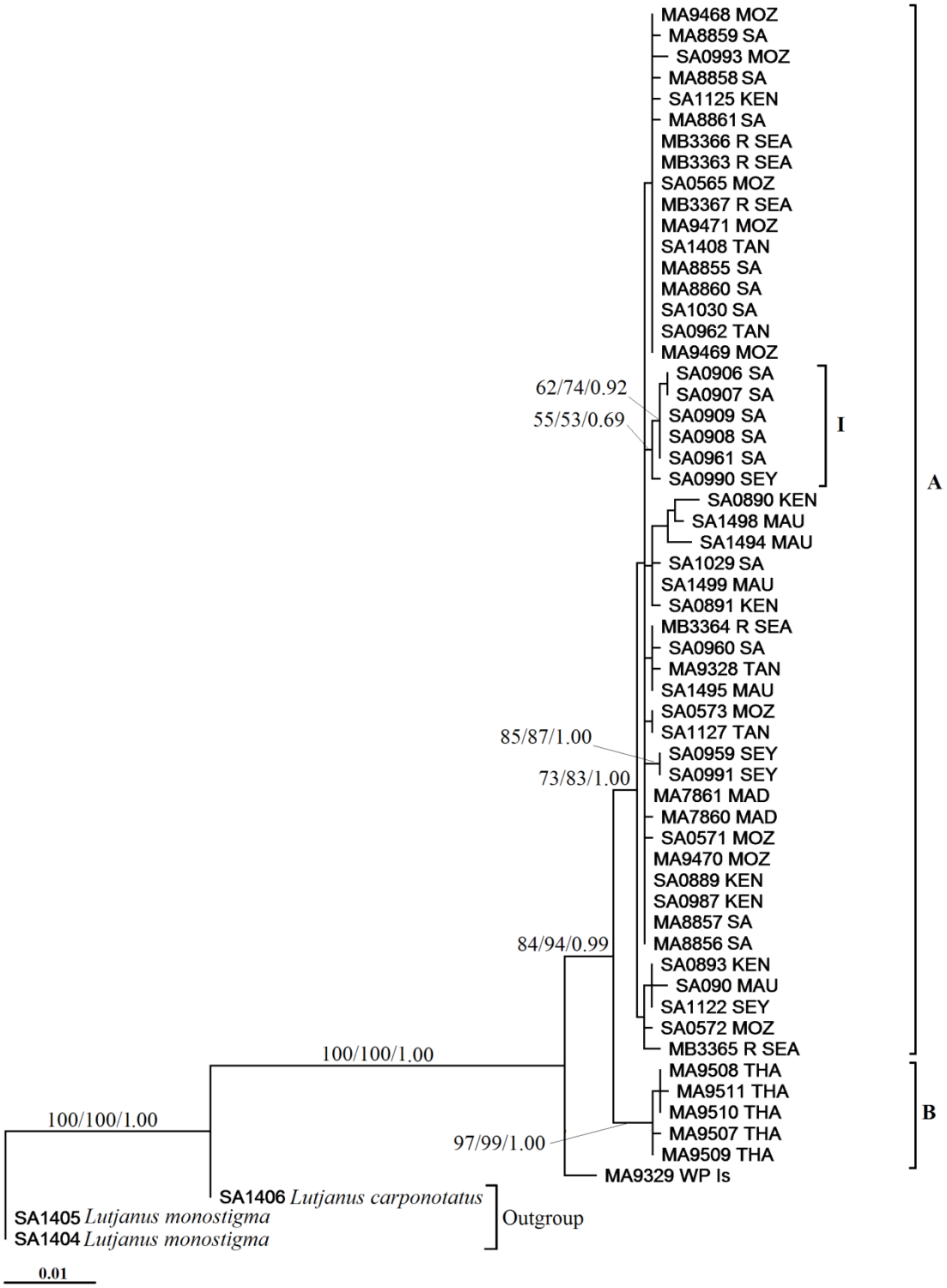


Figure 3.6. Outgroup-rooted maximum likelihood phylogram (-lnL = 1341.84) for the cytochrome *b* data depicting relationships among the sampled *Lutjanus fulviflamma* (Forsskål, 1775) individuals. Bootstrap support (MP and NJ) and Bayesian posterior probabilities respectively are indicated on branches. Localities are indicated on the terminals using the codes used previously in the text. The scale-bar indicates the estimated evolutionary distance.

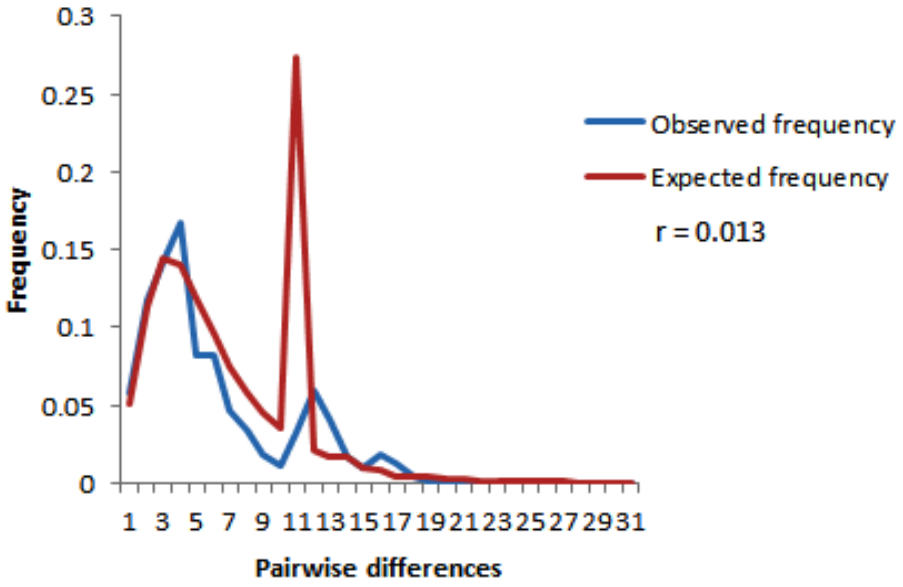


Figure 3.7. Mismatch distribution curve constructed using pairwise differences observed (blue line) among cytochrome *b* sequences of *Lutjanus fulviflamma* (Forsskål, 1775). The red curve represent the expected frequency under a model of population expansion.

3.3.1.2. NADH-2 analyses

The NADH-2 data set contained 49 sequences (633 nucleotides). Of these, 584 sites were conserved, 49 sites were polymorphic of which 21 sites were parsimony informative and 28 sites were autapomorphies. These sequences contained a total of 22 different haplotypes (Table 3.3 & 3.7). The haplotype network (Figure 3.8) clearly showed differentiation of the West-Pacific Island and Thailand individuals from the rest of the WIO localities, as with the cytochrome *b* data. Five haplotypes (H1, H2, H6, H7 and H18) were shared among localities and together they encompassed almost full extent of sampling from South Africa to the Red Sea. The common WIO haplotypes appeared to show a general star-like pattern with less-frequent haplotypes radiating from them. The remaining 17 haplotypes were private. Within the WIO, of those localities with multiple individuas and haplotypes, the highest proportions of private haplotypes were found in the Red Sea (3; 60%) and Seychelles (2; 50%). No private haplotypes were found in Kenya (Table 3.3).

Haplotype diversities (H_D) and nucleotide diversities (π) for the individual localities are presented in Table 3.3. Overall H_D was high (0.906±0.002) and π was low (0.009±0.002).

This H_D is marginally lower than that of cytochrome *b*, with π being marginally higher. The diversity values for each locality were marginally lower, but with π for some of the localities being a little higher, than those of cytochrome *b*.

Table 3.7. The distribution of NADH-2 haplotypes of *Lutjanus fulviflamma* (Forsskål, 1775) from South Africa (SA), Mozambique (MOZ), Tanzania (TAN), Kenya (KEN), Red Sea (R-SEA, Seychelles (SEY), Mauritius (MAU), Thailand (THA) and the West Pacific Island (WP-Is).

	SA	MOZ	TAN	KEN	R-SEA	SEY	MAU	THA	WP-Is	Overall
H1	1	2	4	3						10
H2	1		1		1					3
H3			1							1
H4						1				1
H5		1								1
H6	1					1				2
H7	4	6		1						11
H8	1									1
H9	1									1
H10		1								1
H11									1	1
H12								3		3
H13								1		1
H14								1		1
H15					2					2
H16					1					1
H17					1					1
H18		1		1		1				3
H19							1			1
H20		1								1
H21						1				1
H22			1							1
Total	9	12	7	5	5	4	1	5	1	49

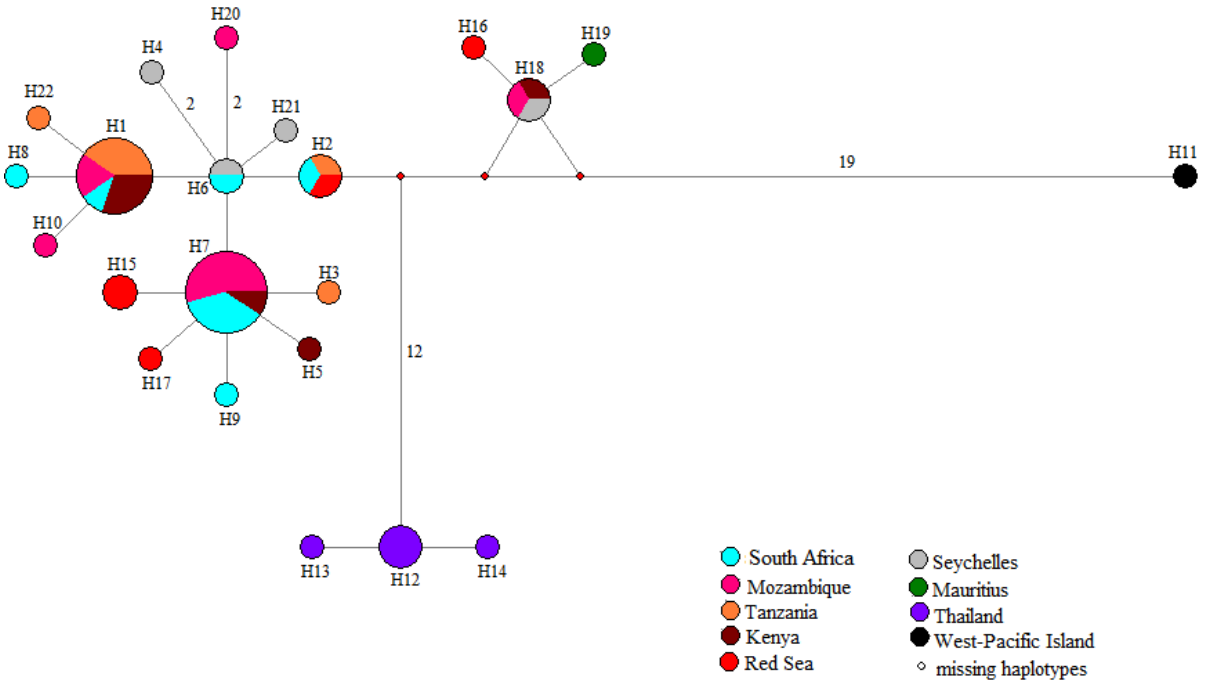


Figure 3.8. The median-joining haplotype network derived from the NADH-2 data for *Lutjanus fulviflamma* (Forsskål, 1775). The size of the node (haplotype) corresponds to the frequency of that haplotype and the colours represent the occurrence of that haplotype at different localities. Numbers on the branches indicates mutational differences if more than one-step.

Among-population comparisons for NADH-2 fragment were also limited to those localities with five or more individuals. These were South Africa (nine individuals), Mozambique (12 individuals), Tanzania (seven individuals), Kenya (five individuals), Red-Sea (five individuals) and Thailand (five individuals). From this analysis, Thailand was significantly differentiated from the WIO localities with Φ_{ST}-values ranging 0.853 to 0.916, $P < 0.01$ (Table 3.8.). Significant differentiation was also observed between Tanzania and the Red Sea ($\Phi_{ST} = 0.233$, $P < 0.05$). Although not significant, $P > 0.05$, there appears to be moderate differentiation between SA (Φ_{ST} values ranging from 0.081 to 0184) and the other WIO localities (except Mozambique). These observations are consistent with the cytochrome *b* results. The Mantel test identified no significant relationship ($r = 0.717$, $P = 0.914$) between genetic and geographic distances (Figure 3.5).

Table 3.8. Φ_{ST} values of among-population differentiation obtained in the comparison of *Lutjanus fulviflamma* (Forsskål, 1775) individuals from South Africa (SA), Mozambique (MOZ), Tanzania (TAN), Kenya (KEN), Red Sea (R-SEA) and Thailand (THA) for the NADH-2 data. The significant Φ_{ST} estimates ($P < 0.05$), as determined from permutation tests, are indicated in bold font.

	SA	**MOZ**	**TAN**	**KEN**	**R-SEA**	**THA**
	-					
SA						
MOZ	-0.052	-				
TAN	0.184	0.117	-			
KEN	0.094	0.007	-0.089	-		
R-SEA	0.081	0.033	**0.233**	0.047	-	
THA	**0.912**	**0.881**	**0.916**	**0.888**	**0.853**	-

The same six biogeographic regions defined for the cytochrome *b* data set were defined for the NADH-2 data set. A large and significant component of variation was due to differentiation among regions (68.71%, $P < 0.001$) and a lower component of variation was due to differentiation within-localities (31.29%, $P < 0.001$) (Table 3.6.). This finding is slightly different to that found with the cytochrome *b* data where most of the variation was found among individuals.

The NADH-2 data set (633 nucleotides) included 49 polymorphic sites; 22 of these were parsimony informative and there were 27 autopomorphies. The Akaike Information Criterion (AIC) selected TrN + I as the best evolutionary model for the data set. A nucleotide composition (A = 0.27, C = 0.35, G = 0.14, and T = 0.24), nucleotide substitution rate matrix of A↔C = A↔T = C↔G = G↔T = 1.00, A↔G =10.63 and C↔T = 3.55, and a proportion of invariable sites ($I = 0.71$) were determined for the data. The ML phylogram depicting relationships among individuals is presented in Figure 3.9 (to ensure consistency with cytochrome *b* data). Unlike cytochrome *b* data, the monophyly of *L. fulviflamma* was statistically not supported with respect to the outgroup, *L. kasmira*. In this tree, a Thailand and WP-Island clade were nested within the WIO clade, but their placement was not supported statistically. However, the branch lengths leading to these individuals were longer than those for the WIO individuls. This observation is in agreement with the haplotype network and

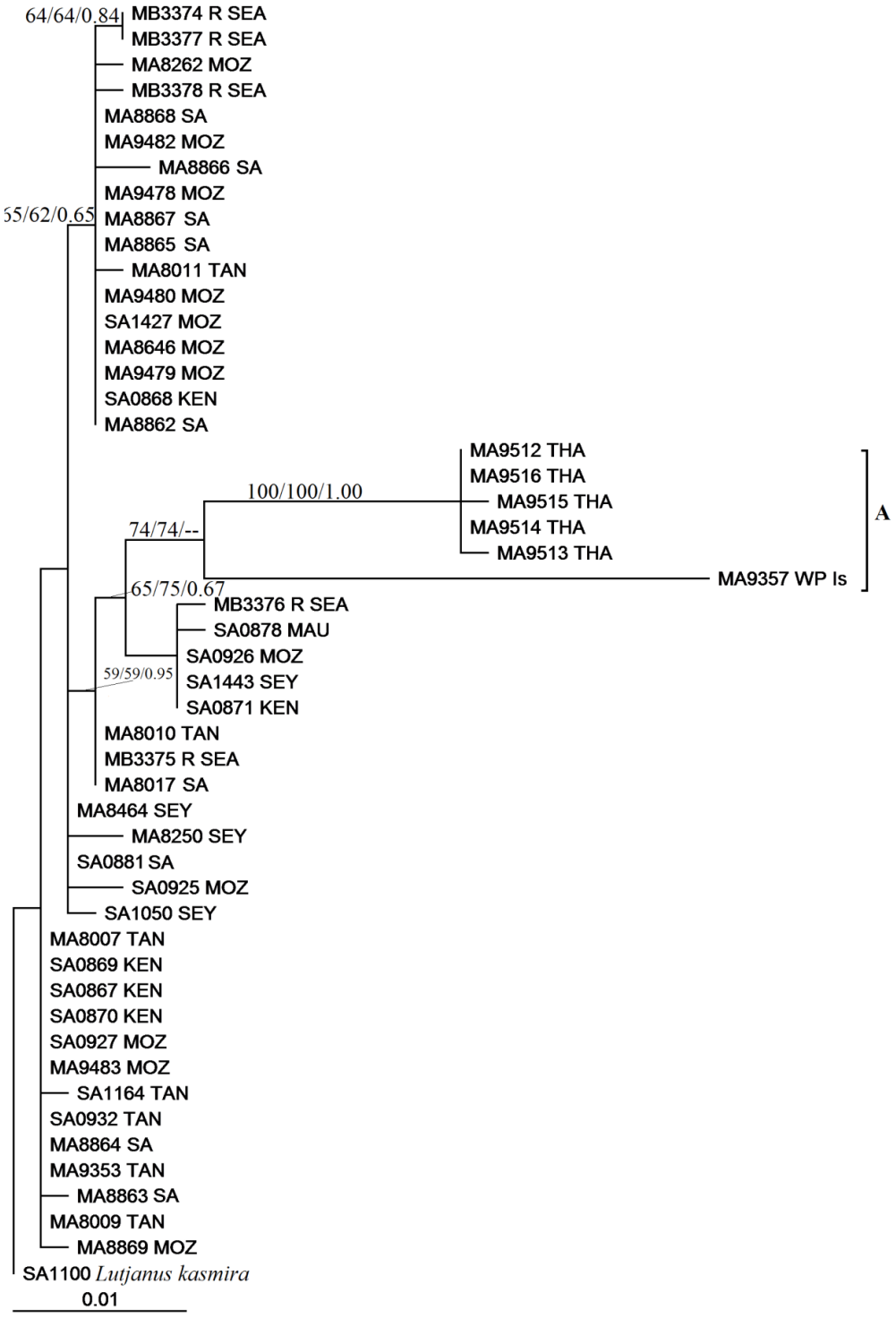


Figure 3.9. Maximum likelihood phylogram (-lnL = 1120.18) derived from NADH-2 data from *Lutjanus fulviflamma* (Forsskål, 1775) individuals, depicting relationships among the WIO localities. *Lutjanus kasmira* (SA1100) is included as an outgroup. Nodal support (bootstrap % for MP and NJ, and BI posterior probabilities, respectively) are indicated on the branches. The scale bar indicates the estimated evolutionary distance.

supports the view that these individuals are divergent from the WIO individuals. No obvious spatial differentiation was observed among WIO localities.

3.3.1.3. S7 intron 1 analyses

The phased S7 intron 1 data set (345 nucleotides, 37 individuals) contained 261 conserved sites, 87 variable sites of which 64 sites were parsimony informative and 23 sites were autapomorphies. This data set contained a total of 38 alleles (Table 3.9). The allele network (Figure 3.10) showed no differentation of the alleles from the West Pacific Islands and Thailand from those from the WIO, disagreeing with the results found using mitochondrial markers. Overall, mitochondrial markers showed differentiation among the WIO and the WP-Island individual, and of Thailand from the WIO, but the nuclear marker showed a lack of differention among the same regions. Although the allele network (Figure 3.10) was complex, three alleles (A2, A5, A10) occurred at a higher frequency and were shared among localities. Together, these alleles occurred across the full extent of spatial sampling in the WIO, with the exception of the Red Sea. Less abundant alleles radiated from these common alleles. Alleles A11, A18, A26 and A34 were shared between proximate localities, while A4, A13 and A30 were shared between geographically-distant localities. Alleles A6 to A9 from Tanzania were very divergent from all other alleles and formed a distinct clade. A16, A17 and A 23 were divergent from other alleles of the same localities (South Africa and the Red Sea) and all other alleles in the region. The highest proportion of private alleles was found in the Red Sea (4, 100%), followed by South Africa (8, 57.14%), and the lowest was found in Mozambique (1, 8.33%) (Table 3.3).

Overall allelic diversity (A_D= 0.946±0.016) and nucleotide diversity were high (π = 0.015±0.003). The diversity values for the individual localities were also high, mostly comparable to each other, and marginally higher than the mitochondrial markers (Table 3.3). In summary, overall allelic/haplotype diversities were high across the markers, with S7 intron 1 (0.946±0.016) being marginally higher than cytochrome *b* (0.942±0.022) and NADH-2 (0.906±0.002). Although π were generally low for mitochondrial markers and highest in S7 intron 1 (0.015±0.003), they were lower in cytochrome *b* (0.008±0.001) than in NADH-2 (0.009±0.002).

Table 3.9. The distribution of S7 intron 1 alleles of *Lutjanus fulviflamma* (Forsskål, 1775) among South Africa (SA), Mozambique (MOZ), Tanzania (TAN), Kenya (KEN), Red Sea (R-SEA), Seychelles (SEY), Madagascar (MAD), Mauritius (MAU), Thailand (THA) and West Pacific Island (WP-Is).

	SA	MOZ	TAN	KEN	R-SEA	SEY	MAD	MAU	THA	WP-Is	Overall
A1				1							1
A2		6	2	2		1	3				14
A3	2										2
A4	1		1								2
A5			4	2		1					7
A6			1								1
A7			1								1
A8			1								1
A9			1								1
A10	2	1				2		1	1		7
A11		1	1								2
A12	1										1
A13	1			1							2
A14	1										1
A15	1										1
A16	1										1
A17	1										1
A18	1	2									3
A19									1		1
A20					1						1
A21					1						1
A22					1						1
A23					1						1
A24		1									1
A25				1							1
A26			1	1							2
A27				1							1
A28				1							1
A29						1					1
A30			1			2					3
A31	1										1
A32	1										1
A33								1			1
A34		1	1								2
A35						1					1
A36							1				1
A37										2	2
A38			1								1
Total	14	12	16	10	4	8	4	2	2	2	74

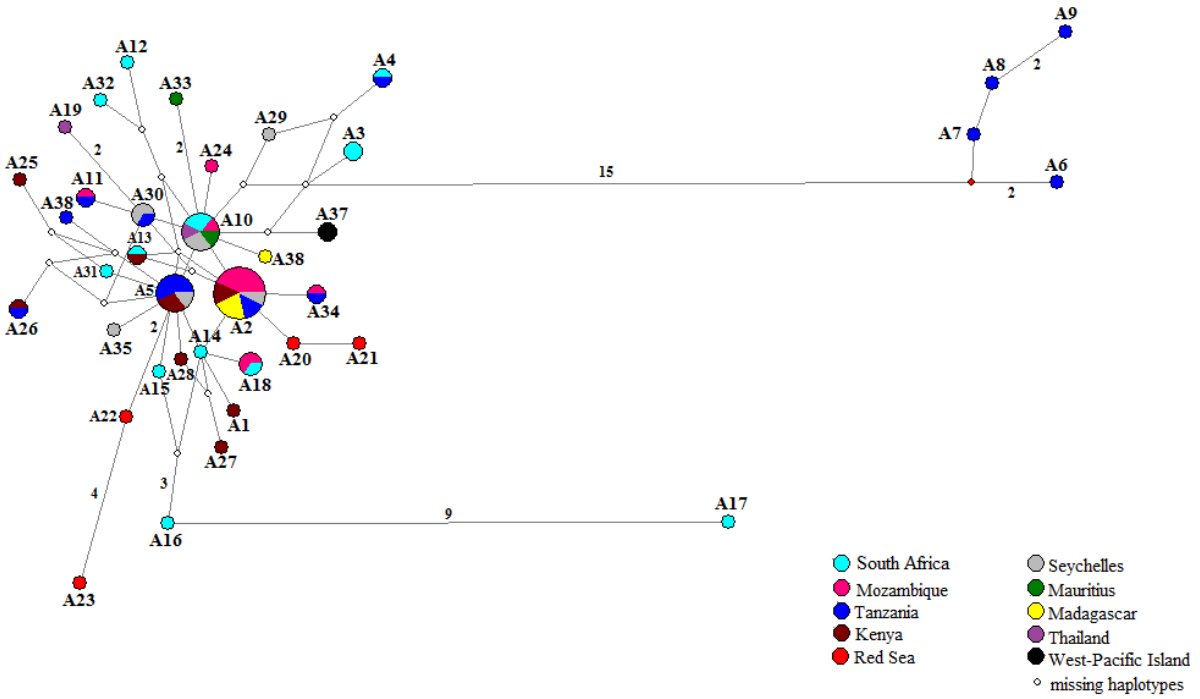


Figure 3.10. Median-joining allele network derived from the S7 intron 1 data set for *Lutjanus fulviflamma* (Forsskål, 1775) alleles. The size of the node (allele) corresponds to the frequency of that allele and the colours represent the occurrence of the allele at different localities. Numbers on the branches indicates mutational differences between alleles where more than one mutational step.

Only four localities within the WIO had sufficient individuals (five or more) to test for population differentiation (Table 3.10). These were South Africa (seven individuals), Mozambique (six individuals), Tanzania (eight individuals), and Kenya (five individuals). Unlike the mitochondrial markers, S7 intron 1 revealed the differentiation of Mozambique from all other localities in the region with Φ_{ST}-values ranging from 0.074 to 0.154, $P < 0.05$. Other comparisons, although they indicated moderate differentiation among the localities considered, particularly Tanzania, were not significant ($P > 0.05$). This could have been influenced by the sample size. Similar to the mitochondrial markers, no significant relationship existed between genetic and geographic distances ($r = 1000$, $P = 0.987$), as determined by a Mantel test.

The same six biogeographic regions (Natal Basin, Somali Basin, Red Sea, Mascarene Plateau, Andaman Basin and Coral Sea groupings) were examined to determine the division of molecular variance (Table 3.6). In contrast to mitochondrial markers, a low (and non-significant) component of variation was due to differentiation among regions (3.78%, $P > 0.05$), with a substantially large and significant component of variation (88.84%, $P < 0.01$) associated with differentiation among individuals.

Table 3.10. Φ_{ST} values of among population differentiation obtained in the comparison of *Lutjanus fulviflamma* (Forsskål, 1775) individuals from South Africa (SA), Mozambique (MOZ), Tanzania (TAN) and Kenya (KEN) for the S7 intron 1 data. Significant Φ_{ST} estimates ($P < 0.05$), as determined from permutation tests, are indicated in bold font.

	SA	**MOZ**	**TAN**	**KEN**
	-			
SA				
MOZ	**0.074**	-		
TAN	0.114	**0.154**	-	
KEN	0.032	**0.126**	0.111	-

The S7 intron 1 data contained 60 polymorphic sites, of which 40 were parsimony informative and 20 were autopomorphies. The Akaike Information Criterion (AIC) identified the most appropriate substitution model for the data set as K81uf + G, with a gamma distribution (α) of 0.67, a nucleotide composition of A = 0.25, C = 0.17, G = 0.28 and T = 0.29, and a nucleotide substitution rate matrix of A↔C = G↔T = 1.00, A↔G = C↔T = 2.83 and A↔T = C↔G = 1.91. In the ML phylogram presented (Figure 3.11), *L. fulviflamma* was statistically supported (100/100/1.00 for MP, NJ and BI) with respect to the outgroup, *L. bohar*. The tree indicated, in agreement with the haplotype network, the presence of two main clades: clade A containing most of WIO alleles, with the West Pacific Island alleles nested within this clade, and the other (Clade B) containing Tanzanian alleles. The latter did not include all alleles from this locality. Both clades were generally well supported by certain

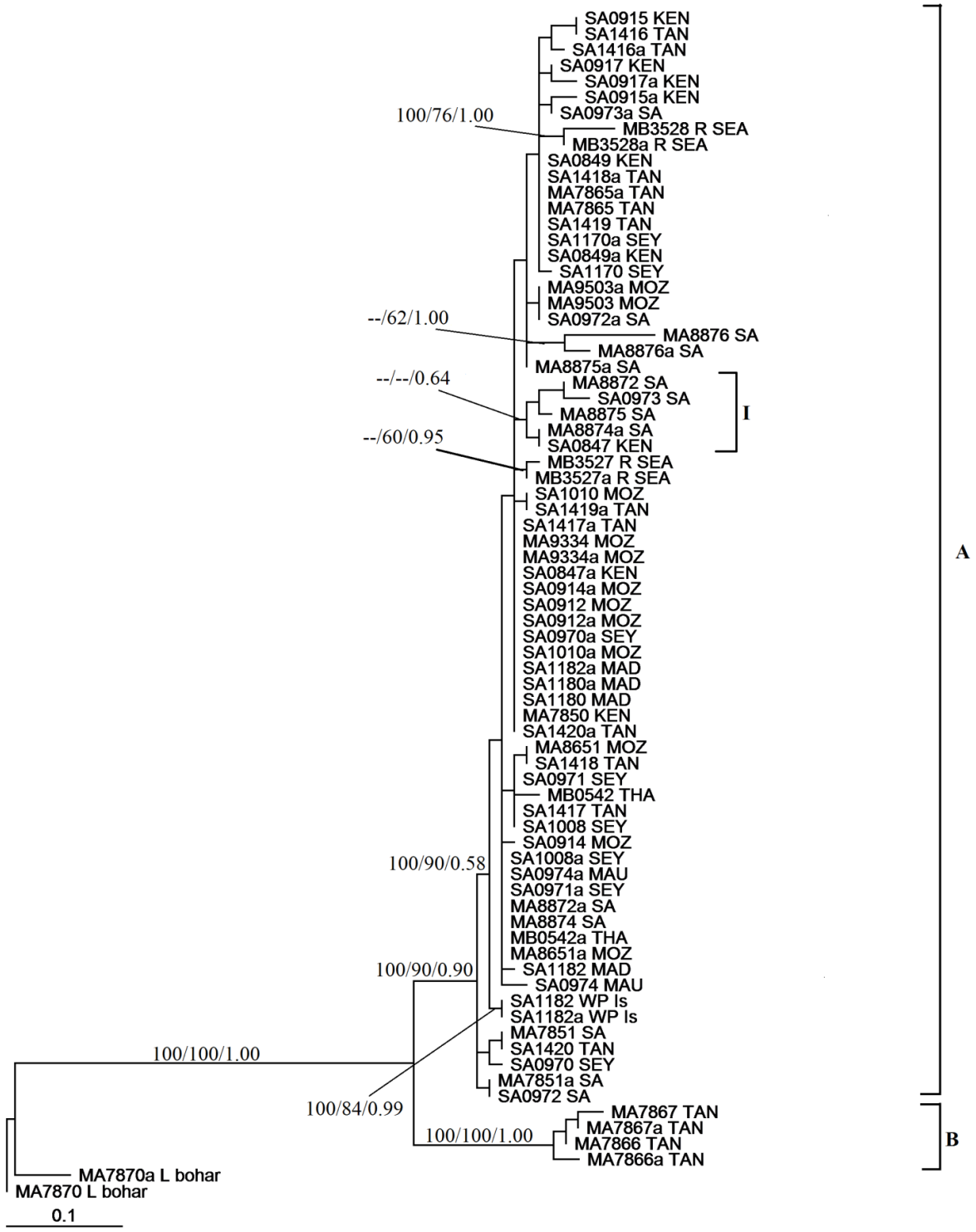


Figure 3.11. Outgroup-rooted ML phylogram (-lnL = 865.14) for the S7 intron 1 data showing relationships among *Lutjanus fulviflamma* (Forsskål, 1775) alleles. Alleles of *L. bohar* (MA7870a and MA7870) were used as outgroups. Nodal support (bootstrap % for MP and NJ, and BI posterior probabilities) are indicated on the nodes. The scale bar indicates the estimated evolutionary distance.

analyses. No regionally differentiated clades were observed within clade A, with the exception of sub-clade I from South Africa. These alleles were from the same individuals that formed a sub-clade in the cytochrome *b* tree. This sub-clade did not include all the alleles from this locality and was poorly supported. Few relationships among other alleles were supported across all analyses.

3.4. Morphometric analyses

Discriminant Function Analysis (DFA) was used to examine differentiation among *Lutjanus fulviflamma* from nine localities across the WIO and peripheral areas, using data from 89 individuals. These were South Africa (33 individuals), Mozambique (16 individuals), Tanzania (11 individuals), Kenya (two individuals), Mauritius (three individuals), Seychelles (14 individuals), Yemen (two individuals), China (two individuals) and Comoros (one individual). Of the 24 morphometric characters available, 17 contributed significantly (Wilks' Lambda: 0.175, $F = 2.579$, $P < 0.000$) to the classification functions that discriminated among individuals from various localities. These were SL, FL, TL, BD, BW, SNL, HL, OD, IO, CPL, CPD, UCL, MCL, DL, AL, PL, VL, PDL, PAL, PVL, D-I-L, D-II-L, D-III-L and D-IV-L (see Table 3.2.). Eight variables (BW, HL, SL, AL, MCL, PVL, D-II-L and D-IV-L) contributed significantly to the variation among individuals and accounted for 88.89% of the variation present in the data set. The re-classification of individuals (Table 3.11) to localities was conducted according to the classification functions for each locality and 50 to 100 %, with an overall of 71.59 %, of individuals were correctly reclassified to their locality of origin. This indicates some degree of morphological differentiation among the localities. These canonical variables were extracted from the morphological measurements. The percentage variation explained by each and the Eigen-values are listed in Table 3.12. The first three canonical variables had an Eigen-value greater than (or equal to) one, which indicates that the combination of variables accounted for more of the overall variation than each variable did independently.

Table 3.11. Classification matrix of *Lutjanus fulviflamma* (Forsskål, 1775) using morphometric characters according to the discriminant functions for the nine sampled localities. The rows present the source locality and the columns present the locality to which the individuals are classified. Localities: Yemen (YEM), Mauritius (MAU), Seychelles (SEY), Madagascar (MAD), Mozambique (MOZ), Kenya (KEN), Tanzania (TAN), South Africa (SA) and China (CHI).

Locality	% Correct	YEM *P*=0.02	MAU *P*=0.03	SEY *P*=0.16	MAD *P*=0.34	MOZ *P*=0.18	KEN *P*=0.03	TAN *P*=0.13	SA *P*=0.39	CHI *P*=0.02
YEM	100.00	2	0	0	0	0	0	0	0	0
MAU	66.67	0	2	1	0	0	0	0	0	0
SEY	57.14	0	0	8	0	2	0	0	4	0
MAD	100.00	0	0	0	3	0	0	0	0	0
MOZ	50.00	0	0	2	0	8	0	0	6	0
KEN	66.67	0	0	0	0	1	2	0	0	0
TAN	72.73	0	0	0	0	1	0	8	2	0
SA	82.35	0	0	1	0	4	0	1	28	0
CHI	100.00	0	0	0	0	0	0	0	0	2
Total	71.59	2	2	12	3	16	2	9	40	2

Table 3.12. Relative contributions of variation and Eigen-values of the canonical variables calculated from the discriminant functions analysis using 17 morphological variables for *Lutjanus fulviflamma* (Forsskål, 1775) individuals.

Canonical variable	Cum. % proportion	Overall %	Eigen-value
Canonical root 1	30.82	30.82	1.819
Canonical root 2	52.28	21.46	1.266
Canonical root 3	69.69	17.41	1.028
Canonical root 4	81.21	11.52	0.680
Canonical root 5	88.34	7.13	0.420
Canonical root 6	93.18	4.84	0.286
Canonical root 7	97.53	4.35	0.257
Canonical root 8	100.00	2.47	0.146

Although canonical variable 1 (canonical root 1) accounted for the highest overall percentage of variation, it was not used for plotting and considering scores and differentiation among individuals from different localities because standard length (SL, r = 4.095) contributed most to this root. Therefore, individuals were plotted two-dimensionally according to their scores along the second and third canonical variables to determine the extent of differentiation among localities (Figure 3.12). A large central cluster was observed with individuals from South Africa, Mozambique, and Mauritius overlapping, reflecting no differentiation among these localities. There was some differentiation of individuals from Kenya, Madagascar, Tanzania, Yemen and China from this central cluster along the 2nd canonical root; this indicated greater anal and pectoral fin lengths, body depth and head length contributing to the

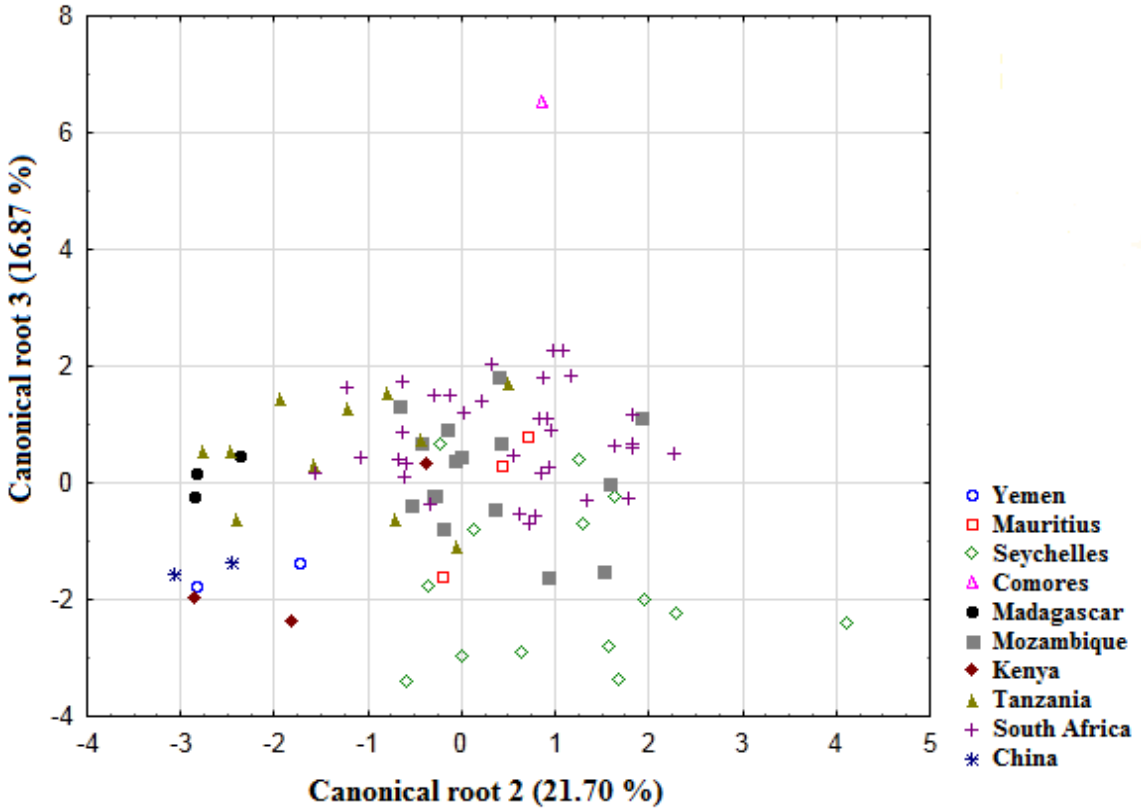


Figure 3.12. Scatter-plot of individuals according to scores along the second and third canonical variables from the discriminant function analyses of 24 morphometric variables from *Lutjanus fulviflamma* (Forsskål, 1775).

differentiation. Among these populations, China, Kenya and Yemen were separated from Madagascar and Tanzania along canonical root 3. Greater body depth, head length, anal length, fork length and a longer third dorsal spine influenced this separation. These characters further drive the separation of a number of Seychelles individuals from the central cluster. The individual from the Comoros was also differentiated from all other localities along canonical root 3. Overall, the results indicate a slight degree of regional morphological differentiation in *L. fulviflamma*.

Most of the meristic counts did not vary much between localities (Table 3.13) and were, thus, not expected to reveal much differentiation among localities when subjected to multivariate statistical analysis. Nonetheless, the DFA were applied on six meristic counts: LLS, UGR, LGR, DS, DR and PR (Table 3.2). Five counts (LLS, UGR, DR, DS and PR) (Wilks' Lambda: 0.366, $F = 2.139$, $P < 0.0002$) contributed significantly to the discrimination among

Table 3.13. Number of individuals (*N*) of *Lutjanus fulviflamma* (Forsskål, 1775) from which meristic data were taken, with median and range counts for each meristic count for each locality sampled (South Africa, Mozambique, Tanzania, Kenya, Comoros, Madagascar, Mauritius, Seychelles, Yemen and China). Abbreviations: LLS (lateral line scales), UGR (upper gill rakers), LGR (lower gill rakers), DS (dorsal spines), DR (dorsal rays) and PR (pectoral rays).

Locality	***N***	**Median, range**					
		LLS	**UGR**	**LGR**	**DS**	**DR**	**PR**
South Africa	34	46, 43-49	6, 6-7	8, 7-10	10	14, 13-14	15, 14-15
Mozambique	16	46, 43-49	7, 6-7	8, 7-11	10	13, 13-14	14, 13-15
Tanzania	11	46, 44-49	6, 6-7	8, 7-10	10	14, 13-14	15
Kenya	3	44, 44-46	6, 6-7	8, 7-9	10	13, 12-13	14, 13-14
Comoros	1	48	7	8	10	14	13
Madagascar	3	47, 45-47	7, 6-7	8, 7-8	10	14, 13-14	14
Mauritius	3	45, 44-50	7	8	10	14, 14-15	14, 14-17
Seychelles	14	44, 43-49	6, 6-7	8, 7-10	10, 10-11	14, 12-14	14, 13-15
Yemen	2	45.5, 45-46	6	7.5, 7-8	10	13.4, 13-14	14.5, 14-15
China	2	44.5, 44-45	6.5, 6-7	7.5, 7-8	10	13.5, 13-14	14

localities. Individuals were reclassified to populations on the basis of the classification functions and the correct classification ranged from 0 to 85.29%, with an average of 53.41 % correctly assigned to their locality of origin (Table 3.14). This percentage was lower in comparison to the analysis of the morphometric characters, suggesting that individuals from most localities are not differentiated in terms of meristics. However, South African individuals were more easily recognisable in comparison to other localities (85.29% correctly assigned).

Table 3.14. Classification matrix of *Lutjanus fulviflamma* (Forsskål, 1775) individuals using meristic characters, according to the discriminant functions. Individuals were sampled from Yemen (YEM), Mauritius (MAU), Seychelles (SEY), Madagascar (MAD), Mozambique (MOZ), Kenya (KEN), Tanzania (TAN) South Africa (SA) and China (CHI). The rows present the source locality and the columns present the locality to which the individuals are classified. Locality abbreviations are the same as in Table 3.11.

Locality	**%**	**YEM**	**MAU**	**SEY**	**MAD**	**MOZ**	**KEN**	**TAN**	**SA**	**CHI**
	Correct	***P*=0.02**	***P*=0.34**	***P*=0.16**	***P*=0.03**	***P*=0.18**	***P*=0.03**	***P*=015**	***P*=0.39**	***P*=002**
YEM	0.00	0	0	0	0	0	0	0	2	0
MAU	33.33	0	1	2	0	0	0	0	0	0
SEY	50.00	0	0	7	0	1	0	0	6	0
MAD	0.00	0	0	0	0	2	0	0	1	0
MOZ	56.25	0	0	0	0	9	1	0	6	0
KEN	33.33	0	0	0	0	0	1	0	2	0
TAN	0.00	0	1	0	0	0	0	0	10	0
SA	85.29	0	0	1	0	4	0	0	29	0
CHI	0.00	0	0	1	0	0	0	0	1	0
Total	53.41	0	2	11	0	16	2	0	57	0

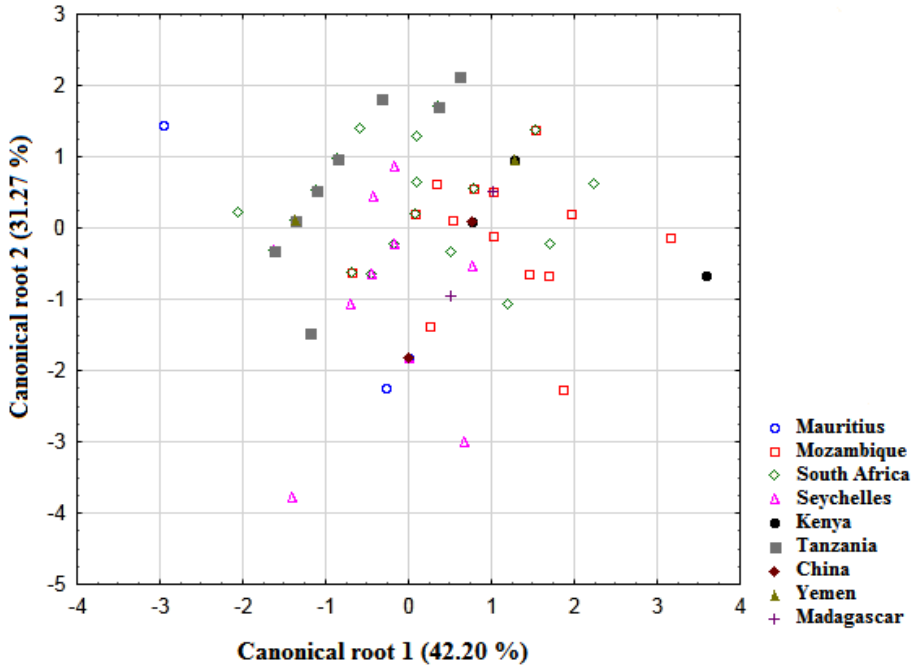
Three variables, UGR, DR and DS, contributed significantly to the canonical roots that explained differentiation among individuals. The percentage variation explained by each canonical root and Eigen-value of each root are displayed in Table 3.15. All Eigen-values were below ≤ 0.5.

Table 3.15. Relative contributions of variation and Eigen-values of canonical variables calculated from discriminant function analysis using five meristic counts taken from *Lutjanus fulviflamma* (Forsskål, 1775) individuals.

Canonical variable	Cum. % proportion	Overall %	Eigen-value
Canonical root 1	42.20	42.20	0.509
Canonical root 2	73.47	30.65	0.372
Canonical root 3	96.65	23.18	0.256
Canonical root 4	99.37	2.72	0.032
Canonical root 5	100.00	0.63	0.008

Individuals were plotted two-dimensionally along the first and second canonical roots (Figure 3.13: A) and second and third canonical roots (Figure 3.13: B). Both plots showed a central cluster of individuals from many localities and no clear separation of any localities. Some differentiation (Figure 3.13: A) was observed with some (but not all) of the individuals from the Seychelles being separated from the main cluster along the 2nd canonical root, influenced by PR and LLS, and the separation of certain individuals from Mozambique, Kenya and Mauritius along the 1st canonical root, influenced by UGR and LLS. The third canonical root revealed the separation of single individuals from Seychelles and Mauritius from the central cluster, driven by UGR and PR. Overall, very little differentiation was observed among localities and the conclusion can be made that meristic counts are not useful in determining differentiation in this species.

A.



B.

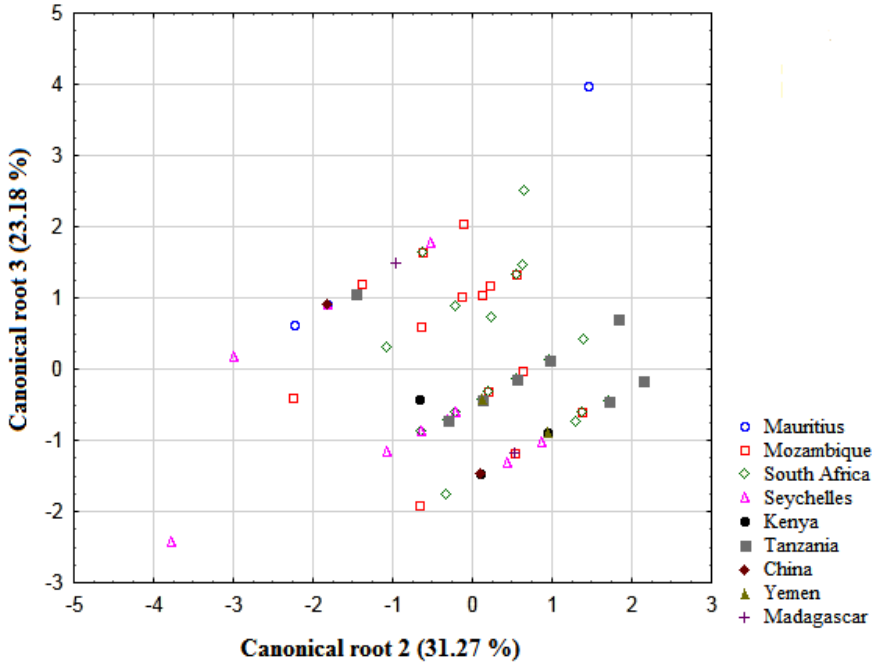


Figure 3.13. Scatter-plot of *Lutjanus fulviflamma* (Forsskål, 1775) individuals from sampled localities according to scores (A) along the first and second canonical roots, and (B) along the second and third canonical roots from discriminant function analyses using six meristic counts.

3.5. Discussion

The results of both mitochondrial and nuclear sequence data analyses indicated a measure of spatial genetic heterogeneity involving some of the localities across the sampling area, depending on the gene region. Both mitochondrial markers revealed the differentiation of Thailand and the Western Pacific Island individuals from those from WIO localities (as revealed by the median joining networks, phylogenetic trees and pairwise estimates of differentiation), but this geographic differentiation could not be observed with the nuclear marker. The separation of localities from the Western Pacific (Western Pacific Island) or Eastern Indian Ocean/Andaman Sea (Thailand) are similar to the results of Bay *et al*. (2004), who found a complex pattern of differentiation in the parrotfish *Chlorurus sordidus* across the IP. These authors also found differentiation of the WIO from other IP localities. McMillan and Palumbi (1995) found differentiation between WIO and IP populations of butterflyfishes *Chaetodon punctatofasciatus* and *Chaetodon rhombochaetodon*. Gaither *et al*. (2010b) observed genetic structuring in *Lutjanus fulvus* between the Indian Ocean and the Pacific Ocean. Craig *et al*. (2007) and Muths *et al*. (2011) also found significant differentiation between the WIO and the Western Pacific populations of soldierfish *Myripristis berndti*. These studies indicate an effective physical mechanism that is blocking gene flow between the WIO and other parts of the IP for some of the species. Fluctuating climatic conditions in the Pleistocene are hypothesised to be responsible for the differentiation between the two regions (McMillan & Palumbi, 1995). Other authors (Craig *et al*., 2007; Gaither *et al*., 2010b) suggested isolation by distance (IBD) to be responsible for the differentiation of the WIO from the IP. However, IBD was not responsible for the differentiation of Thailand in this study, suggesting that other processes could responsible. In contrast to these examples and the current study, Gaither (2010b) found low differentiation of Sodwana Bay and Diego Garcia in *L. kasmira*, which may be influenced by geographical isolation of these localities. The lack of structure in the jobfish *Pristipomoides filamentosus* (Gaither *et al*., 2011a) is influenced by prolonged spawning period lasting up to 10 months maximising the exposure to seasonal currents transport, and the ability of some mature adults to disperse.

Within the WIO, most of the pairwise Φ_{ST} values among localities were low, but a few moderate and significantly large values were observed. This, together with the absence of significant isolation by distance pattern, suggests a general lack of clear population

differentiation within the WIO. However, all three data sets indicated the moderate differentiation of South Africa from most WIO localities. Individual data sets indicated the differentiation of Mauritius from South Africa and Mozambique (cytochrome *b*), the differentiation between Red Sea and Tanzania (NADH-2), and the significant differentiation of Mozambique from other WIO localities (S7 intron 1 data). Other localities showed differentiation with the S7 intron 1 data, but were non-significant. Although the current study found some evidence of genetic connectivity among certain localities in the WIO, as suggested by Dorenbosch *et al*. (2006), the above evidence of local differentiation was also detected. However, no unique lineages were detected in the WIO, with the exception of unique sub-clades of South African individuals recovered in the analyses of the cytochrome *b* and S7 intron 1 data. Several authors have provided evidence of such local differentiation. Gopal *et al*. (2006) found some evidence of differentiation in the spiny lobster *Palinurus delagoae*, along the east African coast (between South Africa and Mozambique), influenced by ocean currents. Muths *et al*. (2011) found differentiation of Kenya individuals in *Myripristis berndti* and suggested that this population may have undergone a different history of colonization from the wider South Western Indian Ocean (SWIO) stock. Visram *et al*. (2010a) also observed differentiation of Kenya and Seychelles versus Tanzania and Mauritius in *Scarus ghobban*.

Morphometric characters indicated some evidence of differentiation of China, Yemen, Madagascar, and certain individuals from Kenya, Seychelles and Tanzania from a central group of WIO individuals. This differentiation indicates the effectiveness of morphometric characters. However, meristics have failed to separate *L. fulviflamma* populations. Failure of meristic counts to separate species has been observed in other species as well (Murta, 2000). Meristic characters are fixed early in development and are not influenced by environmental factors (Murta, 2000; Hermida *et al*., 2005; Sfakianakis *et al*., 2011) and these may be too conserved to detect variation at the intraspecific level. This study found congruence between genetics and morphometrics in that both methods were able to detect differentiation of some localities within the WIO and between WIO and the wider IP.

Analyses of the three markers revealed high haplotype and allelic diversity in the region, with generally low nucleotide diversity. Few haplotypes were shared widely across the region, with the majority of haplotypes being private. Grant and Bowen (1998) suggest that high haplotype

diversity and low nucleotide diversity represent a departure from neutrality, indicating an expanding population. The significant and large negative Fu's *F* and Tajima's *D* values under a model of population expansion (Rogers & Harpending, 1992; Fu, 1997; Chiang *et al*., 2008), suggest that *L. fulviflamma* has experienced population expansion in the WIO. Short branch lengths in the phylogenetic tree further suggest a recent population divergence in this species. The haplotype network showed two abundant/ancestral haplotypes having less abundant haplotypes radiating from them, further suggesting expanding population. This rapid population growth enhances the retention of new mutations (Avise *et al*., 1984).

Dorenbosch *et al*. (2006) proposed three hypotheses that could explain genetic structuring in *L. fulviflamma* along the East African coast when the East African Coastal Current (EACC) is strongest. They suggested that (1) larval exchange between populations will be low, (2) larval exchange would occur over a small geographic scale between populations in conjunction with the flow, or (3) that the EACC, in conjunction with the South Equatorial Current (SEC) and Equatorial Counter Current (ECC), would enable larval exchange over a large geographical scale. The third hypothesis will lead to high genetic connectivity over a large geographic scale, resulting in one gene pool where populations or individual localities cannot be genetically distinguished. They found the third hypothesis to be the most likely for the distribution of genetic variation along the East African coast, but with the differentiation of the Comoros Archipelago. Considering the localities common to the two studies, genetic data (i.e., S7 intron 1, although not significant) found evidence of differentiation of Tanzania, whereas morphometrics detected differentiation of Tanzania and Kenya from other localities. This can possibly be attributable to the influence of the SEC and the ECC systems. The SEC flows towards the main African coast and splits after the northern-tip of Madagascar into a southward-flowing current, which flows through to Mozambique, forming the Mozambique Current, and the northward-flowing component, which forms the EACC (Warren *et al*., 1966; Kemp, 1998; UNEP, 2004; Magori, 2008). This split may be responsible for the differentiation of Tanzania and Kenya observed in the current study. On a smaller spatial scale in Tanzania, Garpe and Öhman (2003) demonstrated that *L. fulviflamma* aggregations were restricted by habitat preference and substrate structure to a small area in Mafia Island Marine Park. By extrapolation, this suggests that habitat preference and habitat fragmentation may also play a role in restricting dispersal of *L. fulviflamma* in this area. The differentiation of South Africa could have resulted from complex oceanographic features between South

Africa and Mozambique. For example, Gopal *et al*. (2006) observed the differentiation of South African specimens from KwaZulu-Natal in the spiny lobster *Palinurus delagoae*. These authors proposed that a combination of life history traits, particularly reproductive migrations, affects the position of larval release relative to oceanic currents. This behaviour has implications for larval dispersal and gene flow. However, larval recruitment to adult habitats and ocean currents result in reduced gene flow between South Africa and Mozambique (Gopal *et al*., 2006). Similar mechanisms are proposed for the differentiation of some South African individuals from other localities in this study.

- Genetic structure may also increase with increasing geographic distance when settlement events decline with increasing distance from the source population (Palumbi, 1994) or decrease with an increasing dispersal ability at any given spatial scale (Bay *et al*., 2004). According to Gold and Richardson (1998), population genetic structure may be attributed to differences in the biology of marine animals (i.e. species life-history, ecology, habitat preference, and reproductive biology), environmental influences (ocean currents and temperature) and species behaviour (breeding strategies). Grimes (1987) described *L. fulviflamma* as a restricted continental, highly-fecund batch-spawning species (with spawning peaks in summer months) in East African waters. Peak spawning occurs in October, a time of high productivity (for eggs and rapid larval development) during the Southeast Monsoon (Nzioka, 1979), which speeds up the EACC and lowers the thermocline (Grimes, 1987). This breeding strategy introduces larvae into a rich food supply in order to grow rapidly and escape predators (Grimes, 1987). The expectation was, therefore, to find high genetic connectivity similar to that found by Dorenbosch *et al*. (2006) in *L. fulviflamma*. Therefore, this study sampled a wider geographic coverage, and found patterns of high connectivity, but also of low but significant differentiation. These patterns are more similar to those of *L. kasmira* in the region (Muths *et al*., 2010). These authors suggested that local processes influenced differentiation. This observation further warrants investigation into early life history of *L. fulviflamma* in order to identify other processes that could potentially have an impact on connectivity and differentiation.

Studies (including fish and invertebrates) in the WIO have focused on few localities and have not considered the region in its entirety. A broader study, like the present, is important for

understanding the patterns of connectivity and differentiation and, in turn, for the conservation and management of coral reef fisheries (Visram *et al*., 2010b). A number of the patterns of connectivity and differentiation find comparison in other taxa studied in the region. This study and those mentioned below present a complex array of patterns of differentiation and connectivity observed in various taxa across the WIO. For instance, Muths *et al*. (2011) found restricted connectivity in the soldierfish *Myripristis berndti*, with localities along the Mozambique Channel being densely connected as a central locality and localities at the extremities, Europa, Kenya and Reunion, being differentiated. Visram *et al*. (2010a) detected isolation by distance among the populations of blue-barred parrotfish *Scarus ghobban* from the east coast Africa (Kenya & Tanzania) and Mascarene Islands (Seychelles & Mauritius). This was due to separation of Kenya and Seychelles from Tanzania and Mauritius through the influence of the SEC and the ECC. Similarly, the differentiation of South Africa from Mozambique and Madagascar observed in the spiny lobster *Palinurus delagoae* (Gopal *et al*., 2006) was attributed to ocean currents, anti-cyclonic eddies along the Mozambique Shelf and East Madagascar Current (EMC) water masses reaching the Agulhas Current (AC) only irregularly. Similar patterns to those observed in fish also apply to crustaceans in the region (WIO). Fratini and Vannini (2002) found greater differentiation of Mauritius from other localities in the WIO, and reduced connectivity, despite the potential for extensive dispersal, in the swimming crab *Scylla serrata* in mangrove swamps of the African tropics (Kenya and Zanzibar). Silva *et al*. (2010) examined population structure of the fiddler crab *Uca annulipes* across the WIO part of its Indo-West Pacific (IWP) distribution. They detected no population differentiation and high gene flow across the East African latitudinal gradient, with evidence for population expansion in this species. Baratti *et al*. (2005) found significant population differentiation among Kenyan and Tanzanian populations of marine isopod *Sphaeroma terebrans* in the WIO, influenced by ecology, reproductive strategy, and geographic isolation between populations. While fish studies typically show a pattern of low population differentiation in the region, crustaceans show significant population differentiation. Therefore, it is inappropriate to extrapolate patterns across different taxonomic groups, assuming that these patterns will be the same.

Several hypotheses have been proposed concerning the biogeographical regions of the WIO and the IP. Hocutt (1987), Pandolfi (1992) and Santini and Winterbottom (2002) considered the vicariant origins of regional faunas and biogeographical regions. The historical events that

have led to the separation of biogeographic regions and those physical features that maintain them may have had an influence on the differentiation observed in the current study. In accordance with Santini and Winterbottom's (2002) hypothesis, differentiation would be expected among localities of the Natal Basin, Somali Basin, Madagascar Plateau, Andaman Basin and the Coral Sea. AMOVAs indicated that much of the observed differentiation was concordant with these biogeographic regions. Other processes, such as dispersal, may be responsible for absence of full concordance with these regions. However, this will require direct methods (tagging and telemetry) to test this hypothesis – this was not covered by the current study.

Management of fisheries is necessary to ensure that present fisheries can continue to be exploited in perpetuity and to assist in the recovery of depleted stocks (Graves, 1998; Ward, 2000). Identifying connectivity and differentiation allows for the identification of localities among which gene flow is restricted. In the current study, these involved South Africa, Mozambique, Mauritius and Thailand localities, although not all were shown to be differentiated with all the markers used. Adults of *L. fulviflamma* are sedentary (Samoilys & Carlos, 2000); hence, connectivity in the WIO is assumed to be maintained by larval dispersal (Dorenbosch *et al*., 2006). Therefore, identifying nursery areas is critical for the conservation and resource management of this species (Nzioka, 1979; Grimes, 1987; Kamukuru & Mgaya, 2004; Vasconcellos *et al*., 2008), but these sites were not identified in the current study. The differentiation of these localities may suggest different stocks for *L. fulviflamma* in the region, but sampling size was inadequate to say with certainty whether they are different stocks. If these represent different stocks, these will then require mostly independent management by the respective countries. This study outlined localities on which to focus conservation efforts.

Several studies in the WIO (Turpie *et al*., 2000; Kamukuru *et al*., 2005; Muths *et al*., 2012), Indo-Pacific (Allen, 2007) and northern Atlantic (Ungfors *et al*., 2009), and a review by Botsford *et al*. (2009) have suggested that a network of Marine Protected Areas (MPAs) is needed for conservation and fisheries management. These MPAs will be effective, especially in lutjanids, due to their high degree of variation in life history strategies and dispersal ability (Grimes, 1987). Generally, short distance dispersers will persist in almost all MPAs, while long distance dispersers require a specific density of MPAs along the coast (Botsford *et al*., 2009). The patterns observed in the current study imply that effective MPAs be developed

and co-managed, as the differentiated localities may also be driven by differences in selection across environmental gradients and larval adaptation in these localities (Grimes, 1987; Rodrigues *et al*., 2008; Visram *et al*., 2010b). Management decisions that will preserve future potential for ecological and evolutionary adaptation will have to implemented to conserve *L. fulviflamma* in localities where the species displayed differentiation. Moritz (1994) described Management Units (MUs) as populations with significant divergence of haplotype/allele frequencies, irrespective of phylogenetic distinctiveness of haplotypes/alleles. However, based on low sampling sizes in the current study, this is a suggestion and will have to be confirmed with more studies, more samples and much wider sampling coverage in the region. Similar management recommendations were suggested for *Lutjanus kasmira* (Muths *et al*., 2012) and the white-spotted rabbitfish *Siganus sutor* (Visram *et al*., 2010b) in the WIO. This is the first large-scale study to address connectivity and differentiation of *L. fulviflamma* in the WIO. The wide sampling in the current study provided patterns that were not detected by Dorenbosch *et al*. (2006) for this species. Comparable phylogeographic studies like the present help identify areas of concern for conservation and help coastal and oceanic nations identify where to focus their conservation efforts.

CHAPTER FOUR

A COMPARISON OF PATTERNS OF DIFFERENTIATION IN *LUTJANUS BOHAR* (Forsskål, 1775) AND *LUTJANUS LUTJANUS* Bloch, 1790 ACROSS THE WESTERN INDIAN OCEAN AS INFERRED FROM THREE DNA MARKERS

4.1. Introduction

The twin-spot red snapper *Lutjanus bohar* (Forsskål, 1775) and the bigeye snapper *Lutjanus lutjanus* Bloch, 1790 are widely distributed in the Indo-Pacific (see Chapter 1 for species-specific details). In the year 2000, the total catch of *Lutjanus bohar* was 127.5 tonnes out of 2875 tonnes of total reef fish caught in the Seychelles. In 2003, this number declined to 44.9 tonnes out of the 2441 tonnes of total fish caught (Marriot & Mapstone, 2006; Marriot *et al*., 2007). In Eritrea, this species contributes an average of 16% to the total artisanal catch (Habte, 2003). *Lutjanus lutjanus* is also common in markets and comprises an estimated 10 – 20% of the snappers in the trawl catch in the Gulf of Suez (Allen, 1985). As evidenced by these statistics, the harvesting of these species for commercial and artisanal purposes is a cause for concern, particularly for *L. bohar*. *Lutjanus bohar* is characterised by delayed maturity, making it less likely to produce sustainable harvest yields (Marriot & Mapstone, 2006) and most likely to be heavily impacted by over-fishing (Musick, 1999; Marriot *et al*., 2007). Therefore, their management is important to ensure that present fisheries can continue to be exploited in perpetuity (Ward, 2000).

These two species have not been studied genetically previously. However, a few studies have examined demographic parameters and age estimates in *L. bohar*, and phylogenetic studies have included *L. bohar* (Newman & Williams, 2001; Marriot & Mapstone, 2006; Marriot *et al*., 2007; Miller & Cribb, 2007), while Martinez-Andrade (2003) looked at age estimates in *L. lutjanus*. Therefore, more studies covering other aspects of life history are needed for a fuller understanding of the biology of these species. The current study will focus on the genetics of these species. Genetic information can be used to make indirect inferences to understand species biology and its integration within an ecosystem context. This knowledge will be useful in making recommendations about the conservation and for management strategies for these species.

Domeier and Colin (1997) reported spawning aggregations of *L. bohar*, but did not provide detailed descriptions. *Lutjanus bohar* spawns during September-October and December-January along the Tanzanian and Kenyan coasts (Nzioka, 1979). *Lutjanus lutjanus* spawning is reported to occur during March and November in the Gulf of Aden and east Africa (Nzioka, 1979) and from January to June in the Gulf of Suez (Allen, 1985). Like other lutjanids, these species spawn pelagic eggs, with the spawning season in east Africa coinciding with Northeast Monsoon period. This time is conducive for dispersal and aids the survival of ichthyoplankton (see Chapter 3). The estimated pelagic larval duration of lutjanids is generally 25-47 days (Allen, 1985; Grimes, 1987; Leis, 1987; Lindeman *et al*., 2001; Zapata & Herron, 2002). However, information about larval dispersal, recruitment behaviour and stock structure of these species is scarce.

The oceanographic nature and the rich biodiversity of the Western India Ocean (WIO) makes it suitable to conduct biogeographic studies to examine factors and processes shaping patterns of species diversity and variation (see Chapters 1 & 3). With reference to previous genetic studies on lutjanids in the WIO, Dorenbosch *et al*. (2006) found low genetic differentiation and high connectivity in *Lutjanus fulviflamma* along the east-African coast (Tanzania, Kenya and the Comoros). In Chapter 3, the same species was studied and similar patterns were found, but with the differentiation of South Africa, Mozambique, Mauritius from other WIO localities and Thailand (depending on the individual analysis). Although only a few haplotypes were shared, together these encompassed almost the full extent of spatial sampling in the WIO; thus indicating genetic connectivity in the region. Muths *et al.* (2012) found differentiation of Moroni and Mauritius from other localities in *Lutjanus kasmira*, influenced by ecological plasticity. In terms of management and conservation of these important fisheries species, we ask if there is genetic differentiation within the two species in the WIO and compare the results to those of *L. fulviflamma* to determine if similar patterns apply to these species. These species have different life history strategies, but they generally have similar reproductive behaviour, dispersal potential and are exposed to similar environmental and oceanographic features. The expectation is to find similar genetic patterns between the two species, and between them and *L. fulviflamma*.

4.2. Material and Methods

Lutjanus bohar specimens were included from Mozambique, Tanzania, Kenya, Seychelles, Madagascar, Red Sea, Maldives and Australia, while *L. lutjanus* specimens were successfully collected from only three localities: Mozambique, Tanzania and Kenya (Figure 4.1). Some of the samples were obtained from the National Fish Collection at SAIAB, while others were obtained from the Australian Museum, Senckenberg Museum and the University of Kansas (USA); see Appendix II.

Total genomic DNA was extracted and three gene regions (cytochrome *b*, NADH-2 and S7 intron 1) were amplified by PCR, purified and sequenced (see Chapter 2 for further details) for individuals of both *Lutjanus bohar* and *L. lutjanus*. PCR cycling-conditions for the amplification of the three regions are detailed in Table 4.1. See Chapter 2 for further details.

Table 4.1. Thermocycling regimes for the amplification of the respective mitochondrial and nuclear gene regions from both *Lutjanus bohar* (Forsskål, 1775) *and Lutjanus lutjanus* Bloch, 1790 individuals.

Gene region	PCR thermocycling profile					
	Stage 1	Stage 2			Cycles	Stage 3
	Initial denaturation	Denaturation	Annealing	Extension		Final extension
Mitochondrial genes						
Cytochrome *b*	94 °C, 4 min	94 °C, 30 sec	50 °C, 30 sec	72 °C, 1.5 min	35	72 °C, 10 min
NADH-2	94 °C, 4 min	94 °C, 45 sec	50 °C, 1 min	72 °C, 1.5 min	35	72 °C, 10 min
Nuclear gene						
S7 intron 1	95 °C, 2 min	95 °C, 45 sec	53 °C, 1 min	72 °C, 1 min	35	72 °C, 10 min

For each of the two species and gene regions, genetic diversity indices were calculated for individual localities and for the overall sample, haplotype networks were constructed, pairwise estimates of population differentiation were determined and AMOVAs were performed as in Chapter 3. For phylogenetic tree construction, *Emmelichthys struhsakeri* (Emmelichthyidae) was used as an outgroup in the construction of MP, NJ, ML and BI trees.

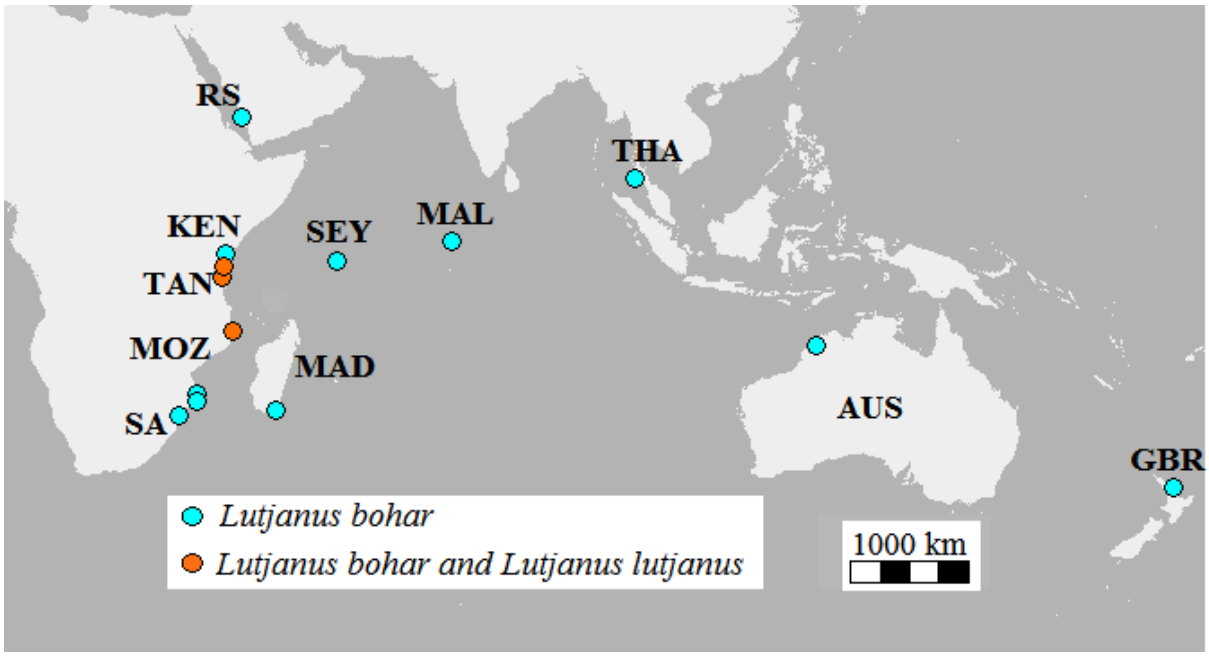


Figure 4.1. Sampling localities of *Lutjanus bohar* (Forsskål, 1775) and *Lutjanus lutjanus* Bloch, 1790 individuals.

Bayesian inference (BI) analysis involved four independent Markov Chain Monte Carlo (MCMC) chains running simultaneously for each data set. These chains were run over one million generations and sampled every 1000th generation. To ensure convergence, each analysis was run three times in parallel until the standard deviation of split frequencies among the runs fell below the 0.05 threshold, then stopped. The first 25% of the trees were discarded as burn-in and a 50% majority-rule consensus tree generated from each analysis to determine the relationships and the posterior probability (support) of each node.

4.3. Results

4.3.1. *Lutjanus bohar* (Forsskål, 1775)

The cytochrome *b* sequence data set (581 bp in length) contained 20 haplotypes from 31 individuals (Table 4.2). The haplotype network (Figure 4.2: A) showed three haplotypes (H1 present in four localities, and H2 and H12 present in two localities each) being shared among localities, but collectively these were not found in all localities. The remaining 17 haplotypes

(54.8%) were private. Within the WIO and of those localities with more than one individual (Mozambique, Tanzania, Kenya, Seychelles, Madagascar and Maldives), the highest proportion of private haplotypes (Table 4.2) was found in Madagascar (2; 100%), followed by Mozambique (5; 62.5%), with a low proportion in Tanzania (1; 50%), Seychelles (2; 40%) and Maldives (2; 25%); the single individual from the Red Sea not being considered. Two other haplotypes are worth consideration. Haplotype H9 from Mozambique was six mutational steps from the most closely-related haplotype and was divergent from other haplotypes from the same locality (Figure 4.3). The other is haplotype H19 from Australia, which was very divergent (12 mutational steps away) from haplotype H20 from Australia. The latter was not divergent from the WIO haplotypes and clustered with them in the network.

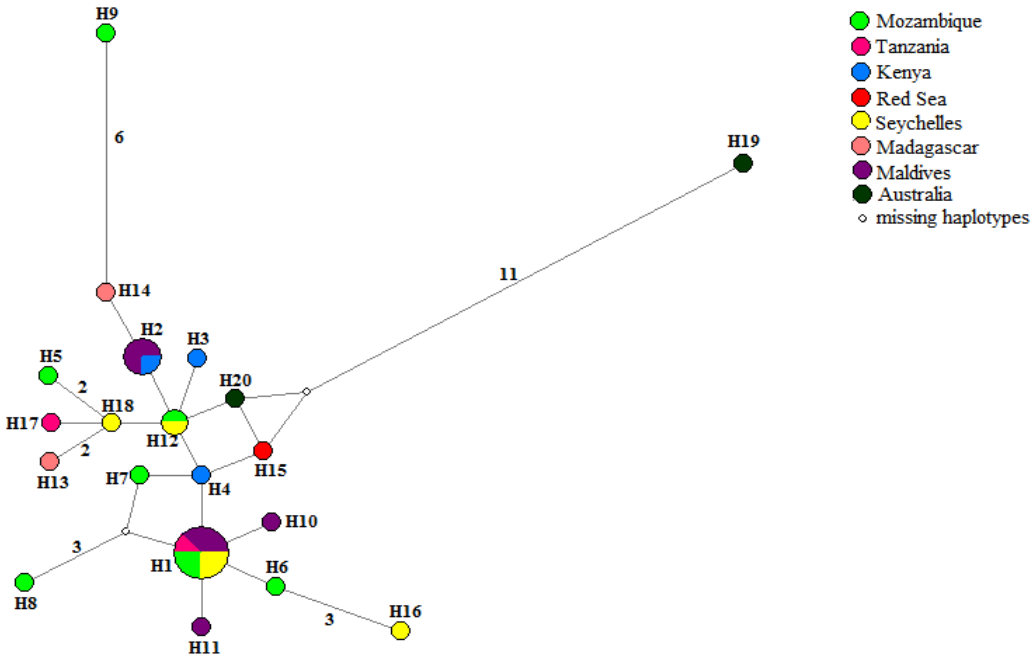
The NADH-2 data set (30 individuals, 632 bp) contained 16 haplotypes (Table 4.2). The Australian samples were not included because they failed to amplify. The haplotype network (Figure 4.2: B) shows two haplotypes (H2 present in three localities, and H3 present in five localities) being more abundant than the others and shared among localities. These two haplotypes encompassed the full spatial extent of sampling from Mozambique to the Maldives. The highest proportion of private haplotypes was found in Kenya (3; 75%), followed by Seychelles (2; 66.7%) and Mozambique (4; 40%). Unlike cytochrome *b* data, no private haplotypes were found in Tanzania. Haplotype H16 from the Seychelles appeared to be slightly more divergent from other haplotypes from the same locality and was four mutational steps away from the closest haplotypes (H8 from Mozambique and the widespread H2).

Fewer individuals (22) amplified for the S7 intron 1 gene fragment than for the mitochondrial gene fragments and some localities (Madagascar, Red Sea and Australia) were not represented. The S7 intron 1 data set (534 bp in length) contained 44 alleles; 39 of these were unique (Table 4.2). Only allele A10 (Figure 4.3) was shared between Mozambique and Tanzania. The highest percentage of private alleles (100%) was found in Kenya and Seychelles, followed by Maldives (15; 93.8%) and Mozambique (8; 66.7%), with a lower percentage in Tanzania (1; 50%) (Table 4.2). Some alleles from Kenya, Mozambique, Seychelles and Maldives were highly divergent from other alleles from the same localities (Figure 4.4).

Table 4.2. The number of individuals/alleles (*N*) and genetic diversity indices at each of the sampling localities for *Lutjanus bohar* (Forsskål, 1775) from Mozambique (MOZ), Tanzania (TAN), Kenya (KEN), Seychelles (SEY), Madagascar (MAD), Red Sea (R-Sea), Maldives (MAL) and Australia (AUS) for the three gene regions (cytochrome *b*, NADH-2, and S7 intron 1) examined. Indices include the number of haplotypes (N_h) or alleles (N_A), the number of private haplotypes (N_{PH}) or alleles (N_{PA}), haplotype (H_D), allelic (A_D) diversity, and nucleotide diversity (π). Standard deviations are presented for H_D, A_D and π.

	Cytochrome *b* (581 bp)					**NADH-2 (632 bp)**					**S7 intron 1 (534 bp)**				
Locality	***N***	***N_h***	***N_{PH}***	***H_D***	***π***	***N***	***N_h***	***N_{PH}***	***H_D***	***π***	***N***	***N_a***	***N_{PA}***	***A_D***	***π***
MOZ	8	7	5	0.964±0.077	0.010±0.006	10	6	4	0.844±0.103	0.003±0.003	12	9	8	0.939±0.058	0.012±0.007
TAN	2	2	1	1.000±0.500	0.007±0.008	2	2	0	1.000±0.500	0.002±0.002	2	2	1	1.000±0.500	0.009±0.010
KEN	3	3	2	1.000±0.272	0.003±0.003	4	4	3	1.000±0.177	0.004±0.003	8	8	8	1.000±0.063	0.027±0.016
SEY	5	4	2	0.900±0.161	0.006±0.004	3	3	2	1.000±0.272	0.006±0.005	6	6	6	1.000±0.096	0.016±0.010
MAD	2	2	2	1.000±0.500	0.009±0.009	1	1	1	---	---	-	-	-	---	---
R-SEA	1	1	1	---	---	1	1	1	---	---	-	-	-	---	---
MAL	8	4	2	0.786±0.113	0.004±0.003	9	5	3	0.806±0.120	0.002±0.002	16	15	15	0.992±0.025	0.010±0.006
AUS	2	2	2	1.000±0.500	0.021±0.021	-	-	-	---	---	-	-	-	---	---
Overall	31	20	17	0.925±0.037	0.007±0.001	30	16	14	0.869±0.047	0.003±0.001	22	39	38	0.994±0.007	0.016±0.002

A



B

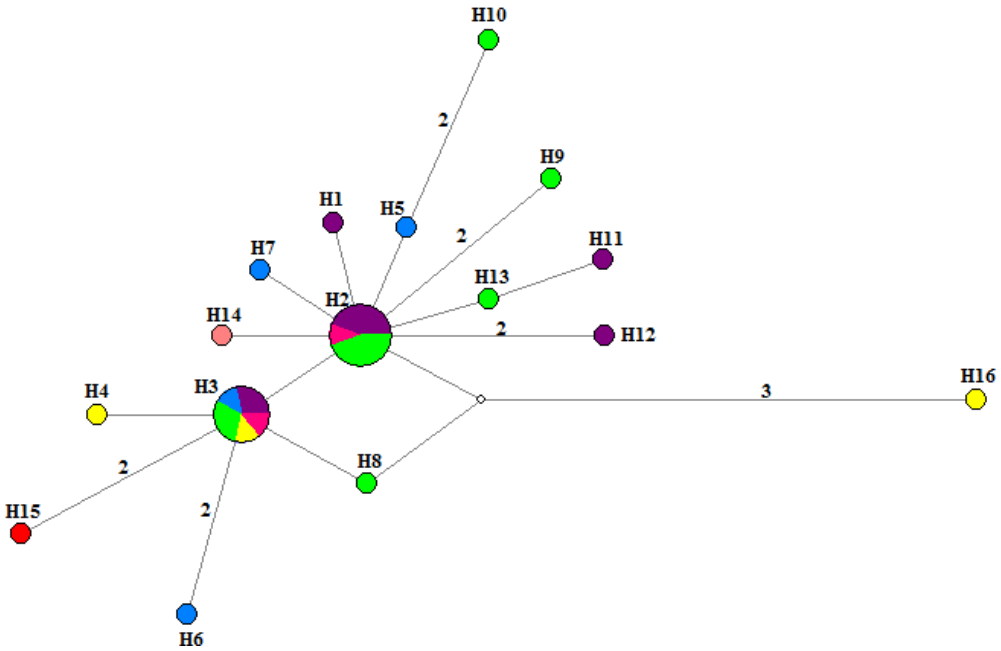


Figure 4.2. Median-joining haplotype networks derived from the cytochrome *b* (A) and NADH-2 (B) data sets for *Lutjanus bohar* (Forsskål, 1775) individuals. The size of the node (haplotype) corresponds to the frequency of that haplotype and the colours represent the occurrence of that haplotype at different localities. The numbers on the branches indicate mutational differences where greater than one.

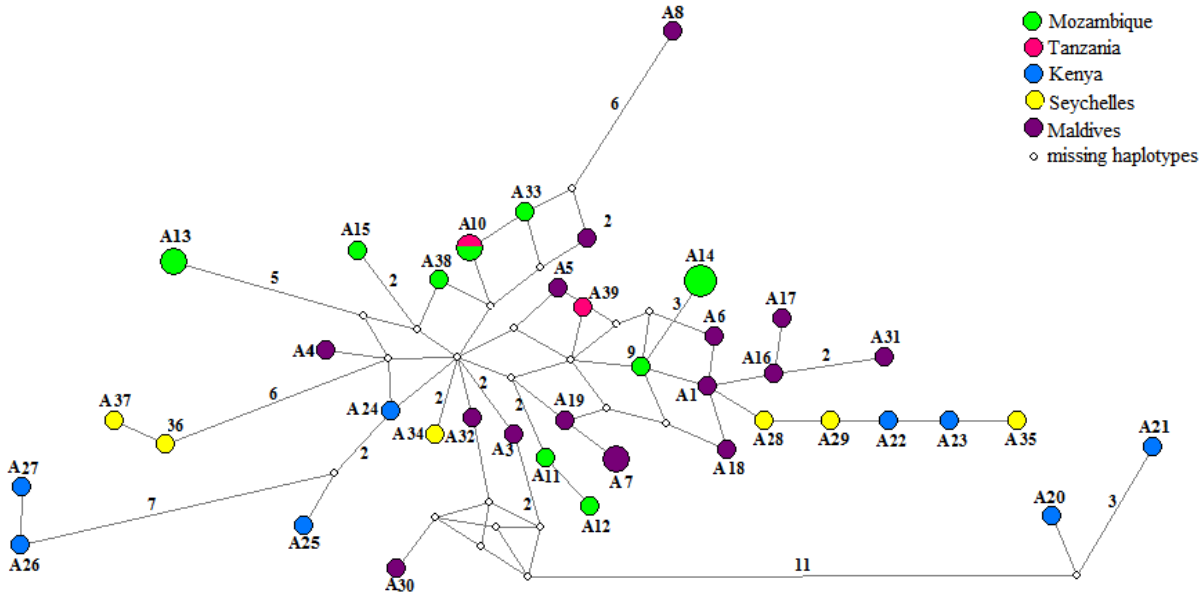


Figure 4.3. Median-joining haplotype network derived from the S7 intron 1 data set for *Lutjanus bohar* (Forsskål, 1775). The size of the node (allele) corresponds to the frequency of that allele and the colours represent the occurrence of that allele at different localities. The numbers on the branches indicate mutational differences greater than one.

Haplotype diversities (H_D), allelic diversity (A_D) and nucleotide (π) diversities are presented in Table 4.2. Overall haplotype diversity was higher for cytochrome *b* (0.925±0.037) than NADH-2 (0.869±0.047), while the allelic diversity for S7 intron 1 (0.994±0.007) was higher than haplotype diversities of both the mitochondrial genes. Nucleotide diversities were highest for S7 intron 1 (0.016±0.002), and slightly higher for cytochrome *b* (0.007±0.001) than for NADH-2 (0.003±0.001). Generally, the diversities at individual localities showed comparable trends across the markers. Within the WIO, Tanzania and Kenya were the most diverse among the localities for all the markers, along with Seychelles for the NADH-2 and S7 intron 1 markers.

Among population comparisons (Table 4.3) were restricted to those localities with five or more individuals (see Table 4.2). For cytochrome *b* these were Mozambique (eight individuals), Seychelles (five individuals) and Maldives (eight individuals). For NADH-2 analysis these were Mozambique (10 individuals) and Maldives (nine individuals), and for the S7 intron 1 they were Mozambique (six individuals) and Maldives (eight individuals). The cytochrome *b* and NADH-2 data sets revealed low to moderate differentiation, but the Φ_{ST} –

Table 4.3. Φ_{ST} values of among population differentiation obtained in the comparison of *Lutjanus bohar* (Forsskål, 1775) representatives from Mozambique (MOZ), Seychelles (SEY) and Maldives (MAL) for the cytochrome *b* (below diagonal) and between Maldives (MAL) and Mozambique (MOZ) for the S7 intron 1 (above diagonal) gene fragments. Significant ($P < 0.05$) estimates, as determined from permutation tests, are indicated in bold font.

Locality	MOZ	SEY	MAL
MOZ	-		**0.121**
SEY	-0.082	-	
MAL	-0.010	-0.014	-

values were all non-significant. The Φ_{ST} values ranged from -0.082 to -0.014 ($P > 0.05$) in cytochrome *b* (Table 4.3), while a value of -0.039 ($P > 0.05$) was obtained between Mozambique and Maldives for NADH-2. However, the S7 intron 1 data (Table 4.3.) highlighted significant differentiation between Mozambique and Maldives with a Φ_{ST} –value of 0.121 ($P < 0.010$). The present data were insufficient to test for patterns of isolation by distance.

Analysis of Molecular Variance (AMOVA) was examined with localities grouped based on Pandolfi's (1992) and Santini & Winterbottom's (2002) biogeographic regions (see Chapter 3). The latter arrangement provided the best description of differentiation, maximising the component of variation attributed to differentiation among regions. There were six regions for the cytochrome *b*: Natal Basin (Mozambique), Somali Basin (Kenya and Tanzania), Red Sea, Mascarene Plateau (Madagascar and Seychelles), Chagos Plateau (Maldives) and the Coral Sea (Australia); five for NADH-2 (as before, but excluding the Coral Sea); and four for the S7 intron 1 data (excluding the Red Sea and Coral Sea). Across the gene regions, the among-region component of variance was low (5.55%, 8.45% and 5.52% for cytochrome *b*, NADH-2 and S7 intron 1, respectively) and non-significant ($P > 0.05$; see Table 4.4). A substantially large and non-significant ($P > 0.05$) for mitochondrial markers, but significant ($P < 0.001$) for the nuclear marker, component of variance (92.09%, 95.18% and 87.94% for cytochrome *b*, NADH-2 and S7 intron 1, respectively) was associated with differentiation among individuals within localities.

Table 4.4. AMOVA results examining the partitioning of differentiation in *Lutjanus bohar* (Forsskål, 1775) among the Natal Basin (Mozambique), Somali Basin (Kenya and Tanzania), Red Sea, Mascarene Plateau (Madagascar and Seychelles), Chagos Plateau (Maldives) and the Coral Sea (Australia), depending on the gene region, at various hierarchical levels based on sequence data of the cytochrome *b*, NADH-2 and S7 intron 1 gene fragments. The biogeographic groupings are defined in the text.

Source of variation	d.f.	Sum of squares	% variation	*P*-value
Cytochrome *b*				
Among regions	5	13.454	5.55	0.438
Among localities within regions	2	4.150	2.36	0.235
Within localities	23	44.525	92.09	0.156
NADH-2				
Among regions	4	5.233	8.45	0.196
Among localities within regions	2	1.833	-3.62	0.821
Within localities	23	22.900	95.18	0.259
S7 intron 1				
Among regions	3	27.697	5.52	0.504
Among localities within regions	1	4.775	6.54	0.739
Within localities	39	150.438	87.94	< **0.001**

Phylogenetic trees were constructed to examine relationships between individuals and localities. The cytochrome *b* data contained slightly more individuals that other data sets and included Australian individuals, allowing examination of differentiation between the WIO and the IP. This alignment (581 bp) had 545 conserved sites and 36 polymorphic sites of which 10 were parsimony informative and 26 were autapomorphies. The AIC identified GTR + I as the appropriate model for the cytochrome *b* data set. The model parameters were a nucleotide composition of A = 0.25, C = 0.31, G = 0.15 and T = 0.29, a nucleotide substitution rate matrix of A↔C = 1.37, A↔G = 7.55, A↔T = 0.28, C↔G = 4.87, C↔T = 3.47 and G↔T = 1.00, and a proportion of invariable sites (I = 0.81). This model was included in the construction of phylogenetic trees. The NJ tree for the cytochrome *b* data is shown in Figure 4.4. This tree was presented because of the resolution of internal nodes, which were poorly resolved with other analyses. In the tree, the monophyly of *L. bohar* with

respect to the outgroup *E. struhsakeri* was not supported. This tree indicated, in agreement with the haplotype network, the presence of a single clade (Clade A) containing all the WIO individuals, with one Australian individual clustered among them. This clade was relatively well supported (bootstraps 96/97/92 for MP, NJ and ML, respectively). The other Australian individual was divergent from the individuals in Clade A. No differentiated clades were observed among the WIO individuals. The ML tree (-lnL = 1127.68, tree not shown) was generally similar to the NJ tree in the positioning of one Australian individual outside the main clade, while the remaining individuals formed a polytomy. The maximum parsimony analysis for the cytochrome *b* resulted in 278 equally parsimonious trees, with the tree parameters: Tree Length (TL) = 25, Consistency Index (CI) = 0.76, Homoplasy Index (HI) = 0.24, Retention Index (RI) = 0.87 and Rescaled Consistency Index (RC) = 0.66. The resulting strict-consensus tree (tree not shown) was generally similar to the NJ tree presented. Bayesian analysis produced a large polytomy, with both Australian individuals within this clade.

A tree generated from combined data was not included becase data sets were not compatible in terms of the number of individuals sequenced; the same individuals were not sequenced for each marker and combining these would result in a loss of information.

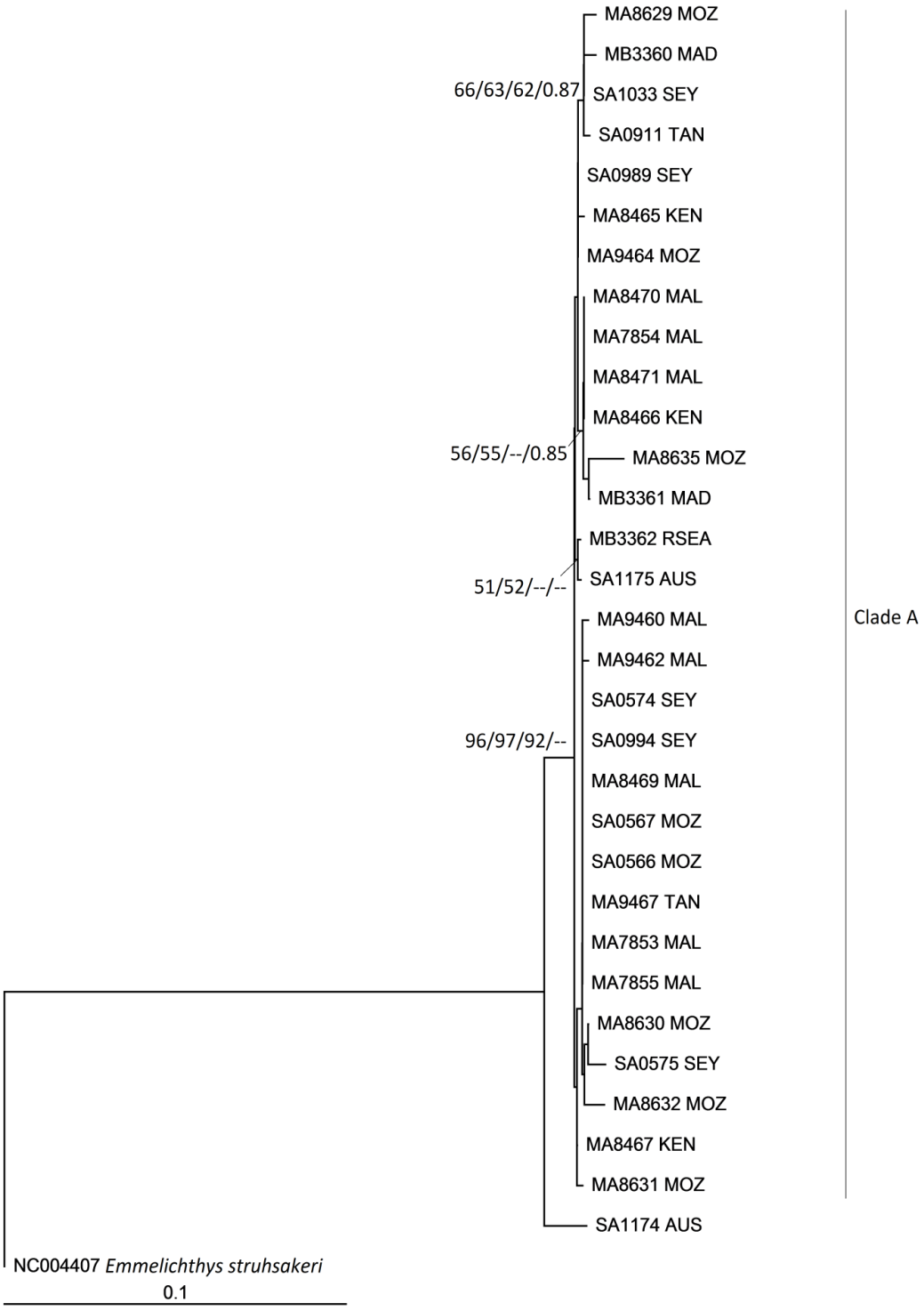


Figure 4.4. Outgroup-rooted neighbor-joining (NJ) tree depicting relationships among *Lutjanus bohar* (Forsskål, 1775) individuals for the cytochrome *b* gene fragment. Bootstrap support (MP, NJ and ML) and Bayesian Posterior Probabilities (BI) are indicated on the branches, respectively. The scale-bar indicates the estimated evolutionary distance.

The NADH-2 data set (632 bp) had 610 conserved sites, 22 polymorphic sites, four parsimony informative characters and 18 autapomorphies. The HKY + I model was the best model for the NADH-2 data set. The model parameters were a nucleotide composition of A = 0.28, C = 0.35, G = 0.13 and T = 0.24, a transition:transversion ration (Ti:Tv) of 1.75 and a proportion of invariable sites (*I* = 0.28). The NJ tree (tree not shown) failed to recover any genetic structure and the monophyly of *L. bohar* was not supported. The ML tree (-lnL = 1025.68, tree not shown) formed a large unsupported polytomy. The MP analysis resulted in five equally parsimonious trees, with tree parameters: TL = 9, CI = 0.67, HI = 0.33, RI = 0.82 and RC = 0.55. The resulting strict-consensus tree (tree not shown) was similar to the NJ tree, forming a polytomy, and was statistically not supported. The results of the BI analysis were similar to the results of the other analyses.

Once phased, the S7 intron 1 data had 482 conserved sites, 52 polymorphic sites, 41 parsimony informative sites and 11 autapomorphies. The F81 + I + G model was the most appropriate evolutionary model for the data set. The parameters for this model were a nucleotide composition of A = 0.24, C = 0.20, G = 0.24 and T = 0.32, a proportion of invariable sites (*I* = 0.71) and a gamma distribution of rate variation ($\alpha = 1.21$). The NJ tree (tree not shown) resulted in a polytomy. However, there were a few alleles from Maldives, Mozambique and Kenya, respectively, that formed moderately supported clades, corresponding to certain clusters in the network. The ML analysis (–lnL = 1025.68, tree not shown) recovered fairly-well supported clades containing Mozambique alleles and clades containing Maldives alleles, but with the majority of the internal nodes forming a polytomy. The MP analysis resulted in 343 647 equally parsimonius trees. These equally parsimonius trees had the parameters: TL = 85, CI = 0.55, HI= 0.45, RI = 0.79 and RC = 0.43. The strict-consensus tree (tree not shown) recovered the same clades as NJ tree, but these were poorly supported. The results of the BI analysis (trees not shown) were similar to the results of the MP analysis. Overall, the trees did not show a clear clustering of individuals from the same locality, thus suggesting little genetic differentiation for this speceis in the study area.

4.3.2. *Lutjanus lutjanus* Bloch, 1790

Lutjanus lutjanus proved difficult to sample, with few individuals obtained from only three localities: Mozambique, Tanzania and Kenya. The cytochrome *b* alignment had 594 nucleotides from each of 21 individuals and contained seven unique haplotypes (Table 4.5). The haplotype network (Figure 4.5: A) showed haplotype H2 as being more abundant and being shared between Tanzania and Kenya, with less frequent haplotypes from these two localities radiating from it. Haplotypes H3 and H4 from Mozambique appeared to be divergent from those from Tanzania and Kenya, but caution should be exercised due to low sample sizes. Six haplotypes were private (Table 4.5), with the highest proportion found in Mozambique (2; 66.7%), followed by Kenya (2; 28.6%) and Tanzania (2; 18.2%).

The NADH-2 alignment (571 nucleotides) from 18 individuals contained five unique haplotypes (Table 4.5). The haplotype network (Figure 4.5: B) showed that only haplotype (H3) was shared among the three localities. Haplotypes H1 and H5 were very divergent from other haplotypes of the same locality and were each 13 mutations steps from H3. The highest proportion of private haplotypes was found in Kenya (3; 50%), followed by Tanzania (1; 11.1%), with none in Mozambique (Table 4.5).

Once phased, the S7 intron 1 data set (392 nucleotides) resulted in 48 constituent alleles (Table 4.5). Three alleles (A2, A9 and A14) were shared among the three localities, while alleles A1, A11 and A16 were shared only between adjacent localities (Figure 4.5: C). Three alleles (A6, A8 and A18) from Tanzania and allele A13 from Kenya were divergent from

Table 4.5. Number of individuals or alleles (*N*) and genetic diversity indices at each of the sampling localities and for the overall sample of *Lutjanus lutjanus* Bloch, 1790 from Mozambique (MOZ), Tanzania (TAN) and Kenya (KEN) for the three gene regions (cytochrome *b*, NADH-2 and S7 intron 1) examined. Indices include the number of haplotypes (N_h) or alleles (N_A), the number of private haplotypes (N_{PH}) or alleles (N_{PA}), haplotype (H_D) or allelic (A_D) diversity, and nucleotide diversity (π). Standard deviations are presented for H_D, A_D and π.

Locality	**Cytochrome *b* (594 bp)**					**NADH 2 (571 bp)**					**S7 intron 1 (392 bp)**				
	N	***N_h***	***N_{PH}***	***H_D***	***π***	***N***	***N_h***	***N_{PH}***	***H_D***	***π***	***N***	***N_a***	***N_{PA}***	***A_D***	***π***
MOZ	3	2	2	0.667±0.314	0.003±0.003	3	1	0	---	---	8	8	3	1.000±0.063	0.012±0.008
TAN	11	3	2	0.345±0.172	0.001±0.001	9	2	1	0.222±0.166	0.005±0.003	22	14	8	0.922±0.045	0.014±0.008
KEN	7	3	2	0.524±0.209	0.001±0.001	6	4	3	0.800±0.172	0.013±0.008	18	9	4	0.837±0.066	0.006±0.004
Overall	21	7	6	0.562±0.121	0.002±0.004	18	5	4	0.405±0.143	0.007±0.003	24	22	16	0.916±0.026	0.011±0.002

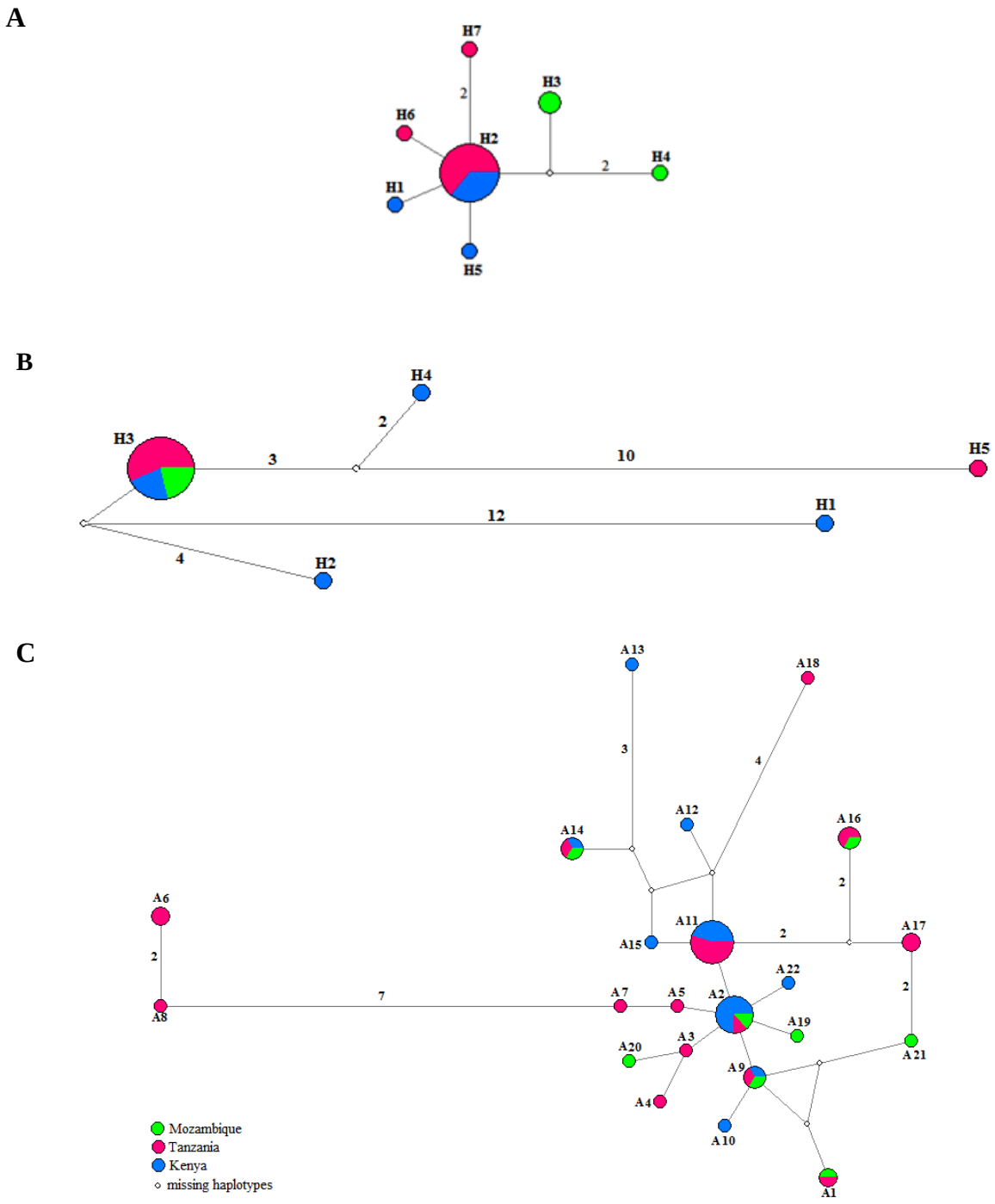


Figure 4.5. Median-joining haplotype networks derived from the cytochrome *b* (A), NADH-2 (B) and S7 intron 1 (C) data sets for *Lutjanus lutjanus* Bloch, 1790. The size of the node (haplotype or allele) corresponds to the frequency of that haplotype or allele and the colours represent the occurrence of that haplotype or allele at different localities. The numbers on branches indicate mutational differences if more than one step.

other alleles in the region, including alleles from the same localities. The highest proportion of private alleles was found in Mozambique (3; 37.5%), followed by Tanzania (8; 36.4%) and then Kenya (4; 22.2%). Although the networks displayed genetic diversity, no clear geographic structuring was observed, with the exception indicated in the cytochrome *b* data.

Genetic diversities haplotype (H_D), allelic (A_D) and nucleotide (π) diversities are presented in Table 4.5. Overall, allelic diversity was high for S7 intron 1 (0.916±0.026), with cytochrome *b* having a slightly higher haplotype diversity (0.562±0.121) than NADH-2 (0.405±0.143). Generally, nucleotide diversity was higher for S7 intron 1 (0.011±0.002) and NADH-2 (0.007±0.002) than cytochrome *b* (0.002±0.004). The diversities for the individual localities were generally high for S7 intron 1 and moderate for cytochrome *b*, while NADH-2 showed a much wider range across the various localities.

Population differentiation was examined between Kenya and Tanzania, as these were the only localities with a sufficient number of samples. Both mitochondrial gene fragments gave non-significant ($P > 0.05$) Φ_{ST}-values (0.008 and 0.020 for cytochrome *b* and NADH-2, respectively), indicating little differentiation among the two localities. However, the more variable S7 intron 1 gene detected moderate differentiation among these localities with a significant Φ_{ST} –value (0.056; $P < 0.05$). Isolation by distance tests could not be performed due to insufficient data.

Analysis of Molecular Variance (AMOVA) was conducted to detect if there was differentiation among localities. For cytochrome *b*, a slightly lower component of variation was due to significant differentiation among localities (45.56%, $P < 0.001$) than within localities (53.44%, $P < 0.001$) (Table 4.6). The former component could be detecting the apparent differentiation of Mozambique as observed in the haplotype network. The NADH-2 data revealed most of the variation to be partitioned within localities. A low but significant component of variation (4.97%, $P < 0.05$) was due to differentiation among localities in the S7 intron 1. This could be due to the divergent alleles from Tanzania as observed in the network. Most of the variation (95.03%; $P < 0.001$) was partitioned within localities (Table 4.6).

Table 4.6. AMOVA results examining the partitioning of variation in *Lutjanus lutjanus* Bloch, 1790 individuals among Mozambique, Tanzania and Kenya at two hierarchical levels based on sequence data of the cytochrome *b*, NADH-2 and S7 intron 1 gene fragments.

Source of variation	d.f.	Sum of squares	% variation	*P*-value
Cytochrome *b*				
Among localities	2	4.606	45.56	**<0.001**
Within localities	18	6.442	53.44	**<0.001**
NADH-2				
Among localities	2	3.167	-4.14	0.630
Within localities	15	30.389	104.14	**<0.001**
S7 intron 1				
Among localities	2	7.524	4.97	**< 0.05**
Within localities	45	95.101	95.03	**<0.001**

Phylogenetic trees were generated to examine relationships among individuals and localities. The cytochrome *b* data (594 bp) included 518 conserved sites and 76 variable sites, with six parsimony informative characters and 70 autapomorphies. The NJ tree is shown in Figure 4.6, representing the relationships among individuals and localities. This tree is represented to ensure a consistent comparison with the results from *L. bohar*. In the NJ tree, the ingroup was not supported with respect to the outgroup. The tree indicated, in agreement with the haplotype network, the presence of Mozambican individuals being differentiated from the main Tanzanian/Kenyan clade.

The model of evolution as determined by AIC in ModelTest 3.8 (Posada & Crandall 1998; Posada 2006) was HKY for cytochrome *b*. This model was enforced in the construction of the ML tree. The model parameters were: base frequencies of A = 0.24, C = 0.32, G = 0.15 and T = 0.29, and a transition:transversion ratio (Ti:Tv) of 2.49. The ML tree (-lnL = 1098.78, tree not shown) failed to recover any clades and all the individuals formed a polytomy. The MP analysis produced nine equally parsimonious trees with parameters: TL = 9, CI = 0.78, HI = 0.22, RI =

Figure 4.6. Outgroup-rooted neighbor-joining (NJ) tree depicting relationships among *Lutjanus lutjanus* Bloch, 1790 alleles for the S7 intron 1 gene fragment. Bootstrap support (MP, NJ and ML) and Bayesian inference (BI) posterior probabilities are indicated on the branches, respectively. The scale-bar indicates the estimated evolutionary distance.

0.71 and RC = 0.56. The resulting strict-consensus tree (tree not shown) was similar to the NJ tree. The results of the BI analysis were similar to the results of the NJ and MP analyses. A tree generated from combined data analysis was not generated because of the reasons stated above.

The NADH-2 data set (571 bp) contained 539 conserved sites and 32 variable sites, with nine parsimony informative characters and 28 autapomorphies. The NJ tree (tree not shown) showed one individual from Kenya (H1 from the haplotype network) being basal to all other individuals, which formed a polytomy. The TIM model was the best model for the data set. The model parameters were base frequencies of A = 0.27, C = 0.35, G = 0.12 and T = 0.25, and a substitution rate matrix of A↔C = G↔T = 1.00, A↔T = C↔G = 1.60, A↔G = 4.86 and C↔T = 2.59. This ML tree (-lnL = 1501.88, tree not shown) was similar to the NJ tree. The MP analysis resulted in nine equally parsimonious trees with parameters: TL = 15, CI = 0.73, HI = 0.27, RI = 0.56 and RC = 0.41. The strict-consensus tree (tree not shown) resulted in a polytomy. The results of the BI analysis were similar to the results of MP analysis.

After phasing, the S7 intron 1 data (392 bp) resulted in 48 constituent alleles, 362 conserved sites and 30 variable sites, where 21 were parsimony informative characters and nine were autapomorphies. The NJ analysis (tree not shown) recovered a few relationships among Tanzanian alleles that were fairly-well supported, but with the majority of alleles forming a polytomy. The model of evolution was F81 + G with model parameters of A = 0.24, C = 0.18, G = 0.26 and T = 0.33, and a gamma distribution of rate variation (α) of 0.39. The ML tree (-lnL = 624.00) was similar to the NJ tree in that the same clades containing Tanzanian alleles were recovered, but these were poorly supported. The MP analysis resulted in 91 equally parsimonious trees with the parameters: TL = 21, CI = 0.62, HI = 0.38, RI = 0.89 and RC = 0.55. The resulting strict-consensus tree (tree not shown) was generally similar to the NJ and ML trees. Bayesian analysis recovered the same clades containing Tanzanian alleles, but these clades were moderately-well supported. Similar to the *L. bohar* phylogenetic results, the trees generally presented no clustering of individuals from the same locality, indicating a general lack of spatial genetic structure among the localities examined with the exception of Mozambique in cytochrome *b* data.

4.4 Discussion

Analyses of mitochondrial markers (cytochrome *b* and NADH-2) and a nuclear marker (S7 intron 1) for *L. bohar* indicated a high level of haplotype and allelic diversity and generally low nucleotide diversity in the region. These diversities are similar to those of crimson snapper *Lutjanus erythropterus* in East Asia (Zhang *et al*. 2006). Only a few haplotypes were widely shared across the region (three in cytochrome *b*, two in NADH-2 and one in S7 intron 1) and these were not always shared among all localities. One haplotype from Australia (cytochrome *b* data) was very divergent from other the Australian haplotype, which was placed among the WIO haplotypes. The BLAST search of both Australian sequences positively identified these sequences as *L. bohar*, however, there were no specimens or photographs to confirm the BLAST results. Most of the pairwise Φ_{ST} –values for mitochondrial genes were low and non-significant. The Φ_{ST} –values for the nuclear gene were higher than for the mitochondrial genes and revealed the significant differentiation between Mozambique and Maldives from each other. AMOVA revealed that a low and non-significant component of variation was associated with differentiation among regions, while most of the variation was partitioned among individuals. From the phylogenetic relationships, cytochrome *b* indicated the divergence of one Australian haplotype from all other haplotypes, with S7 intron 1 recovering clades containing Maldives and Mozambique alleles, respectively.

In *Lutjanus lutjanus*, analyses of the three markers revealed moderate haplotype diversity for the mitochondrial markers (cytochrome *b* and NADH-2) and high allelic diversity for nuclear (S7 intron 1) marker. One haplotype of each mitochondrial marker and three alleles in the nuclear marker were shared among the sampled localities. Similar to *L. bohar*, both mitochondrial genes had low nucleotide diversities with the nuclear gene having higher nucleotide diversity. The pairwise Φ_{ST} –values were very low and non-significant for mitochondrial genes (comparing only Kenya and Tanzania due to sample sizes). The nuclear gene indicated low but significant differentiation between Kenya and Tanzania. Across the markers, AMOVA revealed that a low and significant component of variation was associated with differentiation among regions, with the exception of NADH-2, which was low but non-significant. A large and significant component of variation was associated with differentiation among individuals within localities. Across the

markers, the haplotype networks and phylogenetic trees indicated the differentiation of Mozambique with cytochrome *b*, while S7 intron 1 recovered clades containing some Tanzanian alleles. This differentiation may be a signal picked up by the AMOVAs, which indicated a high and significant proportion for the among region component of variation in cytochrome *b* and a very low proportion for the S7 intron 1. Overall, these results suggest that there is differentiation between Maldives and Mozambique in *L. bohar* and between Tanzania and Kenya, as well as Mozambique, in *L. lutjanus*. Although every effort was made to collect as many samples of the latter as possible from across the region, samples could only be found in three localities and this warrants extreme caution when interpreting these results.

The high haplotype diversities observed in *L. bohar* are generally similar to those observed in other lutjanids such as *L. fulviflamma* (Chapter 3), *L. kasmira* (Gaither *et al*., 2010a, b; Muths *et al*., 2012) and *L. fulvus* (Gaither *et al*., 2010b). The low genetic diversities observed in *L. lutjanus* are similar to those of the jobfish *Pristipomoides filamentosus* (Gaither *et al*., 2011a). The few studies previously conducted in the WIO demonstrated high levels of genetic connectivity (Dorenbosch *et al*., 2006; Ragionieri *et al*., 2010; Visram *et al*., 2010a; Muths *et al*., 2012). In each of these studies, however, there were cases of restricted differentiation. Accordingly, this study demonstrated pattern of differentiation of Mozambique (observed in both *L. bohar* and *L. lutjanus*) and Maldives (observed in *L. bohar*). Differentiation of Mozambique from South Africa has been found in the spiny lobster *Palinurus delagoae* (Gopal *et al*., 2006). Besides currents, local adaptation may influence and contribute to the differentiation of Mozambique in these species, including *L. fulviflamma* (see Chapter 3). The differentiation of Maldives in *L. bohar* is probably due to isolation by distance, but this hypothesis could not be confirmed by a Mantel test due to insufficient data. This differentiation could also result from local processes or species behaviour. Similarly, this differentiation of Maldives is in agreement with the results found for *Abudefduf vaigiensis* and *Epinephelus merra* (Gouws *et al*., 2011), and four species of damselfishes: *Stegastes nigricans*, *Chrysiptera biocellata*, *C. glauca* and *C. leucopoma* (Lacson & Clark, 1995). Overall, both snapper species demonstrated high levels of genetic connectivity in the region. Lutjanids are characterised by high fecundity, spawn pelagic eggs and have pelagic larvae with the ability to swim (Grimes, 1987; Fisher *et al*., 2005). These characteristics and the fact that spawning occurs during the Northeast Monsoon period could enhance and influence

dispersal ability. The pelagic larval stage of lutjanids can last up to 38 days in some species (Zapata & Herron, 2002), which is sufficient for eggs and larvae to be carried by ocean currents to distant localities, maintaining connectivity. This pelagic duration further allows new recruits to reach considerable size before settlement, increasing survival (Gaither *et al*., 2011a).

Pandolfi's (1992) and Santini and Winterbottom's (2002) vicariant biogeographic hypotheses were tested as distinct scenarios. Considering Santini and Winterbottom's (2002) hypothesis, differentiation would be expected among localities of the Mozambique Basin, Somali Basin, Mascarene Plateau and Maldives/Chagos Laccadive Ridge. In accordance with this hypothesis, differentiation of Mozambique (representing Mozambique Basin) and Maldives (representing Maldives/Chagos Laccadive Ridge) was observed. Santini and Winterbottom (2002) proposed that the central Indian Ocean (Maldives in this case) is inaccessible to many reef fish species because of the large expanses of water towards this region. Complex oceanographic features within the Mozambique Channel (Procheş & Marshall, 2002; Bourjea *et al*., 2007) may influence the differentiation of the Mozambique locality. Biogeographic hypotheses for *L. lutjanus* were not tested because samples only came from three localities.

Catch statistics from the artisanal, recreational and commercial fisheries are generally scarce for both *L. bohar* and *L. lutjanus*. Total catch statistics for Madagascar and Mozambique have not been kept for over a decade, while South African, Seychelles' and Tanzanian catches are generally declining, and Kenyan catches are fluctuating (FAO, 2011b). In all these countries, 45% of total marine landings are unidentified, representing the poor quality of catch data in the WIO (FAO, 2011b). With regard to the current species, information shows that *L. bohar* landings have declined to 44.9 tonnes in the Seychelles (Marriot & Mapstone, 2006; Marriott *et al*., 2007). *Lutjanus lutjanus* catch landings have not been recorded since Allen's (1985) statistics from the FAO. The difficulty in catching these species in the WIO for this study could result from many factors, singly or in combination, including using inappropriate fishing gear or sampling approaches, sampling from incorrect habitats or at the incorrect time of the year. To manage these species, information about their spawning grounds, ecology, and their stock structure is critical. In the present study, the stock structure could not be determined accurately due to low sample sizes. As observed above, this study identified differentiation between Mozambique and

Maldives in *L. bohar*, and between Tanzania and Kenya in *L. lutjanus*, but these were not recovered with all the markers. Structure among these localities and others may suggest different stocks for these species. However, independent management of these stocks (if present) will be required, especially for *L. bohar*, which is long-lived and slow-growing, and very susceptible to overfishing. It is also necessary to understand the intrinsic features (life history, reproductive biology and ecology) of these species to make informed management decisions. This knowledge is scant and more direct studies (tagging, telemetry and propagule movement) are needed to understand the intrinsic features in order to have more confidence in the patterns observed.

CHAPTER FIVE

PHYLOGENETIC RELATIONSHIPS IN THE GENUS *LUTJANUS* WITH A FOCUS ON THE POSITION OF THE WESTERN INDIAN OCEAN SNAPPERS IN RELATION TO THE INDO-PACIFIC

5.1. Introduction

Fishes of the family Lutjanidae (snappers) are widely distributed throughout the world's marine environments (see Chapter 2). Snappers are mostly reef-dwelling marine fishes, with three Indo-Pacific (IP) species inhabiting freshwaters and the juveniles of several species inhabiting mangroves, estuaries and the lower reaches of freshwater streams (Allen, 1985). Most lutjanids are carnivorous and feed primarily on fishes, crustaceans, molluscs and urochordates (Allen, 1985, Smith & Heemstra, 1986). Miller and Cribb (2007) considered there to be six subfamilies, Lutjaninae, Paradicichthyinae, Etelinae, Apsilinae, as well as the Caesioninae and Gymnocaesioninae, and 21 genera and 123 species in the family. However, taxonomic uncertainty exists regarding this family. Much debate stems around the relationship between the Lutjanidae and Caesionidae, whether they should be treated as separate families or whether the Caesionidae should be placed as a subfamily within the Lutjanidae (Johnson, 1980, 1993; Carpenter, 1990). Many authors (Leis, 1987, 2005; Johnson, 1993; Reader & Leis, 1996) disagree with the separation of the two families because it renders the Lutjanidae paraphyletic. Johnson (1980) and Carpenter (1990) separated the two families based on the Caesionidae's adaptation to a planktivorous feeding mode and modifications in the upper jaw structure. Based on larval morphology and jaw musculature, the Caesionidae has been placed as a subfamily within the Lutjanidae (Johnson, 1993; Reader & Leis, 1996). However, there are not many clear synapomorphies characterising either group (Allen, 1985). Miller and Cribb's (2007) analysis of genetic data placed the Caesionidae within the Lutjanidae, further expanding this debate.

The genus *Lutjanus* is the largest in the subfamily, with 67 recognised species (Guo *et al*., 2007). Twenty-seven species of this genus are known to inhabit the Western Indian Ocean (WIO) (Allen, 1985). Most species in this genus are long-lived, slow growing and important to artisanal,

recreational and commercial fisheries (Allen, 1985). They provide a significant food source for developing countries (Blaber *et al*., 2005).

Guo *et al*. (2007) and Miller & Cribb (2007) looked at phylogenetic relationships among snapper species. Guo *et al*. (2007) determined the relationships of the 12 snappers (genus *Lutjanus*) from the South China Sea (SCS) using sequence data from the cytochrome *b* (cyt-*b*) and cytochrome *c* oxidase II (COII) genes. The monophyly of the Lutjanidae was not supported, but the monophyly of individual species was strongly supported. These authors also found unexpected relationships among *L. ophuysenni* and *L. vitta*, and among *L. argentimaculatus* and *L. erythropterus* and concluded that these were due to introgression caused by hybridisation. Miller and Cribb (2007) examined the relationships of the IP snappers, including the subfamilies Lutjaninae, Paradicichthyinae, Apsilinae and Caesioninae, using cytochrome *b* and 16S rDNA gene fragments. Their results nested the Caesioninae within the Lutjanidae, supporting the view that Caesioninae should be treated as subfamily within the Lutjanidae. The subfamilies Apsilinae + Paradicichthyinae formed a clade and this was placed as a sister taxon to the Lutjaninae. They further found evidence that the western Atlantic snappers (*Lutjanus campechanus, L. synagris*, and *Rhomboplites aurorubens*) included in their analysis were derived from the IP. Allen (1985) and Allen and Talbot (1985) inferred lutjanid relationships and identified groups based on general morphology and external colouration. These groups included the "blue-lined group" (*Lutjanus kasmira*, *L. bengalensis*, *L. notatus* and *L. quinquelineatus*), the "black-spot group" (*Lutjanus fulviflamma, L. ehrenbergii*, *L. johnii*, *L. fuscescens, L. russellii* and *L. monostigma*), the "yellow group" (*Lutjanus adetii*, *L. lutjanus, L. madras, L. vitta* and *L. mizenkoi*) and the "red group" (*Lutjanus erythropterus, L. gulcheri*, *L. malabaricus, L. sanguineus* and *L. timorensis*). *Lutjanus bohar*, *L. gibbus* and *L. sebae* are considered "red group" species (Newman & Dunk, 2002; Marriot & Mapstone 2007), but phylogenetic relationships do not always put them among the members of the "red group" (Miller & Cribb, 2007). Nevertheless, Miller and Cribb's (2007) study supported these relationships, where members of the "black-spot" and the "blue-lined" groups each clustered together.

Regional genetic differentiation among lutjanids has been demonstrated in the WIO. This includes differentiation of South Africa, Mozambique and Mauritius from other WIO localities in

L. fulviflamma (Chapter 3), the differentiation between Mozambique and Maldives in *L. bohar* and between Tanzania and Kenya in *L. lutjanus* (both in Chapter 4). Muths *et al*. (2012) detected differentiation of Mauritius and Moroni in *L. kasmira*, while Gaither *et al*. (2010b) did a genetic evaluation of *L. kasmira* across the IP and detected low but significant differentiation of Sodwana Bay and Diego Garcia within the WIO. These studies illustrate differentiation over a range of spatial scales in the WIO for a few snapper species. It will be interesting to see if there will be evidence of genetic differentiation over a larger spatial scale, between WIO and IP conspecifics. Differentiation of the WIO from the larger IP has been observed in many reef fishes (McMillan & Palumbi, 1995; Planes & Fauvelot, 2002; Bay *et al*., 2004; Leray *et al*., 2010), shrimps (Duda & Palumbi, 1999) and mangrove crabs (Ragionieri *et al*., 2009).

This study included the same taxa and the data generated in the studies by Guo *et al*. (2007) and Miller and Cribb (2007), and additional data and some taxa not included in their studies. The primary aims of this study were to (1) determine the placement of the WIO representatives of the widespread taxa and WIO endemics, (2) determine the phylogenetic relationships among snappers and the placement of newly included taxa, (3) determine the extent of differentiation between conspecifics from the WIO and the IP, and (4) determine the position of the Caesioninae. This study will further examine if there is concordance between morphology and genetics through the examination of the complexes discussed above. This study also aims to determine if the WIO endemics form a monophyletic clade similar to the Atlantic species (Miller & Cribb, 2007).

5.2. Material and Methods

5.2.1. Sampling and data analysis

Tissue, primarily muscle and fin clips, of *Lutjanus* species collected from the WIO were obtained from the National Fish Collection (SAIAB) and by active sampling at various localities in the WIO (see previous chapters). Tissues of specimens from regions outside the WIO were obtained from the Australian Museum, the Northern Territory Art Gallery and Museum (Australia), and the University of Kansas (Appendix III). The same sample was used to generate both the 16S

rDNA and COII data for all taxa sequenced. Miller and Cribb's (2007) and Guo *et al*.'s (2007) sequence data were downloaded from GenBank (Appendix V). Sequences of an additional gene fragment, cytochrome *c* oxidase I (COI), were downloaded from the Barcode of Life Database (BOLD; Appendix IV) and GenBank databases (Appendix V). These included *Lutjanus* and representatives of the Caesionidae from the IP. This gene fragment was included because data were available for a wide range of snappers collected from across the WIO. Species included here but not included in Guo *et al*.'s (2007) and Miller and Cribb's (2007) studies are *L. bengalensis*, *L. ehrenbergii*, *L. gulcheri*, *L. lutjanus*, *L. notatus* (a WIO endemic), *L. rivulatus*, *L. sanguineus* (WIO endemic) and *L. timorensis*. Species included in the previous studies for which WIO representatives are included here are *L. argentimaculatus*, *L. bohar*, *L. erythropterus*, *L. fulviflamma*, *L. fulvus*, *L. gibbus*, *L. johnii*, *L. kasmira*, *L. monostigma*, *L. quinquelineatus*, *L. russellii*, *L. sanguineus*, *L. sebae* and *L. vitta*. This represents 22 of the 27 species known to occur in the WIO.

Genomic DNA was extracted using commercial kits from samples of the included taxa. Each gene region (16S rDNA and COII) was amplified by PCR, purified and sequenced (see Chapter 2 for details). PCR conditions for the amplification of the gene regions are listed in Table 5.1. These gene fragments and primers were used to complement and expand Guo *et al*.'s (2007) and Miller and Cribb's (2007) data sets. See Chapter 2 for details of sequence editing and alignment.

Table 5.1. Thermocycling regimes for the amplification of the respective mitochondrial gene regions (16S rDNA and COII) amplified specifically for this study for *Lutjanus* species.

Gene region	PCR thermocycling profile					
	Stage 1	Stage 2				Stage 3
	Initial denaturing	Denaturing	Annealing	Extension	Cycles	Final extension
16S rDNA	96 °C, 3 min	94 °C, 50 sec	50 °C, 30 sec	72 °C, 50 sec	30	72 °C, 10 min
CO-II	94 °C, 1.2 min	94 °C, 45 sec	54 °C, 45 sec	72 °C, 1 min	35	72 °C, 10 min

The relationships among individuals were determined by constructing phylogenetic trees. *Lethrinus obsoletus* (Lethrinidae) and *Emmelichthys struhsakeri* (Emmelichthyidae) were used as outgroups for both the 16S and COII analyses, while *E. struhsakeri* was used as an outgroup for the COI analyses because *L. obsoletus* COI data could not be found in GenBank. These species were used as outgroups based on their hypothesised close relationship to the Lutjanidae (Johnson 1980). Support for the relationships was determined by bootstrapping (as before), using 10 replicates for ML analysis. Bayesian analyses were run over 5 million generation. See Chapter 3 for further details.

5.3. Results

5.3.1. Cytochrome *c* oxidase I (COI)

The COI sequences from 43 individuals were 517 nucleotides long. This data included sequences from individuals from the WIO downloaded from the BOLD database, and IP individuals downloaded from GenBank. The BI tree (Figure 5.1) shows the ingroup was not supported with respect to the outgroup, *Lethrinus obsoletus*. The Apsilinae (*A. rutilans* and *A. virescens*) appears paraphyletic with respect to Lutjaninae + Caesioninae, but this is not statistically supported. The monophyly of Lutjaninae + Caesioninae was poorly supported (67/86/--/0.73 for MP, NJ, ML and BI, respectively). Although species clades were strongly supported with 100% bootstraps for MP, NJ and ML, respectively, and 1.00 BI support, there was no statistical support for relationships among species. These supported clades included the *Lutjanus argentimaculatus*, *L. bohar*, *L. gibbus*, *L. lutjanus* and *L. sebae* clades. *Lutjanus analis* and *L. madras* clades were strongly supported, but they included individuals from one ocean region only. A *Lutjanus ehrenbergii* clade was retrieved, but not supported. *Lutjanus bengalensis* individuals did not form a clade.

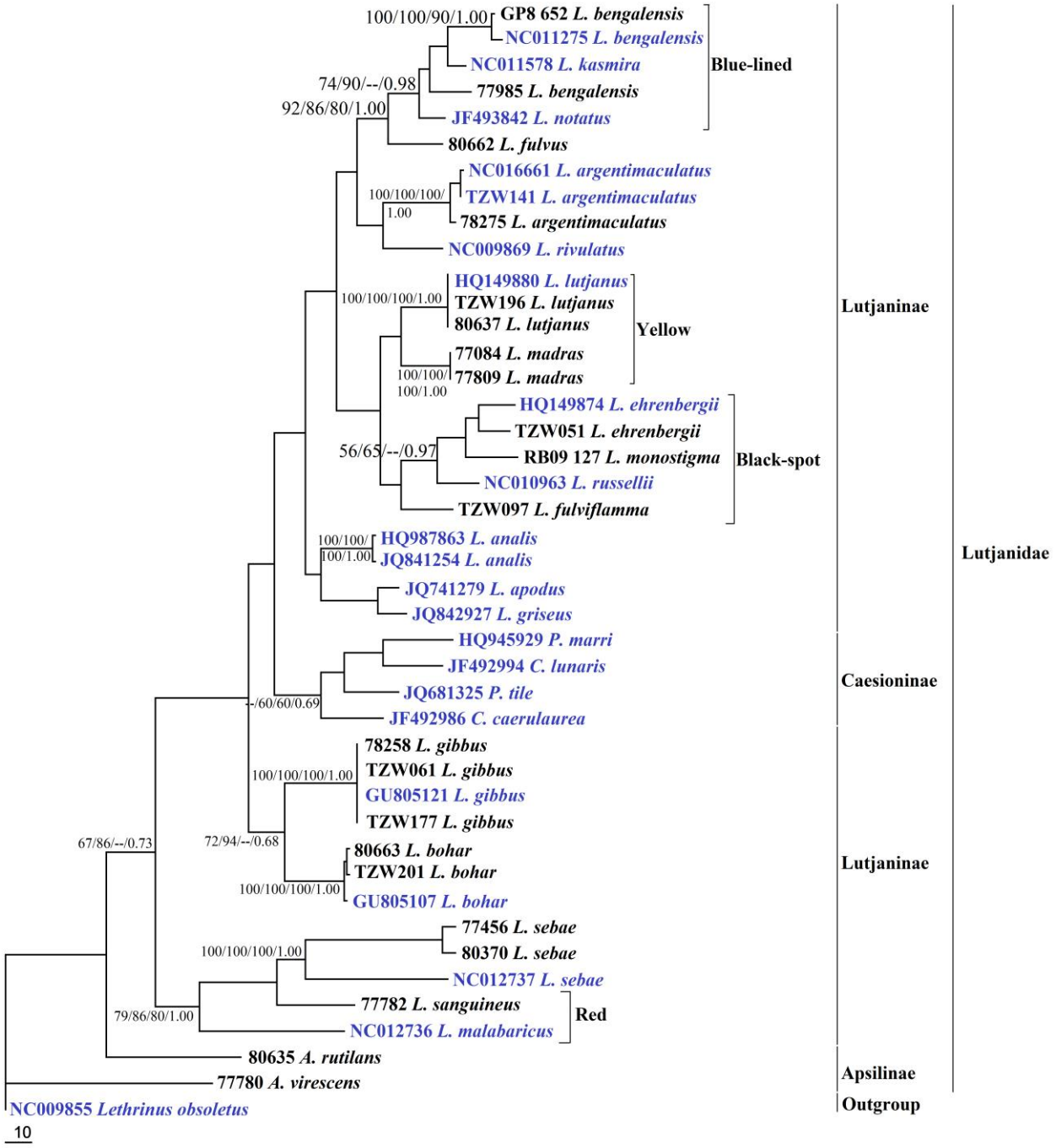


Figure 5.1. Bayesian inference tree derived from the COI data depicting relationships among snappers. Bootstrap support (MP, NJ, and ML) and Bayesian Posterior Probabilities (BI) are indicated on the branches, respectively. The blue text indicates individuals from the Indo-Pacific and the black text represents the Western Indian Ocean individuals. Morphological/external colouring groupings are indicated. The scale bar indicates the estimated evolutionary distance.

Some of the genetic relationships corresponded to morphology/external coloration. The WIO endemic (*L. notatus*) belonged to the "blue-lined group". This clade was moderately-well supported (74/90/--/0.98 for MP, NJ, ML and BI, respectively). *Lutjanus fulvus* was closely related to this clade (see below). The clade containing *L. ehrenbergii*, *L. russellii*, and *L. monostigma* and *L. fulviflamma* (the "black-spot group") was only supported by BI (0.97), but with the *L. fulviflamma* representative found outside this clade. *Lutjanus sanguineus* (WIO endemic) formed a clade with *L. malabaricus* ("red group"), as well as with *L. sebae*; this clade was moderately-well supported (79/86/80/1.00). *Lutjanus bohar* and *L. gibbus* formed a poorly supported clade (72/94/--/0.68) away from the "red group" clade.

The ML tree (-lnL = 2019.16, tree not shown) was constructed to examine the relationships among individuals. The Akaike Information Criterion (AIC) identified TrN+I+G as the best evolutionary model for the data set. Model parameters were as follows: gamma parameter (α) = 2.16, with nucleotide composition of A = 0.29, C = 0.31, G = 0.12 and T = 0.29, a nucleotide substitution rate matrix of A↔C = G↔T = 1.00, A↔G = C↔T = 15.90 and A↔T = C↔G = 1.80, and a proportion of invariable sites (I = 0.66). The relationships among species were poorly supported (see below for subfamily relationships). The NJ tree (tree not shown) was similar to the BI tree presented.

The MP alignment yielded 206 variable sites, with 193 parsimony informative characters and 13 autopomorphies. This analysis resulted in nine equally parsimonious trees with these parameters: Tree Length (TL) = 955, Consistency Index (CI) = 0.30, Retention Index (RI) = 0.64 and Rescaled Consistency Index (RC) = 0.33. These low values indicate that characters could be saturated and homoplasious. The resulting strict consensus tree yielded poor resolution of internal nodes, resulting in a large polytomy of the ingroup taxa (tree not shown).

Genetic differentiation was observed between certain WIO and IP conspecifics. These included *L. bohar* (0.27% sequence divergence), *L. argentimaculatus* (0.37%), *L. sebae* (0.77%), *L. bengalensis* (4.2%) and *L. ehrenbergii* (7%). There was no genetic differentiation observed in *L. lutjanus* and *L. gibbus* between the two regions.

The clade containing the Caesioninae was monophyletic, but poorly supported (--/60/60/0.69), and clustered within the Lutjaninae. Within this clade, the monophyly of each of *Pterocaesio* and *Caesio* was not resolved, since individuals from same genus did not cluster together. There was no support for these relationships.

5.3.2. Cytochrome c oxidase II (COII)

Guo *et al*.'s (2007) sequences from 12 *Lutjanus* species (37 individuals) and the outgroup (*Emmelichthys struhsakeri*) were 690 nucleotides long. The present study included 13 species not included previously: *L. bengalensis*, *L. ehrenbergii*, *L. gibbus*, *L. gulcheri*, *L. kasmira*, *L. lutjanus*, *L. monostigma*, *L. notatus*, *L. quinquelineatus*, *L. rivulatus*, *L. sanguineus* and *L. timorensis*, and *Lethrinus obsoletus* as an outgroup. Eight species, *L. argentimaculatus*, *L. bohar*, *L. fulviflamma*, *L. fulvus*, *L. johnii L. malabaricus*, *L. sebae* and *L. vitta* were common to both data sets.

The molecular phylogeny produced using Bayesian inference depicting relationships among snappers is presented in Figure 5.2. The Lutjanidae was moderately-well supported (89/79/--/0.96 for MP, NJ, ML, and BI, respectively), but included the single individual from the Caesioninae (*P. tile*). The WIO snappers clustered with the IP representatives of the same species, with additional taxa grouping in accordance with general morphology and/or external colouration. This inclusion of additional taxa did not alter the groupings found by Guo *et al*. (2007). Generally, individual species were strongly supported with 100% bootstraps for MP, NJ and ML, respectively, and 1.00 BI support. These included the *Lutjanus fulviflamma*, *L. johnii*, *L. russellii*, *L. sebae* and *L. stellatus* clades. Although the *L. argentimaculatus* clade was supported, it included an *L. erythropterus* individual (see below). Those species that were not retrieved as monophyletic included *L. bohar*, *L. fulvus*, *L. vitta* and *L. malabaricus*.

Some concordance between genetics and morphology was observed. The clade containing *L. monostigma*, *L. ehrenbergii*, and *L. fulviflamma* (the "black-spot group") was not supported, and *Lutjanus johnii and L. russellii* were excluded from this clade. *Lutjanus notatus* (WIO endemic)

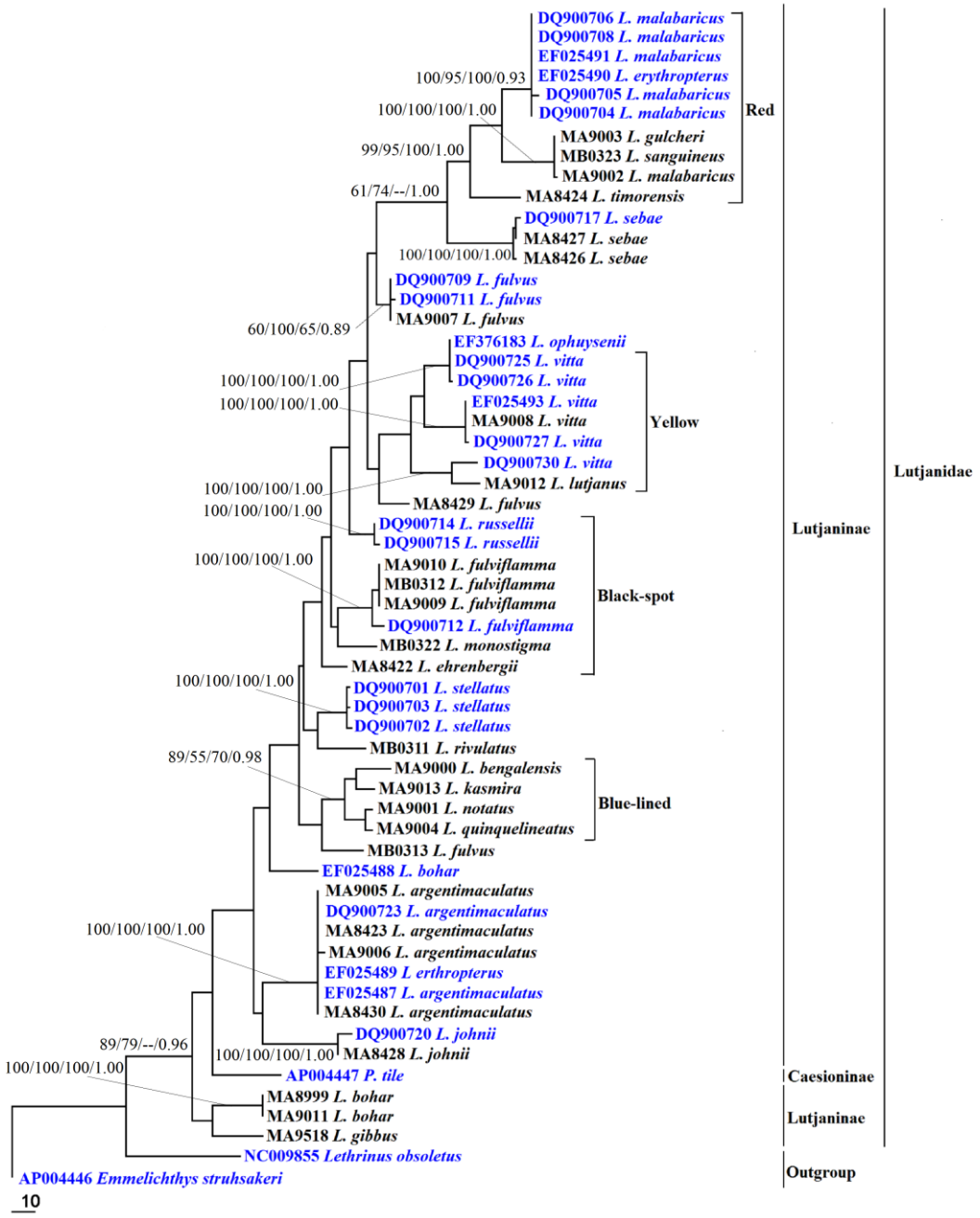


Figure 5.2. Bayesian inference tree representing relationships among Western Indian Ocean and South China Sea snappers derived from the COII data. Bootstrap support and Bayesian Posterior Probabilities (BI) are indicated on the branches (as MP, NJ, ML, and BI, respectively). The blue text indicates Guo *et al.*'s (2007) specimens and the black text represent the Western Indian Ocean individuals, with morphological/external coloration groupings indicated. The scale bar indicates the estimated evolutionary distance.

and one *L. fulvus* (a possible misidentification) individual were placed in a clade containing the "blue-lined group" species, *L. bengalensis*, *L. kasmira* and *L. quinquelineatus*. This clade was relatively well supported (89/52/70/0.98). The remaining *L. fulvus* individuals formed a moderately-well supported clade (60/100/65/0.89). *Lutjanus sanguineus* (a WIO endemic) was placed in a strongly supported (99/95/100/1.00) clade containing the "red group" species, including *L. gulcheri*, *L. malabaricus*, *L. timorensis* and an *L. erythropterus* individual. The inclusion of *L. erythropterus* among the *L. malabaricus* individuals, as well as the inclusion of a *L. erythropterus* individual in the *L. argentimaculatus* clade is dealt with below. Unlike the COI data, the relationship between *L. bohar* and *L. gibbus* was not supported, and one *L. bohar* individual was positioned outside this clade.

For the ML analysis, the AIC was used to identify the best model of evolution in ModelTest. The K81uf+I+G model was identified as the most appropriate model for the data set. The model parameters were a gamma parameter (α) of 1.044, nucleotide composition: A = 0.32, C = 0.31, G = 0.12 and T = 0.26, a nucleotide substitution rate matrix: A↔C = G↔T = 1.00, A↔G = C↔T = 22.64 and A↔T = C↔G = 1.96, and proportion of invariable sites (*I* = 0.59). The ML phylogram (-lnL = 1921.75, tree not shown) had poorly resolved internal nodes. The NJ tree (tree not shown) was similar to the BI tree presented.

The MP alignment (570 bp) of 58 individuals provided 199 variable characters of which 166 were parsimony informative and 33 were autopomorphies. This analysis yielded four equally-parsimonious trees with the parameters: TL = 784, CI = 0.32, RI = 0.75 and RC = 0.38. The resulting strict consensus tree resolved the internal nodes similarly to the ML tree (tree not shown). See below for the positioning of *L. johnii* across the analyses.

Genetic differentiation was observed between WIO and SCS conspecifics. These included *L. sebae* (0.12% sequence divergence), *L. argentimaculatus* (0.12%), *L. johnii* (0.26%), *L. fulviflamma* (0.38%), *L. fulvus* (2.8% among all individuals), *L. vitta* (2.8%) and *L. malabaricus* (6.5%). The highest sequence divergence (10.2%) was observed between *L. bohar* individuals which were in different parts of the tree (see above).

Pterocaesio tile was the only member of the Caesioninae with data for the COII gene fragment available from GenBank , and was nested within the Lutjaninae.

5.3.3. 16S ribosomal-DNA (16S rDNA)

The original data set of Miller and Cribb (2007) contained 572 bp sequences from 29 species from the Lutjanidae (including the subfamilies Lutjaninae, Apsilinae, Paradicichthyinae and Caesioninae) and a wide range of outgroup taxa from the Nemipteridae, Sparidae, Lethrinidae and Haemulidae. However, this study used similar outgroups (*Lethrinus obsoletus* and *Emmelichthys struhsakeri*) to those in the COI and COII analyses for consistency. This study included eight species (*L. bengalensis, L. ehrenbergii, L. gulcheri, L. lutjanus, L. malabaricus, L. notatus, L. rivulatus* and *L. timorensis*) not included in Miller and Cribb's (2007) study. Thirteen species (*L. argentimaculatus, L. bohar, L. erythropterus, L. fulviflamma, L. fulvus, L. gibbus, L. johnii, L. kasmira, L. monostigma, L. quinquelineatus, L. russellii, L. sebae and L. vitta*) were represented in both studies.

In the BI tree presented (Figure 5.3), the monophyly of the Lutjanidae was resolved, but with poor support (--/62/--/0.85 for MP, NJ, ML and BI, respectively). The inclusion of additional taxa did not alter the groupings found by Miller and Cribb (2007). Conspecifics of widespread taxa formed individual clades. Most of these were resolved with good support, with 100% bootstraps for MP, NJ and ML, respectively, and 1.00 BI support. These included *L. argentimaculatus, L. bohar, L. gibbus, L. johnii* and *L. sebae*. There was fair support for the *L. fulviflamma* and *L. fulvus* clades in some analyses. Some individual species did not form monophyletic clades or were not supported. These included *L. erythropterus, L. kasmira, L. quinquelineatus, L. russellii* and *L. vitta*.

As with previous data sets, some concordance was found between genetic relationships and general morphology/external colouration. *Lutjanus notatus* (WIO endemic) was placed in a poorly supported clade (61/64/--/0.56) containing the "blue-lined group", *L. bengalensis, L. kasmira* and *L. quinquelineatus*. Similar to the COI data set, *L. fulvus* was included in a larger clade

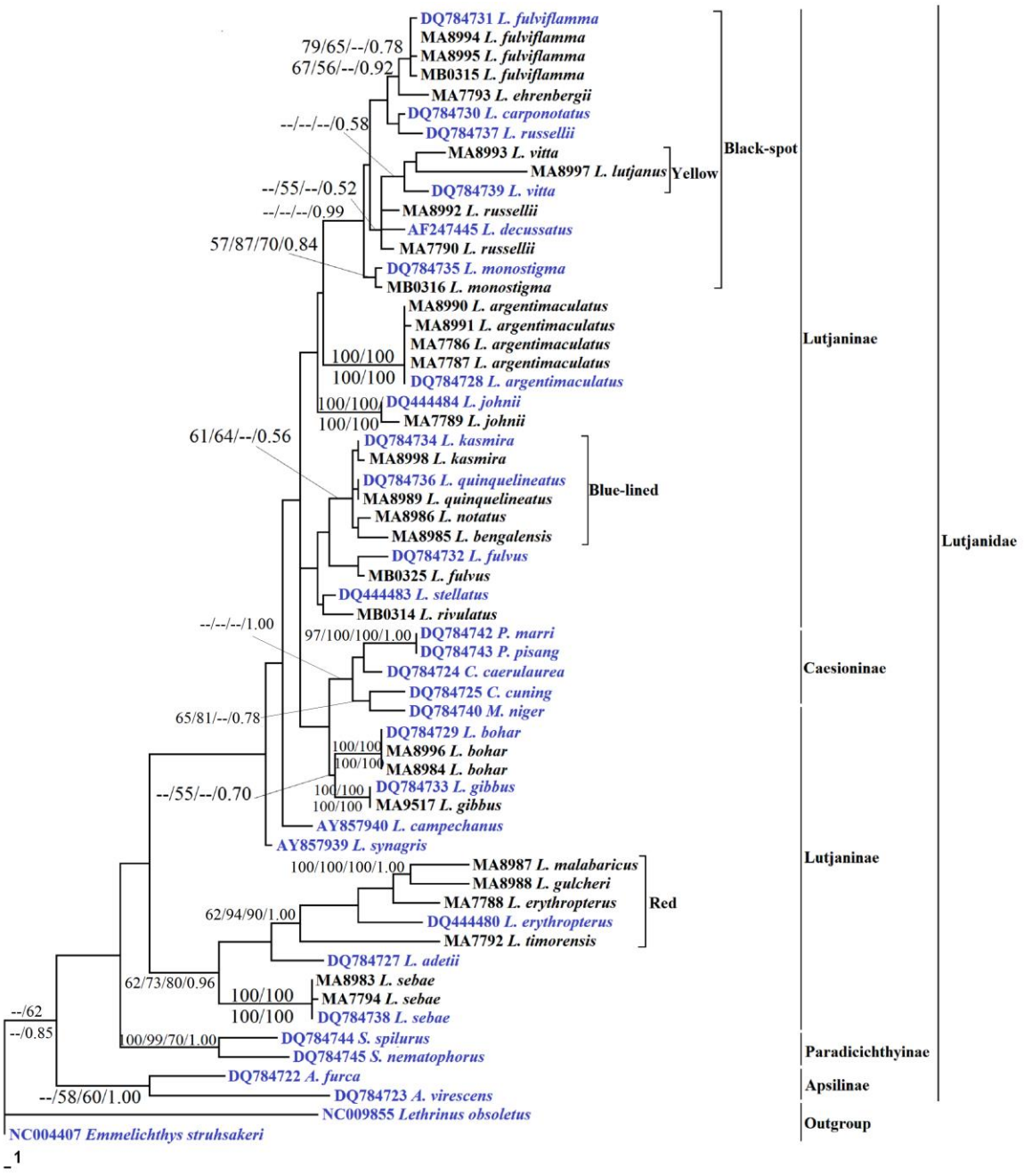


Figure 5.3. Bayesian inference tree-representing relationships among snappers from the Western Indian Ocean and Indo-West Pacific from the 16S rDNA data. Bootstrap support (MP, NJ, ML, and BI, respectively) and Bayesian Posterior Probabilities (BI) are indicated on the branches. The blue text indicates Miller and Cribb's (2007) specimens while the black text indicates the Western Indian Ocean individuals, with morphological/external colouration groupings indicated. The scale bar indicates the estimated evolutionary distance.

containing the "blue-lined group" and two other species (*L. stellatus* and *L. rivulatus*) not belonging to this group. A clade containing *L. malabaricus*, *L. gulcheri*, *L. erythropterus* and *L. timorensis*, as well as *L. sebae* (the "red group"), was moderately-well supported (62/94/90/1.00). Similar to previous data sets, *L. bohar* and *L. gibbus* formed their own clade outside the "red group" and were sister taxa to the caesios, a finding that is consistent across the markers. The clade containing the "black- spot group", *L. fulviflamma*, *L. ehrenbergii*, *L. russellii* and *L. monostigma*, was neither monophyletic nor supported. *Lutjanus johnii* was not included in this clade, while some *L. russellii* individuals formed a clade with *L. carponotatus* (not a "black-spot" member). *Lutjanus dessucatus* (not a "black-spot" member) and members of the "yellow group" (*L. lutjanus* and *L. vitta*) were included in this clade. The relationship between the "yellow group" species was not supported.

In the ML analysis, the AIC selected TVM + I + G as the best evolutionary model for the data set in ModelTest. The model parameters were a gamma distribution (α) of 0.491, nucleotide composition of A = 0.31, C = 0.25, G = 0.21 and T = 0.24, a nucleotide substitution rate matrix of A↔C = 2.65, A↔G = C↔T = 15.29, A↔T = 2.98, C↔G =0.34 and G↔T = 1.00, and a proportion of invariable sites (*I* = 0.473). The ML phylogram (-lnL = 1485.42, tree not shown) resolved some of the internal nodes poorly, resulting in polytomies. The NJ analysis (tree not shown) resolved internal nodes similar to the BI tree presented.

The MP alignment, 484 nucleotides from 59 individuals, provided 142 variable sites, 97 parsimony informative sites and 44 autapomorphies. This analysis resulted in 859 equally parsimonious trees with parameters: TL = 431, CI = 0.37, RI = 0.67 and RC = 0.30. These low values could result from the reason stated above. The resulting strict consensus tree resolved the internal nodes poorly (tree not shown).

Genetic differentiation was observed among some conspecifics from the two regions. These included *L. argentimaculatus* (0.05% sequence divergence), *L. sebae* (0.06%), *L. fulviflamma* (0.15%), *L. kasmira* (0.21%), *L. monostigma* (0.42%), *L. johnii* (0.63%), *L. fulvus* (1.3%), *L. russellii* (2%), *L. vitta* (2.3%) and *L. erythropterus* (2.5%). No differentiation was observed in *L. bohar*, *L. gibbus* and *L. quinquelineatus* between the two regions.

The monophyly of the Caesioninae was not resolved and they appeared paraphyletic, since a close relationship was found between *Macolor niger* (a lutjanid) and *Caesio cuning*. This relationship was fairly-well supported in certain analyses (65/81/--/0.78). The genus *Pterocaesio* was well-supported (97/100/100/1.00); this is contrary to the results found with the COI data. Although the Caesioninae clade included *Macolor*, this clade was supported by a high posterior probability. The inclusion of this clade within the Lutjaninae rendered the Lutjaninae paraphyletic

5.4. Discussion

The phylogenetic analyses in the current study were based on sequences of the COI, COII and 16S rDNA mitochondrial gene fragments. This study included species previously not included in Guo *et al.*'s (2007) and Miller and Cribb's (2007) data sets. Of the 27 lutjanid species (genus *Lutjanus*) occurring in the WIO, 22 were represented in the current study, including two endemic species, *L. notatus* and *L. sanguineus*. This data set also contained representatives of the widespread taxa in order to examine population differentiation between the WIO and the wider IP.

The results presented clearly indicate concordance among the three markers with regard to the general relationships among subfamilies (see below). The inclusion of additional taxa did not alter relationships found by Guo *et al*. (2007) or Miller and Cribb (2007). The WIO individuals are part of the wider IP lutjanid fauna with most conspecifics from the two regions clustering together.

All the markers were able to recover most individual species groups with good support, with the exceptions stated below. This study indicated some evidence of genetic differentiation between IP and WIO conspecifics. All the taxa differentiated in the COII data (except *L. malabaricus*) were also differentiated with the 16S rDNA data (see above). Across the markers, the species that showed differentiation between the WIO and IP included *Lutjanus argentimaculatus, L. fulviflamma, L. fulvus, L. johnii, L. sebae* and *L. vitta*. The species that showed no differentiation

were *L. bohar*, *L. gibbus L. lutjanus* and *L. quinquelineatus*. It appears that an environmental mechanism may be impeding gene flow between WIO and IP across various species (McMillan & Palumbi, 1995; Williams & Benzie, 1996; Avise, 2000). However, it has not been established where this barrier is located or which processes are responsible (Bay *et al*., 2004; Horne *et al*., 2008). More focused studies are needed to examine what these barriers are and where are they most likely to be in reducing free movement of most species between the two regions. This knowledge is further impeded by limited information available about the life history features of many lutjanids. Nonetheless, Gaither *et al*. (2010b) demonstrated that the Indo-Pacific Barrier is not effective in restricting gene flow in *L fulvus* and *L. kasmira*. Besides lutjanids, other reef fishes have shown differentiation between WIO and the IP (see Chapter 3). The lack of differentiation observed in other species could result from life history traits of these species. Generally, some of these species are long-lived (*L. bohar* and *L. gibbus*), have long pelagic larval durations (PLDs) and the larvae have strong swimming abilities allowing for extensive gene flow across the IP. Alternatively, temporal rather than spatial partitioning with periods of expansion and re-colonisation to distant habitats resulting in widespread secondary contact among previously isolated populations could be a result of this connectivity across the IP, as suggested by Klanten *et al*. (2007) and Horne *et al*. (2008).

There were some discrepancies in the COII data. *Lutjanus bohar* was positioned away from its WIO conspecifics, which could possibly have been a misidentification in Guo *et al*.'s (2007) data. Similarly, the inclusion of *L. erythropterus* in the *L. argentimaculatus* clade and *L. erythropterus* in the *L. malabaricus* clade could have been a misidentifications or introgressive hybridisation as suggested by Guo *et al*. (2007). These sequences from Guo *et al*.'s (2007) data were the only COII sequences on GenBank, and this study could not determine the true origin or identity of these species. Nonetheless, hybridisation among lutjanids is a common phenomenon (Loftus, 1992).

According to distribution records of lutjanids (Allen, 1985; Allen & Talbot, 1985), *L. notatus* and *L. sanguineus* are WIO endemics and the expectation was that these individuals would form a monophyletic clade similar to the Atlantic species (Miller & Cribb, 2007), but they did not. Across the data sets, *L. notatus* was included in the "blue-lined group" + *L. fulvus* clade, whereas

L. sanguineus was included in the "red group" + *L. sebae* clade (*Lutjanus sebae* did not amplify for 16S rDNA data). This observation suggests that placement of these WIO endemics is in accordance with general morphology/external colouration.

The inclusion of the Caesioninae within the Lutjaninae renders the Lutjaninae paraphyletic. However, the caesios themselves are not monophyletic. In the 16S rDNA data, *Macolor niger* was nested within the Caesioninae and was a sister taxon to *C. cuning*. Leis (2007) found *Macolor* larvae to possess all the characteristics of lutjanids, but its systematic relationship has not been investigated thoroughly (Miller & Cribb, 2007). *Pterocaesio* was well supported and was sister to *Caesio/Macolor*. This sister-group relationship was supported by a high BI posterior probability. The Caesioninae clade was not supported by COI data. In both the COII and 16S rDNA data, the Caesioninae was a sister taxon to the *L. bohar*/*L. gibbus* clade. The main morphological trend separating the Caesioninae from the Lutjaninae is the separate ossification of the ascending process of the pre-maxilla and the absence of the ethmo-maxillary ligament (Carpenter, 1987, 1990). Other characters include decreased dentition, which is correlated with a specialisation for small prey size, and the forked caudal fins and a fusiform body shape related to mid-water schooling (Carpenter, 1990). However, Allen (1985) found no unique characters distinguishing either the Lutjanidae or Caesionidae. Hence, these adaptations in feeding mode may be taxonomically insignificant. This is because the current results, in agreement with Miller & Cribb's (2007) results, place the caesionids among the lutjanids. It is highly unlikely that their position will change with additional taxa and analyses. Based on the current results, this study supports the views of Johnson (1993), Reader and Leis (1996), and Miller and Cribb (2007) that the Caesionidae should be placed as a subfamily within the Lutjanidae.

Phylogenetic relationships from these mitochondrial data were consistent with morphology/external colouration as suggested by Allen (1985). For example, in all analyses the "blue-lined group" (*L. bengalensis*, *L. kasmira*, *L. notatus* and *L. quinquelineatus*) was monophyletic. The "yellow-group" (*L. vitta*, *L. madras* and *L. lutjanus*) was monophyletic in the analyses of the COI and 16S rDNA data sets. As for the "red group" (*L. erythropterus*, *L. gulcheri*, *L. malabaricus*, *L. sanguineus* and *L. timorensis*) and the "black-spot group" (*L. ehrenbergii*, *L. fulviflamma*, *L. johnii*, *L. monostigma* and *L. russellii*), certain taxa meant to be

included in these clades fell outside, rendering these groups paraphyletic or polyphyletic. For instance, *Lutjanus bohar*, *L. gibbus* and *L. sebae* are regarded as red species (Marriot & Mapstone, 2006; Marriott *et al*., 2007; Nanami & Yamada, 2009); however, these species were not always included in the "red group" clades across the markers. *Lutjanus sebae* was included in the "red group" in the COI data only, while *L. bohar* and *L. gibbus* were always excluded from this group. The latter species pair showed a close relationship to the members of the Caesioninae in both the COII and 16S rDNA analyses. Similarly, in all analyses *L. johnii* did not group with the "black-spot" group. *Lutjanus johnii* is characterised by a large black-spot above the lateral line below the anterior soft dorsal rays, sometimes absent in adults (Allen, 1985), suggesting a close relationship to members of the "black-spot group". The close association of *L. fulvus* to members of the "blue-line complex" is somewhat surprising. Morphologically, *Lutjanus fulvus* is yellowish-tan to brown with a series of narrow yellow or golden-brown stripes (Allen, 1985), which superficially suggests a close relationship to members of the "yellow group". Apart from external colouration, meristic characters appear to reflect the separation of these groups (Allen & Talbot, 1985). In most cases, most members of each of these respective groups have the same number of dorsal and anal spines and rays (see Allen, 1985). These characters are usually fixed early in the development and are independent of fish size (Murta, 2000). The combination of meristic characters with other methods is useful in population differentiation studies (Turan *et al*. 2006). These observations lead to a conclusion that, while external coloration is reliable for inferring phylogenetic relatedness in some lutjanids, it should not be used exclusively without other methods (e.g. genetics) to infer evolutionary history.

CHAPTER SIX

GENERAL DISCUSSION

This study examined population differentiation within Western Indian Ocean (WIO) species of the snappers (Family Lutjanidae) from the genus *Lutjanus*: *Lutjanus bohar*, *L. fulviflamma* and *L. lutjanus*. Both mitochondrial and nuclear genes were employed, and a morphometric component included to examine differentiation in *L. fulviflamma*, to check for concordance between these approaches. The combination of mitochondrial and nuclear genes allows for comparison of the extent of population differentiation recovered with each marker, the discrimination of past colonisation from recent connectivity, and for the detection of deeper phylogenetic relationships due to different mutation rates (Von der Heyden *et al*., 2008; Muths *et al*., 2011). Across the markers, the diversities for the nuclear marker were always higher than mitochondrial markers. *Lutjanus bohar* and *L. fulviflamma* had high genetic diversity indices, whereas *L. lutjanus* was characterised by low genetic diversity indices. The high genetic diversities observed in *L. fulviflamma* and *L. bohar* are similar to those found in *Lutjanus erythropterus* (Zhang *et al*., 2006) and *Lutjanus kasmira* (Gaither *et al*., 2010a; Muths *et al*., 2012), while low diversities observed in *L. lutjanus* are similar to those observed in jobfish (*Pristipomoides filamentosus*: Lutjanidae) (Gaither *et al*., 2011a).

The results of this study clearly presented some evidence of genetic differentiation between WIO and IP. This was most evident in *L. fulviflamma* (Chapter 3) and members of the "blue-lined group" (*L. bengalensis* and *L. kasmira*), "red group" (*L. erythropterus* and *L. malabaricus*), "yellow group'' (*L. vitta*) and "black-spot group" (*L. ehrenbergii*, *L. johnii*, *L. monostigma* and *L. russellii*), as well as in *L. argentimaculatus*, *L. bohar*, *L. fulvus* and *L. sebae*, which do not belong to these groups. The differentiation of the WIO from the IP has been observed in many other reef fishes and invertebrates. This differentiation is generally accepted to be historical, coinciding with fluctuating sea-levels during the Pleistocene, which exposed the Sunda Shelf between Malaysia and northern Australia. This exposure limited water exchange between the Indian and Pacific Oceans (McMillan & Palumbi, 1995; Barber *et al*., 2000; Bay *et al*., 2004; Jeffrey *et al*.,

2007; Gaither *et al*., 2010b, 2011b; Leray *et al*., 2010). In this study, varying levels of differentiation were observed between WIO and IP, these included high levels of differentiation in *Lutjanus fulviflamma, L. fulvus* and *L. vitta*, moderate differentiation in *L. johnii*, and low differentiation in *L. argentimaculatus* and *L. sebae*. Species that showed no differentiation between the two regions included *L. bohar, L. gibbus, L. lutjanus* and *L. quinquelineatus*.

In examining patterns of connectivity and differentiation within the WIO, the current study presented evidence for the differentiation of South Africa, Mozambique and Mauritius in *L. fulviflamma* (Chapter 3). *Lutjanus bohar* showed differentiation between Mozambique and Maldives, while *L. lutjanus* indicated differentiation between Tanzania and Kenya (Chapter 4). Similar patterns of differentiation within the WIO have been established in other species. For instance, Visram *et al*. (2010a) found the differentiation of Kenya and Seychelles from Tanzania and Mauritius in the blue-barred parrotfish *Scarus ghobban*. The authors found that this differentiation was influenced by the South Equatorial Current (SEC) and Equatorial Counter Current (ECC) systems. Ridgway *et al*. (2008) observed differentiation of South Africa from Mozambique in the coral *Pocillopora verrucosa*. This differentiation was over an ecological time scale and was coupled with a genetic diversity gradient from north to south. Gouws *et al*. (2011) demonstrated differentiation of Maldives from other localities of the WIO in *Abudefduf vaigiensis* and *Epinephelus merra*. The authors suggested that this differentiation might be influenced by the geographical isolation of this locality. Muths *et al*. (2011) found restricted connectivity in *Myripristis berndti*, with individual localities along the Mozambique Channel being densely connected as a central region and localities at the extremities, Europa, Kenya and Reunion, being differentiated. In contrast, other studies have demonstrated high connectivity between East Africa (Tanzania and Kenya) and Comoros (e.g. *Lutjanus fulviflamma*: Dorenbosch *et al*., 2006) and across the WIO (e.g. *Lutjanus kasmira*: Muths *et al*., 2012). These and the current study illustrate the complex array of patterns of connectivity and differentiation that are observed in various taxa across the region. Overall, these studies show that oceanic currents, local adaptation and, to some extent, species behaviour enhance and influence differentiation in the WIO. For example, species which are resident as adults, such as *L. kasmira, L. fulviflamma, L. quinquelineatus, L. monostigma* and *L. fulvus*, would enhance population differentiation, whereas roving species like *L. argentimaculatus, L. bohar, L. gibbus* and *L. sebae* (Samoilys & Carlos,

2000) would promote connectivity among populations. Thus, population structure results from the interaction of the intrinsic features of the species, such as ecology, life-history, habitat preference and reproductive biology, and the extrinsic physical features of the environment, such as currents and temperature (Gold & Richardson, 1998).

Lutjanids are highly fecund and spawns pelagic eggs (Grimes, 1987), which would promote connectivity among localities. In addition, larval movement, recruitment and settlement behaviour and adult movement can promote connectivity or differentiation. However, little is known about the early life history and ecology of these species and genetic connectivity is, thus, assumed to be maintained by egg and larval dispersal and by adult migration in *L. bohar* (especially to spawning aggregations: Domeier & Collin, 1997). Several studies (Bernardi *et al*., 2001; Fauvelot & Planes, 2002; Dorenbosch *et al*., 2006; Craig *et al*., 2007; Gaither *et al*., 2011a) have shown that the pelagic larval stage is the main mode of connectivity among localities for many species. Muths *et al*. (2012) proposed that ecological features of *Lutjanus kasmira*, such as habitat preference, competition for space and trophic behaviour, influence and contribute to the genetic connectivity of this species in the WIO. However, information regarding larval dispersal and recruitment behaviour of the three species (*Lutjanus bohar*, *L. fulviflamma* and *L. lutjanus*) is lacking and it would provide more confidence in the patterns observed and inferences made in the current study. Thus, understanding the components of connectivity and differentiation that are attributable to intrinsic and extrinsic features is a challenge (Gouws *et al*., 2011), but are necessary for understanding connectivity and for implementing conservation and management strategies.

Several authors (e.g. Hocutt, 1987; Pandolfi, 1992; Santini & Winterbottom, 2002), considered the vicariant origins for regional faunas and proposed biogeographical regions for the IP, as well as the WIO. The physical features or the historical events that have led to the separation of the biogeographic regions may have influenced population differentiation observed in the current study. According to Santini and Winterbottom's (2002) biogeographic hypothesis, differentiation would be expected among the localities of the Mozambique Basin, Somali Basin, Mascarene Plateau, Maldives/Chagos/Laccadive Ridge, Red Sea and the Arabian Basin. The three species (*Lutjanus bohar*, *L. fulviflamma* and *L. lutjanus*) demonstrated patterns of differentiation

corresponding to these biogeographic regions. Differentiation was observed among the Mozambique Basin (represented by South Africa and Mozambique), the Mascarene Plateau (represented by Mauritius) and the Maldives/Chagos Laccadive Ridge (represented by Maldives). Absence of full concordance with Santini and Winterbottom's (2002) proposed biogeographic regions suggests that, for the studied taxa, other processes may have been significant and perhaps dispersal is an overriding process across certain areas. Other hypotheses (Hocutt, 1987; Pandolfi, 1992) provided a poor fit to the data.

To effectively manage and conserve fisheries resources in the region, an understanding of population genetic structure is necessary. Accordingly, the differentiation observed, as discussed above, may suggest different stocks in the mentioned localities. However, this was not the aim of this study and the present data cannot effectively examine stock structure of these species. If there are different stocks, this would then require further examination of population dynamics for each locality for each species. This would then provide information regarding the ecological independence of subpopulations within each species in these localities. Independent management will then have to be put in place to conserve these species and preserve the genetic integrity of these stocks (if present). *Lutjanus bohar* is most likely to be impacted by over-fishing because the species is long-lived and takes time to reach sexual maturity, while *L. fulviflamma*, which grows rapidly and attains sexual maturity early, may be resilient to exploitation (Kamukuru & Mgaya, 2004; Marriott & Mapstone, 2006; Marriott *et al*., 2007). With the increased fishing pressure, most species are exposed to over-exploitation (Kamukuru *et al*., 2005). This fishing pressure can be alleviated by establishment of Marine Protected Areas (MPAs), which have been suggested for the conservation and management of many fisheries species (Turpie *et al*., 2000; Kamukuru *et al*., 2005; Allen, 2007; Botsford *et al*., 2009; Ungfors *et al*., 2009; Muths *et al*., 2011, 2012), including lutjanids. As illustrated by these authors, MPAs offer a range of benefits including alleviating declining and recovering populations. However, these MPAs will work only if all kinds of fishing, commercial, recreational and artisanal, are carefully managed (see below) (Denny & Babcock, 2004). Although WIO MPAs are largely ineffective (Mora *et al*., 2006), McClanahan *et al*. (2007) demonstrated fish recovery in Kenyan MPAs, influenced by migration of individuals from outside into the reserve. Of the three species, *L. bohar* will have a competitive advantage over the other two species in a no-take MPA due to its longevity. Munga

et al. (2012) observed high abundances of long-lived species, including members of the Lutjanidae, in a no-take Mombasa MPA. Periodic fishing closures and bag limits for fishery sensitive species have been proposed for population recovery in Kenyan MPAs (McClanahan *et al*., 2007). In the Atlantic, Carson *et al*. (2011) demonstrated that MPAs are critical in preserving *Lutjanus analis* spawning aggregations to maintain genetic diversity in this species.

Caution should be taken when interpreting the results of this study due to (1) limitations in sample sizes, particularly for *L. bohar and L. lutjanus*, (2) some of the samples amplified for mitochondrial markers, did not amplify for nuclear markers, and (3) the spatial coverage for these two species, which is a cause for concern. Nonetheless, the results of this study contribute substantially to the understanding of the WIO biogeography. It is clear from the results (particularly for *L. bohar* and *L. fulviflamma*) that increasing sample size or more data may not necessarily change the patterns observed. This study further demonstrated that utilising multiple markers (mitochondrial and nuclear), which provide different properties of evolution and transmission, is important in uncovering the patterns observed. The concordance between the markers strengthens the confidence in the patterns observed, while contradicting patterns could result from features such as hybridisation and introgression, demographic changes or sex-biased dispersal.

According to Allen (1985), these three species of lutjanids (*Lutjanus bohar*, *L. fulviflamma* and *L. lutjanus*) are abundant in the WIO. However, during this study, fewer individuals were collected, particularly of *L. bohar* and *L. lutjanus*. A range of factors (as outlined in Chapter 4) could have resulted in the study not acquiring the desired sample numbers. As such, reservations have to be made over some of the conclusions drawn from certain analyses. Therefore, future research should increase sampling sizes to have a more comprehensive understanding of the phylogeography and biogeography of these species. To make accurate inferences about connectivity, the interaction between the species' intrinsic features (life history, reproductive biology and ecology) and extrinsic features (the environmental features) will need to be given careful consideration in future studies. This, however, will require other methodologies, such as tag-recapture experiments, telemetry and studies of propagule movement, to have greater confidence in what caused the patterns observed. Alternatively, if funding, sample size and time

were adequate, different marker sets could have been examined. These would most likely be microsatellites. These markers are codominant and distributed throughout the genome, offering an advantage over nuclear markers. This suggestion is made because across the data sets, while all samples amplified for cytochrome *b*, fewer individuals amplified for NADH-2 and the fewest for S7 intron 1. Cytochrome *b* was more sensitive to detecting patterns of differentiation, which in most cases, were not observed with NADH-2. The S7 intron 1 provided patterns of differentiation in agreement with cytochrome *b*, and in some instances recovered differentiation not observed with cytochrome *b*. The inclusion of microsatellites would add more confidence and recover more patterns of differentiation, as observed in *Lutjanus kasmira* (Muths *et al*., 2012) study. A suggestion for future studies is to use mitochondrial markers (particularly cytochrome *b*) with microsatellites to recover patterns of differentiation /or connectivity in lutjanids. Adequate sample sizes are needed in order to do more analyses for better confidence in the patterns observed.

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APPENDICES

Appendix I: *Lutjanus fulviflamma* (Forsskål, 1775) samples collected across the Western Indian Ocean and borrowed from Kansas University of Technology and analysed in this study. These samples were used to generate sequence data for Cytochrome *b*, NADH-2 and S7 intron 1 genes. Samples for the morphometric study came from various localities across the Indo-Pacific.

Species	Sample size	Locality name	Country	GPS position	
				Latitude	Longitude
Genetics samples					
Lutjanus fulviflamma	5	Sodwana Bay	South Africa	-27.864999	35.383333
Lutjanus fulviflamma	4	Mthatha River mouth	South Africa	-31.933333	29.183333
Lutjanus fulviflamma	6	Mhlathuze estuary	South Africa	-28.449999	32.416667
Lutjanus fulviflamma	4	Inhambane	Mozambique	-23.864999	35.383333
Lutjanus fulviflamma	2	Pemba Bay	Mozambique	-12.960833	40.507778
Lutjanus fulviflamma	2	Lumbo	Mozambique	-15.010556	40.666389
Lutjanus fulviflamma	4	Maputo Bay	Mozambique	-25.948203	32.679537
Lutjanus fulviflamma	3	Shimoni	Kenya	-4.649999	39.383333
Lutjanus fulviflamma	3	Dabaso	Kenya	-2.683333	39.600000
Lutjanus fulviflamma	2	Anosy	Madagascar	-25.067000	46.931300
Lutjanus fulviflamma	7	Zanzibar	Tanzania	-6.166667	39.183333
Lutjanus fulviflamma	5	Port Louis	Mauritius	-20.185219	57.408478
Lutjanus fulviflamma	2	Baie Ternay	Seychelles	-4.633333	55.366667
Lutjanus fulviflamma	2	Mahe	Seychelles	-4.616667	55.450000
Lutjanus fulviflamma	5	Farasān Island	Saudi Arabia	16.800000	41.900000
Lutjanus fulviflamma	5	Phuket	Thailand	7.883333	98.400000
Lutjanus fulviflamma	1	Tongatapa	Tonga	-21.166667	-175.166666
Morphometric samples					
Lutjanus fulviflamma	1	Kosi Bay	South Africa	-33.599998	26.899999
Lutjanus fulviflamma	3	Kosi Bay	South Africa	-26.883333	32.866665
Lutjanus fulviflamma	6	Durban Bluff	South Africa	-29.850000	31.000000
Lutjanus fulviflamma	2	Bizana Coast	South Africa	-31.566668	29.399999
Lutjanus fulviflamma	4	Sodwana Bay	South Africa	-27.516666	32.683334
Lutjanus fulviflamma		Six Mile Reef	South Africa	-27.625000	32.656666
Lutjanus fulviflamma	2	Xora	South Africa	-32.150002	29.000000
Lutjanus fulviflamma	1	Swartkops River mouth	South Africa	-33.866665	25.633333
Lutjanus fulviflamma	1	Kariega River	South Africa	-33.683334	26.683332
Lutjanus fulviflamma	1	Whale Rock	South Africa	-31.933332	29.216667
Lutjanus fulviflamma	1	Preslies Bay	South Africa	-31.883333	29.266666
Lutjanus fulviflamma	1	Between Goss Bay & Goss Point	South Africa	-31.399999	29.883333
Lutjanus fulviflamma	9	unspecified	South Africa		
Lutjanus fulviflamma	2	Maputo	Mozambique	-25.966667	32.583332
Lutjanus fulviflamma	1	Tekomaji Island	Mozambique	-10.783334	40.650001
Lutjanus fulviflamma	1	Mozambique Island	Mozambique	-15.033334	40.650002
Lutjanus fulviflamma	1	Delagoa Bay	Mozambique	-25.966667	32.583332
Lutjanus fulviflamma	1	Moebase lower mouth	Mozambique	-17.066668	38.683334
Lutjanus fulviflamma	1	Inhaca Island	Mozambique	-26.016666	32.966667
Lutjanus fulviflamma	1	Ponta Milibangalala	Mozambique	-26.450001	32.933334
Lutjanus fulviflamma	1	Baixo Sao Joan	Mozambique	-26.399999	32.916668
Lutjanus fulviflamma	2	Larde Estuary - Lower reaches	Mozambique	-16.524723	39.711113
Lutjanus fulviflamma	1	Inhambane	Mozambique	-23.793283	35.527117
Lutjanus fulviflamma	4	Unspecified	Mozambique		
Lutjanus fulviflamma	1	Zanzibar Island	Tanzania	-6.166667	39.183334
Lutjanus fulviflamma	2	Tanga	Tanzania	-5.116667	39.083332

Lutjanus fulviflamma	2	Nyama Reef 7	Tanzania	-5.100000	39.100000
Lutjanus fulviflamma	5	Chwaka Bay Landing Site	Tanzania	-6.129500	39.466167
Lutjanus fulviflamma	1	Paje	Tanzania	-6.266667	39.550000
Lutjanus fulviflamma	1	Shimoni	Kenya	-4.650000	39.383335
Lutjanus fulviflamma	1	Mombasa	Kenya	-4.083333	39.666668
Lutjanus fulviflamma	2	Mauritius	Mauritius	-20.299999	57.583332
Lutjanus fulviflamma	1	La Morne	Mauritius	-20.469833	57.344300
Lutjanus fulviflamma	4	Mahe	Seychelles	-4.616667	55.450001
Lutjanus fulviflamma	1	Assumption Island	Seychelles	-9.750000	46.500000
Lutjanus fulviflamma	3	Aldabra Island	Seychelles	-9.433333	46.333332
Lutjanus fulviflamma	2	Off Baie Ternay	Seychelles	-4.636600	55.375198
Lutjanus fulviflamma	1	Baie Ternay	Seychelles	-4.641699	55.376099
Lutjanus fulviflamma	3	Seychelles	Seychelles	-3.950000	54.533332
Lutjanus fulviflamma	2	Karaman Island	Yemen	15.350000	42.583333
Lutjanus fulviflamma	2	Makung market	China	23.566668	119.58334
Lutjanus fulviflamma	1	Grande Comore	Comoros	-11.816666	43.016666

Appendix II: *Lutjanus bohar* (Forsskål, 1775) and *Lutjanus lutjanus* Bloch, 1790 samples analysed in the current study collected from various localities in the Western Indian Ocean and borrowed from Kansas University of Technology. These samples were used to generate Cytochrome *b*, NADH-2 and S7 intron 1 sequence data for both species.

Species	Sample size	Locality name	Country	GPS position	
				Latitude	Longitude
Lutjanus bohar	2	Pomene	Mozambique	-22.925278	35.552222
Lutjanus bohar	2	Inhambane	Mozambique	-23.864999	35.383333
Lutjanus bohar	4	Incaha	Mozambique	-26.046883	32.851090
Lutjanus bohar	2	Zanzibar	Tanzania	-6.166667	39.183333
Lutjanus bohar	4	Dabaso	Kenya	-2.683333	39.600000
Lutjanus bohar	2	Picard Island	Seychelles	-9.399486	46.197911
Lutjanus bohar	1	Denis Island	Seychelles	-3.799998	55.555556
Lutjanus bohar	1	Anosy	Madagascar	-25.067000	46.931300
Lutjanus bohar	1	Farasān Island	Saudi Arabia	16.800000	41.900000
Lutjanus bohar	9	Gan	Maldives	7.883333	98.400000
Lutjanus bohar	1	Lizard Island	Australia	-15.933333	124.416667
Lutjanus bohar	1	Great Barrier Reef	Australia	-36.166667	175.416667
Lutjanus lutjanus	3	Off Mozambique	Mozambique	-13.818056	40.618611
Lutjanus lutjanus	11	Zanzibar	Tanzania	-6.166667	39.183333
Lutjanus lutjanus	7	Shimoni	Kenya	-4.649999	39.383333

Appendix III: Samples analysed for the phylogeny chapter (Chapter 5) from various localities in the Western Indian Ocean, and samples borrowed from Art Gallery and Museum of the Northern Territory (NT), Darwin, in Australia. These samples were used to generate COII and 16S sequence data.

Species	Sample size	Locality name	Country	GPS position	
				Latitude	Longitude
Lutjanus argentimaculatus	1	Anosy	Madagascar	-25.061000	46.931300
Lutjanus argentimaculatus	1	Robinson River	Australia	-16.049999	137.266667
Lutjanus argentimaculatus	2	Phuket	Thailand	7.883333	98.400000
Lutjanus bengalensis	1	Cape Vidal	South Africa	-46.333333	51.733333
Lutjanus bohar	1	Pomene	Mozambique	-22.925278	35.552222
Lutjanus ehrenbergii	1	Zanzibar	Tanzania	-6.166667	39.183333
Lutjanus erythropterus	1	Darwin Harbour	Australia	-12.471111	130.847500
Lutjanus fulviflamma	1	Sodwana Bay	South Africa	-27.864999	35.383333
Lutjanus fulviflamma	1	Inhambane	Mozambique	-23.864999	35.383333
Lutjanus gibbus	1	Shimoni	Kenya	-4.649999	39.383333
Lutjanus gulcheri	1	Off Mozambique	Mozambique	-16.384444	40.033333
Lutjanus johnii	1	Barron Island	Australia	-12.166667	132.350000
Lutjanus kasmira	1	Mascare-6	Mauritius	-16.843333	59.593333
Lutjanus lemniscatus	1	Darwin Harbour	Australia	-12.471111	130.847500
Lutjanus lemniscatus	1	Phuket	Thailand	7.883333	98.400000
Lutjanus lemniscatus	1	Pomene	Mozambique	-22.925278	35.552222
Lutjanus lutjanus	1	Off Mozambique	Mozambique	-16.384444	40.033333
Lutjanus madras	1	Phuket	Thailand	7.883333	98.400000
Lutjanus malabaricus	1	Off Mozambique	Mozambique	-16.384444	40.033333
Lutjanus notatus	1	Off Mozambique	Mozambique	-16.384444	40.033333
Lutjanus quinquelineatus	1	Quissico & Zavora	Mozambique	-24.659932	35.083704
Lutjanus sebae	1	Zanzibar	Tanzania	-6.166667	39.183333
Lutjanus sebae	1	Lynedoch Bank	Australia	-10.016667	130.816667
Lutjanus timorensis	1	Lynedoch Bank	Australia	-10.016667	130.816667

Appendix IV: Sequences downloaded from the BOLD database used in the construction of the COI phylogeny in Chapter 5.

Family	Species	Sample size	Sequence name
Lutjanidae	*Aphareus rutilans*	1	80635
	Aprion virescens	1	77780
	Lutjanus argentimaculatus	1	TZW141
		1	78275
	Lutjanus bengalensis	1	GP8625
		1	77985
	Lutjanus bohar	1	80663
		1	TZW201
	Lutjanus ehrenbergii	1	TZW051
	Lutjanus fulviflamma	1	TZW097
	Lutjanus fulvus	1	80662
	Lutjanus gibbus	1	78258
		1	TZW061
	Lutjanus lutjanus	1	80637
		1	TZW196
	Lutjanus madras	1	77084
		1	77809
	Lutjanus monostigma	1	RB09_127
	Lutjanus sanguineus	1	77782
	Lutjanus sebae	1	77456

Appendix V: Sequences downloaded from GenBank with their accession numbers for COI, COII and 16S mitochondrial DNA gene fragments for the construction of phylogenies in Chapter 5.

Family	Species	GenBank accession number		
		COI	COII	16S
Emmelichthyidae	*Emmelichthys struhsakeri*		AP004446	AP004446
Lethrinidae	*Lethrinus obsoletus*	NC009855	NC009855	NC009855
Lutjanidae	*Aphareus furca*			DQ784722
	Aprion virescens			DQ784723
	Caesio caerulaurea	JF492986		DQ784724
	Caesio cuning			DQ784725
	Caesio lunaris	JF492994		
	Lutjanus adetii			DQ784727
	Lutjanus analis	HQ987863		
		JQ841254		
	Lutjanus apodus	JQ741279		
	Lutjanus argentimaculatus	NC016661	DQ900723	DQ784728
			EF025487	
	Lutjanus bengalensis	NC011275		
	Lutjanus bohar	GU805107	EF025488	DQ784729
	Lutjanus campechanus			AY857940
	Lutjanus carponotatus			DQ784730
	Lutjanus decussatus			AF247445
	Lutjanus ehrenbergii	HQ149874		
	Lutjanus erythropterus		EF025489	DQ444480
			EF025490	
	Lutjanus fulviflamma		DQ900712	DQ784731
	Lutjanus fulvus		DQ900709	DQ784732
			DQ900711	
	Lutjanus gibbus	GU805121		DQ784733
	Lutjanus griseus	JQ842927		
	Lutjanus johnii		DQ900720	DQ444484
	Lutjanus kasmira	NC011578		DQ784734
	Lutjanus lutjanus	HQ149880		
	Lutjanus malabaricus	NC012736	DQ900704	
			DQ900705	
			DQ900706	
			DQ900708	
			EF025491	
	Lutjanus monostigma			DQ784735
	Lutjanus notatus	JF493842		
	Lutjanus ophuysenii		EF376183	
	Lutjanus quinquelineatus			DQ784736
	Lutjanus rivulatus	NC009869		
	Lutjanus russellii	NC010963	DQ900714	DQ784737
			DQ900715	
	Lutjanus sebae	NC012736	DQ900717	DQ784738
	Lutjanus stellatus		DQ900701	DQ444483
			DQ900702	
			DQ900703	
	Lutjanus synagris			AY857939
	Lutjanus vitta		DQ900725	DQ784739
			DQ900727	
			DQ900730	
			DQ900756	
			EF025493	

Macolor niger			DQ784740
Pterocaesio marri	HQ945929		DQ784742
Pterocaesio pisang			DQ784743
Pterocaesio tile	JQ681325	AP004447	
Symphorus nematophorus			DQ784745
Symphorus spilurus			DQ784744