Molecular phylogeny and seascape genetics of the *Panulirus homarus* subspecies in the Western Indian Ocean

By

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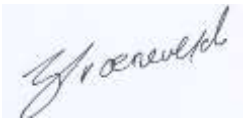
Submitted in fulfilment of the academic requirements for the degree of Doctor of Philosophy in

The School of Life Sciences

University of KwaZulu-Natal

Durban

As the candidate's supervisors we have approved this thesis/dissertation for submission.

Signed:  Name: Johan Groeneveld Date: 6 December 2017

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Preface

The work described in this dissertation was carried out at the Oceanographic Research Institute (ORI), which is affiliated with the School of Life Sciences at the University of KwaZulu-Natal, Westville. The study was undertaken between February 2015 and December 2017, under the supervision of Professor Johan Groeneveld and co-supervision of Dr Sandi Willows-Munro. It represents original work by the author and has not otherwise been submitted in any form for any degree or diploma to any tertiary institution. Where use has been made of the work of others it is duly acknowledge in the text.

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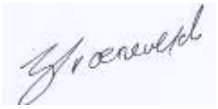
Prof. Johan Groeneveld Signed  Date 6 December 2017

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Declaration 2 – Publications

Detail of contribution to publications that form part and/or include research presented in this thesis.

Chapter 2

1. Singh SP, Groeneveld JC, Al-Marzouqi A, Willows-Munro S. (2017) A molecular phylogeny of the spiny lobster *Panulirus homarus* highlights a separately evolving lineage from the Southwest Indian Ocean. *PeerJ* 5:e3356 https://doi.org/10.7717/peerj.3356.

2. Singh SP, Groeneveld JC, Willows-Munro S. (2017) A molecular phylogeny of the spiny lobster *Panulirus homarus* highlights a separately evolving lineage from the Southwest Indian Ocean. Oral presentation at the Southern African Marine Science Symposium, July 2017, Port Elizabeth, South Africa.

3. Singh SP, Groeneveld JC, Willows-Munro S. (2017) A molecular phylogeny of the spiny lobster *Panulirus homarus* highlights a separately evolving lineage from the Southwest Indian Ocean. Oral presentation at the Western Indian Ocean Marine Science Symposium, October/November 2017, Dar-es-Salaam, Tanzania.

Chapter 3

4. Singh SP, Groeneveld JC, Willows-Munro S. (2017) Comparative population genetics and phylogeography of the *Panulirus homarus* subspecies complex in the Southwest Indian Ocean. Oral presentation at the Southern African Marine Science Symposium, July 2017, Port Elizabeth, South Africa.

Abstract

The oceans are one of the world's most dynamic environments. To cope with the constantly changing conditions brought about by climatic and physical forcing, its living inhabitants must adapt continually, or else they may not survive. This basic tenet of evolutionary change can explain the rich diversity of marine biota – a diversity which is also captured as genetic information at a molecular level. The present study represents a comprehensive genetic assessment of the spiny lobster *Panulirus homarus* from the Western Indian Ocean, relying on a multidisciplinary approach and different, though complementary lines of evidence. Phylogenetic-, phylogeographic- and population genetics analyses were performed, augmented with a seascape approach, to account for the influences of ocean currents, environmental variables and life history characteristics. Molecular markers appropriate for different timescales were chosen – to provide insights spanning from evolutionary epochs to fine-scale recent and contemporary events.

In Chapter 2, a panel of mitochondrial and nuclear intron DNA markers were used in conjunction with fossil data, to examine the phylogenetic relationships between three *Panulirus homarus* subspecies. Concatenation of mitochondrial and nuclear markers strongly supported the status of *P. h. rubellus* (the red southern form from the Southwestern Indian Ocean) as a distinct species. Divergence dating analysis estimated that *P. h. rubellus* and the other two subspecies (*P. h. homarus* from the Indo-West Pacific and *P. h. megasculptus* from the Arabian Sea combined) diverged from a common ancestor approximately 26 million years ago (MYA), during the Oligocene. *P. h. rubellus* is estimated to have arisen between 10 and 16 MYA during the Miocene, possibly in response to the final closure of the Tethys Sea and associated changes in global ocean circulation patterns.

An analysis of demographic history in Chapter 3 indicated that *P. homarus* underwent a population decline and subsequent expansion during the Pleistocene, when sea-level rise, warming and strengthened ocean currents after the last glacial maxima would have promoted a southerly expansion of *P. h. rubellus*. Faster-evolving microsatellite data revealed incipient genetic structure between *P. h. homarus* and *P. h. megasculptus*, which could not be observed from the more conserved mitochondrial loci. This suggests that larvae of *P. h. megasculptus* are retained in the contemporary Arabian Sea circulation, influenced by the seasonally reversing Somali Current and eddies off Oman and Somalia.

Empirical genetic data were correlated with ocean circulation based on a particle simulation model of larval dispersal, using a CBOCM in Chapter 4. Particle simulations over a 120 day period broadly supported the patterns observed in the genetic data. Spatially explicit genetic clustering methods using the microsatellite data identified a genetic discontinuity/ transition zone near Zavora in south-central Mozambique, with *P. h. homarus* occurring to the north and *P. h. rubellus* to the south. Migration analysis using information from all markers in Chapter 3 showed that gene flow across the Mozambique Channel, from Madagascar to the African shelf, occurs from east to west, and that the population in southeast Madagascar is self-recruiting. The results are congruent with the 'suitcase hypothesis', that larvae become entrained in eddies where the East Madagascar Curent rounds southern Madagascar, and are then transported across the Mozambique Channel towards the African mainland shelf. Both the genetic data and CBOCM larval dispersal simulations supported this pattern.

The CBOCM suggests that *P. h. rubellus* larvae that stray off the mainland continental shelf and into the Agulhas Current will rapidly be displaced southwestwards, beyond the range of benthic *P. homarus* populations. These larvae will most likely be lost. The narrow continental shelf off eastern South Africa may therefore form a bottleneck for dispersing larvae. The Seascape analyses of effects of environmental variables on genetic differentiation suggested that sea surface temperature along a latitudinal gradient can partially explain the diversification into the subtropical *P. h. rubellus* lineage and the more tropical *P. h. homarus* lineage.

The recurrent patterns emerging from all lines of evidence in this study (genetic, oceanography and environment) are that climatic fluctuations, which affect ocean currents and other oceanic variables, are in turn responsible for genetic connectivity or diversity in *P. homarus*. These observations can facilitate more informed predictions on how changing climates may impact the diversity of marine life.

Acknowledgements

This thesis is dedicated to the memory of my late grandfather, who always wanted me to become a doctor.

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Chapter One – General Introduction

1.1 Introduction

1.1.1 The marine environment

Oceans cover 70% of the Earth's surface, making them our world's largest ecosystem and home to a vast number of species ranging from microbes to whales. Marine ecosystems are pivotal to the Earth's biology, harbouring rich biodiversity, much of which still needs to be fully documented (Kaiser et al. 2011). These ecosystems are also vital resources, responsible for producing atmospheric oxygen and reducing carbon dioxide by acting as a massive heat sink (Hoegh-Guldberg and Bruno 2010). From a human viewpoint, they also provide essential social and economic goods and services (Costanza 1999). The ecosystems within oceans and seas are stochastic because of the spatial heterogeneity of environmental conditions (Riginos and Liggins 2013). These conditions create a myriad of ecological niches that a variety of organisms can utilize, thereby enhancing species richness. However, anthropogenic mediated climate change is having unprecedented effects on marine ecosystems, such as shifts in species distributions, reduced numbers of habitat-forming species like corals and seagrasses, and decreases in ocean productivity (Hoegh-Guldberg and Bruno 2010). It has now become crucial to evaluate these ecosystems and understand the variables that influence change and adaptation, so that we can better manage and protect our marine resources.

The primitive ocean was the birthplace of life on Earth, around 3.5 billion years ago. Along with the desire to find out when and where life began, marine researchers have also been interested in the life histories and evolution of marine organisms, because they can provide clues as to what has shaped the current patterns of diversity and speciation. These insights can potentially predict future patterns, as a response to climate change. Life history traits are linked to the overall success of a population, and understanding them make an important contribution towards the disciplines of genetics, ecology and resource management within the marine environment.

1.1.2 Life history strategies of marine organisms

Many life history traits of an organism are determined by its life cycle. The life cycle begins with birth and ends in death, and the two main stages within it are growth and reproduction (Flatt and Heyland 2011). The ontogeny, or the developmental history of an organism, differs among species. At the core of evolution and life history, are the concepts of fitness and natural selection (Dingle 1990). Fitness is a measure of an organism's reproductive success, or how many descendants or genotypes make it to the next generation when compared to other organisms or members of the same species (Futuyma 1995, Stearns and Hoekstra 2000, Ridley 2004). Natural selection is the process by which the traits that favour fitness are expressed over time, and are continually adapted to the organism's environment to ensure evolutionary success (Ridley 2004, Gregory 2009). Therefore, evolution will advance life history strategies which maximise fitness in a given environment, such as size at birth, pattern of growth, age and size at maturity, how many offspring are produced and their sex ratio, age and size specific reproductive investments and mortality rates, and longevity (Stearns 1992).

In order to categorize different life history strategies, MacArthur and Wilson (1967) introduced the r- and K- selection models, where 'r' refers to the maximal intrinsic rate of natural increase and K refers to carrying capacity.Organisms classified as r-selected are generally suited to unstable environments, with fast growth rates, high fecundity, and a shorter lifespan. They mature early, produce many offspring, and offer little or no parental care. Some examples of r-selected species include bacteria, many fish species and marine invertebrates, such as spiny lobsters. On the other hand, K-selected organisms usually exploit stable environments. They produce fewer offspring with a high rate of survival and provide parental care. Humans are a prime example of this strategy. Because many organisms occupy a gradient between these strategies, and exhibit variation in life history traits due to trade-offs (traits that are prioritized over another trait and are usually negatively associated with each other), the r- and K- selection concepts are now considered to be overly simplistic (Stearns 1977). New models have been proposed that attribute mortality patterns (extrinsic or intrinsic) as drivers of life history evolution, and they also incorporate features of the r- and K- models, such as density-dependent population regulation, changes in the environment, and the availability of resources (Stearns 1977, Reznick et al. 2002). It is important to understand variation in life history traits, because they affect the ecological and evolutionary responses of populations to changes in the environment (Snell-Rood et al. 2015).

Organisms in the marine environment have diverse life histories, because of the many available ecological niches and variable environmental conditions. Some organisms have evolved to occupy completely different ocean environments during different life history stages. The existence of some marine organisms is entirely benthic, while others are entirely planktonic (pelagic), but many organisms exhibit bipartite life histories consisting of a pelagic and benthic stage (Riginos and Liggins 2013). An early planktonic stage of development is thought to be paramount for the dispersal of marine species, especially when adults are sedentary (Weersing and Toonen 2009). Due to the small size of larvae, and because they are generally weak swimmers, the length of the pelagic larval duration (PLD), ocean currents, eddies and gyres influence dispersal patterns and hence genetic connectivity or population structure (Scheltema 1971, Grantham et al. 2003). The life history strategies adopted by marine organisms are crucial to their survival, proliferation and genetic differentiation (Duffy and Stachowicz 2006, Riginos et al. 2011, Rocliffe et al. 2014).

Theoretically, it is predicted that marine species with high dispersal capabilities would be genetically homogenous, so dispersal is cited as a critical factor in the structuring of marine organisms (Waples 1998, Hellberg 2009). Though the ocean may seem vast, open and continuous, dispersal is affected by variables such as temperature, nutrient availability, and physical-oceanographic features (Galindo et al. 2006, Weersing and Toonen 2009, Kelly and Palumbi 2010, Teske et al. 2011, Amaral et al. 2012). These physical, chemical and environmental elements can have profound effects on the life history of an organism and affect genetic variation and speciation. Temperature has a key effect on the organism's metabolic rate and influences the timing of the different ontogenetic stages (Harley et al. 2006). The harmful effects of thermal change due to global warming can indeed be seen in the recent coral bleaching events (Hughes et al. 2003, McWilliams et al. 2005). The planktonic larval stage in some organisms may be vulnerable to temperature change as it can lead to variation in the timing and duration of this stage in some species (Pechenik 1990, Houde and Zastrow 1993). Changes in temperature and wind patterns may alter ocean currents, and hence influence dispersal patterns and nutrient availability (Brierley and Kingsford 2009, García Molinos et al. 2017). Understanding the patterns of dispersal and the factors that affect them is key for informing conservation and management decisions, yet a knowledge gap still exists for many species because they are difficult to track and observe (Cowen et al. 2006, Fogarty and Botsford 2007, Weersing and Toonen 2009).

1.1.3 The hybridization problem

A challenge that faces conservation in the marine environment is hybridization (Winkler et al. 2011). Climate change may influence the evolutionary trajectory of species through hybridization, as shifts in climate may alter species distributions to bring closely related species into contact. These may then reproduce, giving rise to hybrids (Chunco 2014). Understanding hybridization among species is important for management of commercial stocks and biodiversity conservation, because hybridization could lead to genetic introgression, the extinction of the original parent species or the emergence of new species of hybrid origin (Rhymer and Simberloff 1996, Allendorf et al. 2001). Hybridization may be a disadvantage to organisms which have developed local adaptation, but may also produce organisms that are genetically more adaptable (Chunco 2014).

1.1.4 Study taxon: *Panulirus homarus* (Linnaeus, 1958)

The scalloped spiny lobster *Panulirus homarus* is an example of a marine organism with a bipartite life history; adults are benthic, and highly fecund females carry thousands of eggs attached to their abdomen (Berry 1971a). The eggs hatch into phyllosoma (leaf-like) larvae with a planktonic existence, which drift in the water column for 4 – 6 months, before settling on the seafloor to resume their benthic existence, as juveniles and adults (Berry 1971b, 1974a). *P. homarus* occurs throughout the Indo-West Pacific, in tropical and subtropical waters (Holthuis 1991).

1.2 *Panulirus homarus* taxonomy, ecology and distribution

First described by Linnaeus in 1758, *Panulirus homarus*, also known as the East Coast rock lobster in South Africa or the shallow water scalloped spiny lobster, is a decapod crustacean from the family Palinuridae (George 1964, Smale 1978, Kemp and Britz 2008). The family Palinuridae are divided into two groups; the ‘stridentes’ which possess a sound-producing stridulating organ, and the ‘silentes’ which do not have the organ (Berry 1971a, Pollock 1990, Tsang et al. 2009, Chan 2010). *P. homarus* falls into the group that possesses the

stridulating organ, which they utilize for communication and as a defence mechanism (Moulton 1957, Berry 1971a, Palero et al. 2009).

Within the spiny lobster genus *Panulirus*, *P. homarus* can be identified by its broad antennular plate consisting of four large spines, dense shorter spines present on the carapace, elevated eyes which are protected by supra-orbital horns, strong legs and a fan-like tail (Figure 1.1) (George and Main 1967, Heydorn 1969, Berry 1971a, Pollock 1990, George 2005). There are transverse grooves present on the second to the fifth abdominal segments and the size of the squamae on the margin of the abdominal grooves range from large and even, to small and irregular (Berry 1971a).

Figure 1.1 *Panulirus homarus* (Linnaeus in 1758).

Panulirus homarus is further divided into three sub-species (Figure 1.2), grouped into two forms based on the size of the squamae, sculpturing pattern on the abdominal carapace, colour and geographic distribution. *P. homarus homarus* is the nominotypical microsculpta form, with small squamae on the transverse abdominal grooves. It is mostly dark green/ brownish in colour, and is widely distributed throughout the Indo-West Pacific (Berry 1971a, Lavery et al. 2014). *P. homarus megasculptus* has large squamae (megasculpta) on the abdominal area, and is olive green with lateral yellow markings on abdominal segments (Lavery et al. 2014). *P. homarus rubellus* also has large squamae on the abdomen, but it is

distinguished from *P. h. megasculptus* by its reddish colour (Berry 1971a, Lavery et al. 2014).

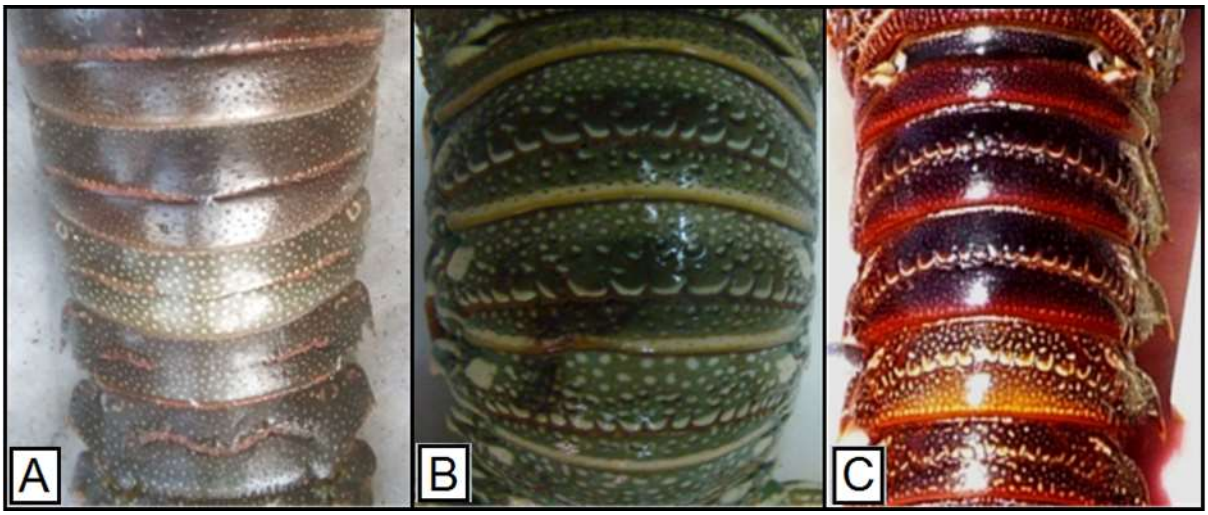

Figure 1.2 (A) *Panulirus homarus homarus* form with microsculpta, (B) *P. homarus megasculptus* form with megasculpta, and (C) *P. homarus rubellus* form with megascultpa and red colouration.

The geographic range of *P. homarus* extends from southeast Africa to Japan, Indonesia, Australia, New Caledonia and the Marquesas Archipelago (Figure 1.3) (Holthuis 1991). *P. h. homarus* occurs throughout the Indo-West Pacific (Holthuis 1991), *P. h. rubellus* is found along the southeast African and Madagascar coasts (Berry 1971b, 1974a, Holthuis 1991) and *P. h. megasculptus* occurs along the Somali coast and in the Arabian Sea (Fielding and Mann 1999, Kulmiye et al. 2006).

Adult *P. homarus* are found in reef habitats, in particular they prefer coral and rocky reefs within 200 m of the shore (Berry 1971b, Tsang et al. 2009). They occur at depths of 1–90 m, but are most common in waters shallower than 20 m, along with its main food source, the brown mussel *Perna perna* (Berry 1971b, Holthuis 1991, Kulmiye et al. 2006). They are gregarious, active nocturnally, and prefer a temperature range of 17–24 °C and turbid water (Berry 1971b). During the day, they retreat into cracks, crevices and reef overhangs to avoid predators (Berry 1971b).

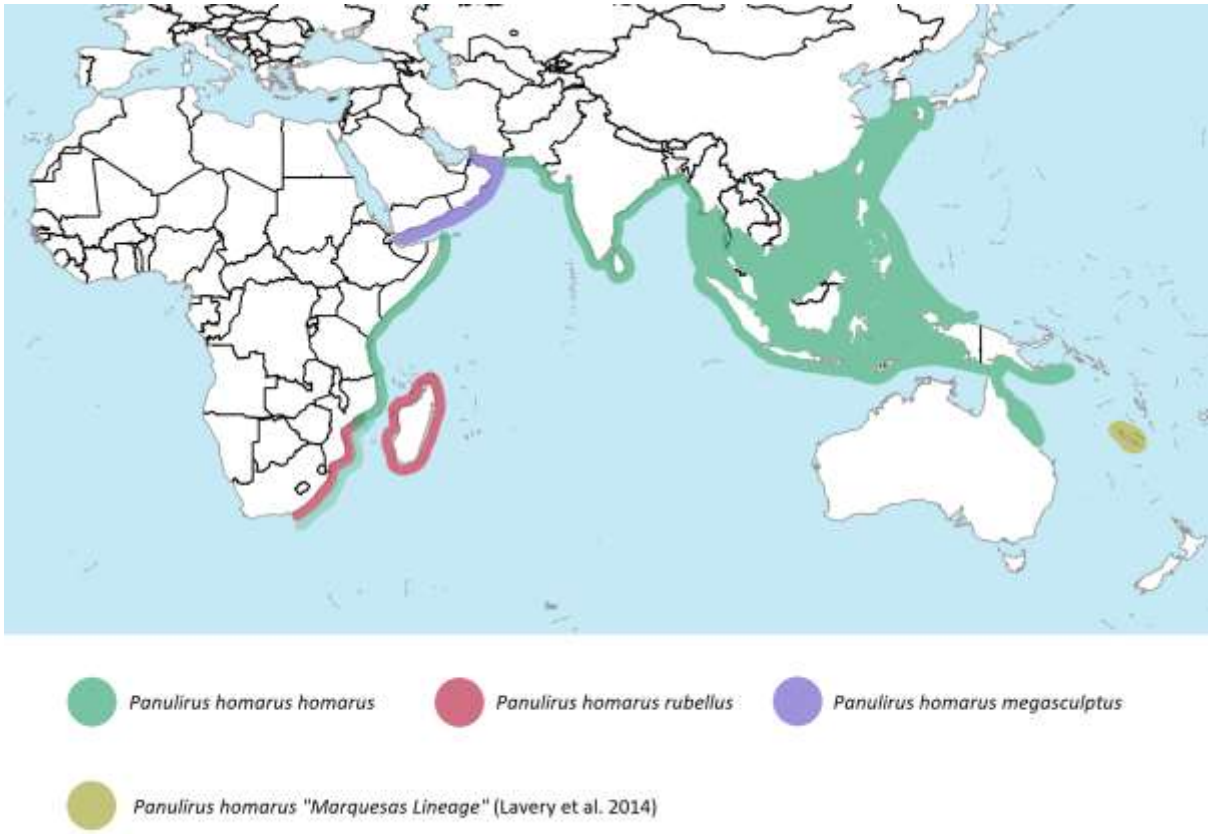

Figure 1.3 The distribution of *Panulirus homarus* throughout the Indo-West Pacific, adapted from the range map obtained on the IUCN redlist webpage (http://www.iucnredlist.org/details/170062/0).

1.3 Life cycle and growth

After reaching sexual maturity, (females at carapace length > 54 mm; males at carapace length 50–59 mm), *P. homarus* breed all year round, but the maximum breeding activity occurs during the summer months between November and March in the southwestern Indian Ocean (SWIO) (Berry 1971b, Kemp and Britz 2008) and during May and June in the northwestern Indian Ocean (NWIO) (Al-Marzouqi et al. 2008). They exhibit polygamous mating behaviour, and males that are larger in size are more aggressive, produce more sperm and are able to mate many more times than smaller males, making them reproductively dominant (Berry 1971a, George 2005).

Fertilization occurs when the male deposits a spermatophore on the sternum of the female (Berry 1970, Kemp and Britz 2008). The spermatophore hardens on the female's sternum, and its protective layer is scraped away to release the spermatozoa when oviposition begins

around a week later (Berry 1970). Fertilization is then external. An egg-bearing female (also called a 'berried' female) carries eggs externally, on ovigerous setae attached to the pleopods on the ventral abdomen (Berry 1971). Like the males, larger females are more fecund and can produce up to four broods of eggs during a breeding season (Berry 1971a). Depending on female size, up to 900 000 eggs can be produced per batch, and are then incubated for 29 – 50 days, thereafter the larvae hatch at night (Berry 1971b, George 2005). The large number of eggs produced is essential because they are subject to high predation rates during their larval life in the plankton (Strathmann 1985).

The first stage after hatching in the *P. homarus* life-cycle is the phyllosoma larval stage (Phillips and Booth 1994, Sekiguchi and Inoue 2002). When they hatch, they are only 1 – 2 mm long, dorso-ventrally flattened and transparent (Berry 1974a). The larvae spend 4 – 6 months in the phyllosoma phase where they move vertically in the water column and utilize currents, gyres and eddies to assist them with horizontal movement (Cobb 1997, George 2006, Phillips et al. 2006). During this period, they moult through a series of 9 to 10 developmental stages, while increasing in size (Berry 1974a). The larval stage is considered to be a selective advantage because it allows for wide dispersal, and changes in circulation patterns of gyres and eddies may isolate some parts of a population, eventually resulting in speciation (Cobb 1997).

Towards the end of the pelagic phase, phyllosoma larvae close to the coast undergo a metamorphic moult into a puerulus stage (Pollock 1990, George 2005). The puerulus resembles a tiny, transparent lobster that is capable of swimming horizontally and crossing the shelf, to reach shallow habitats close to the shore (Pitcher 1993, Pollock and Melville-Smith 1993, Phillips 2013). After settlement to the seafloor, the puerulus phase moults into a juvenile spiny lobster. Moulting is a growth process whereby a crustacean sheds its old exoskeleton to form a new one, while increasing in size or mass. After several moults spanning over years, juveniles reach adulthood, and become sexually mature. The average size at which maturity is achieved is a carapace length of > 54 mm in *P. homarus* individuals (Berry 1971a, Jayakody 1989), although this varies by area.

1.4 Commercial value and management

Spiny lobsters rank high among the most valuable seafood, and they are therefore intensely exploited worldwide (Holthuis 1991, Sweijd et al. 2000, Chan 2010). Over 90 000 tonnes of spiny lobsters are harvested annually around the globe (FAO 2017). Even though most spiny lobster species are not presently endangered, continued overharvesting may result in depletion of spiny lobster stocks. Therefore, it is crucial to understand the biology, habitat and genetic population structure of spiny lobsters, so that individual stocks may be managed effectively. Managing stocks in marine species is difficult as stocks may be shared among neighbouring countries and are not confined to clearly demarcated political boundaries (Carvalho and Hauser 1994, von der Heyden 2009). The genetic identification of shared stocks could prove invaluable in monitoring, management and conservation of marine species (Knowlton 1993 and 2000).

According to the IUCN red list, *P. homarus* is classified as least concern because of its widespread distribution (Cockcroft et al. 2013). However, there have been reports of catches declining worldwide (van der Elst et al. 2005), especially in countries such as India (Radhakrishnan et al. 2005), Somalia (Fielding and Mann 1999) and Oman (Mohan 1997, Al-Marzouqi et al. 2007). Each subspecies of *P. homarus* is exploited by fisheries along the entirety of its distribution. *P. h. homarus* is targeted by fisheries in Mozambique, Tanzania, Kenya, Somalia, Phillipines, Taiwan, Thailand and India (Steyn et al. 2008, Steyn and Schleyer 2011). In South Africa, coastal communities in the Eastern Cape province depend on the *P. h. rubellus* fishery, and on the KwaZulu-Natal coast it is caught in a recreational fishery (Steyn et al. 2008). In Madagascar, *P. h. rubellus* is fished in the economically disadvantaged region of Fort Dauphin (Sabatini et al. 2007), and in Mozambique it is an artisanal fishery. Lobsters caught by these fisheries are prone to overfishing during the holiday seasons as they are sold to tourists, irrespective of lobster size (Fielding et al. 1994; Steyn and Schleyer 2008). *P. h. megasculptus* is the subject of tangle net and trap fisheries in several countries around the Arabian Sea, including Oman, Yemen and Iran (Mohan 1997, Al-Marzouqi et al. 2007).

In South Africa, lobster fisheries contribute to the Gross Domestic Product (GDP) as well as providing employment to a large number of people (Griffiths et al. 2010). The lobster fisheries are managed according to the Marine Living Resources Act, through the Department

of Agriculture, Forestry and Fisheries (DAFF). However, the current information base on which management relies is incomplete and outdated for *P. homarus* fisheries along the east coast, requiring more extensive biological and population parameters to be determined (Al-Marzouqi et al. 2007, van der Elst et al. 2005). Genetic stock structure and identification of management units are important for implementing sustainable management strategies at the appropriate levels, and also for the conservation of genetic diversity (Spencer et al. 2010). This makes it important to distinguish between species, sub-species or populations so that better, and more specific, management strategies can be devised. At present, fisheries management strategies for shallow-water spiny lobsters are restricted to national levels in all countries across the Western Indian Ocean, despite the fact that populations of *P. homarus* may be shared by neighbouring countries (Al-Marzouqi et al. 2007, Radhakrishnan et al. 2005, Steyn et al. 2008).

1.5 Molecular phylogenetics, phylogeography and population genetics

The advances in molecular technology and the utilization of genetic techniques have revolutionized the fields of biodiversity, population and conservation biology, particularly those studies focused on marine ecosystems. Genetic techniques have been instrumental in understanding speciation, evolution, and biodiversity within the marine environment, and as such, are informative for developing conservation strategies (Palsbøll et al. 2007). Phylogenetic methods can be used to understand the evolutionary history and relationships among species. Phylogenetic trees generated with these methods have an important role in conservation, because they can be used to assess phylogenetic diversity and to identify lineages that are at risk of extinction (Webb et al. 2002, Shaffer et al. 2014).

Population genetics studies seek to understand the microevolutionary processes that shape population genetic structure and enable the identification of genetic structure within a species or identify patterns of connectivity (Allendorf et al. 2013). Microevolutionary processes such as mutation, genetic drift and selection could, in time, result in disruption of gene flow leading to genetic structuring, whereas gene flow and stabilizing selection can act to conserve species integrity through genetic homogenizing (Leese and Held 2011).

Phylogeography studies seek to understand the historical processes that shape genetic diversity (Allendorf et al. 2013), thus accounting for past geographical or hydrographic drivers of evolutionary processes. This molecular technique is valuable to conservation and biodiversity studies because it provides accurate and consistent information based on sophisticated methods of data analysis (Excoffier and Heckel 2006).

The molecular markers commonly used in phylogenetic, population genetic and phylogeographic studies are mitochondrial DNA markers such as the DNA barcoding marker cytochrome oxidase c subunit I (COI), 16S rDNA, the hypervariable control region and cytochrome *b* (Patwardhan et al. 2014). Mitochondrial genes are ideal markers at this taxonomic level because the mitochondrial genome is compact, and present in high copy numbers in nearly all animals. The mitochondrial genome is also generally maternally inherited and haploid, and it comprises fast and slow evolving regions which makes it useful at different taxonomic levels (Avise et al. 1987). Mutations in mtDNA are more rapidly fixed leading to greater genetic drift, due to the four-fold reduction in the effective population size of mtDNA, compared to nuclear DNA (Montooth et al. 2009, Neiman and Taylor 2009). However, there are limitations to working with mtDNA – because it is uni-parentally inherited, it is likely that only one facet of the evolutionary history of an organism is observed. These oversimplified evolutionary relationships and underestimation of genetic diversity result from faster lineage sorting rates and high allele extinction rates in mtDNA lineages (Zhang and Hewitt 1996, 2003).

The existence of nuclear copies of mitochondrial genes (numts) or pseudogenes that have been translocated from the mitochondrial genome and have become non-functional, further complicate analyses and data interpretation (Zhang and Hewitt 1996, Hazkani-Covo et al. 2010, Pink et al. 2011). Supplementing mtDNA data with the use of other classes of molecular markers is therefore necessary for more accurate interpretation of diversity patterns. Other candidate markers for use in phylogenetic, population genetic and phylogeographic studies are nuclear introns and microsatellites.

Introns are regions of genomic DNA that are untranslated and non-coding. These markers are now routinely used as independent markers in molecular systematics, particularly to study the evolutionary relationships between organisms that are closely related (Creer et al. 2005, Willows-Munro et al. 2005, Matthee et al. 2007). The major hurdles associated with using

intron markers are the technical difficulties associated with resolving haplotypes and recombination, which can affect the analysis of the data (Zhang and Hewitt 2003).

Microsatellites are stretches of DNA that are repeats of 2 – 6 nucleotides and are also called simple sequence repeats (SSR), variable number tandem repeats (VNTR) and short tandem repeats (STR) (Selkoe and Toonen 2006, Holderegger and Wagner 2008, Selkoe et al. 2008). They are co-dominant, selectively neutral and they exhibit Mendelian inheritance. Microsatellites are the markers of choice in population genetics because they allow for the examination of fine-scale genetic structure. Microsatellites also have their limitations; in particular, primers have to be designed specifically for a species and these cannot be applied across a wide taxonomic range. They have complex mutational mechanisms and suffer from problems associated with homoplasy, and amplification can be challenging (Selkoe and Toonen 2006).

The three subspecies of *P. homarus* are morphologically distinct, and have partially overlapping geographic distribution ranges. Recent studies using molecular approaches have sought to clarify genetic relationships among them. A study using the hypervariable control region demonstrated that *P. homarus* lobsters from Tanzania were genetically different from their *P. h. megasculptus* counterparts in Iran and Oman (Farhadi et al. 2013). However, in a subsequent study using three mitochondrial markers (COI, CR and 16S rDNA) and two nuclear markers (18S rDNA and ITS-1), *P. h. homarus* could not be distinguished from *P. h. megasculptus* (Lavery et al. 2014). The latter study found *P. h. rubellus* to be genetically distinct from the other two subspecies. A study of *P. h. rubellus* lobsters on the southeast coast of South Africa, Mozambique and Madagascar, using a COI pseudogene, suggested that the Madagascan *P. h. rubellus* lobsters were genetically distinct from those on the east African shelf (Reddy et al. 2014).

Molecular techniques have been applied successfully to many *Panulirus* lobsters. Examples include; the assessment of genetic variability and structure using mtDNA in *P. argus* (Diniz et al. 2005, Naro-Maciel et al. 2011), *P. inflatus* (García-Rodríguez and Perez-Enriquez 2008), *P. pencillatus* (Abdullah et al. 2014), *P. homarus* (Farhadi et al. 2013, Lavery et al. 2014, Senevirathna et al. 2016) and *P. h. rubellus* (Reddy et al. 2014). Using mitochondrial markers to identify genetic stocks have been applied successfully to *P. interruptus* (García-Rodríguez and Perez-Enriquez 2006). The relationship between pelagic larval drift and

genetic structure has been examined in *P. argus*, using COI and the control region (Naro-Maciel et al. 2011). The use of microsatellites has also been explored to understand population genetic structure and connectivity in *Panulirus* lobsters. Microsatellite markers have been designed and optimised for *P. cygnus* (Kennington et al. 2010), *P. argus* (Diniz et al. 2004), *P. interruptus* (Ben-Horin et al. 2009), *P. ornatus* (Dao et al. 2013) and *P. homarus* (Delghandi et al. 2015). Collectively, these studies have shown that the role ocean currents play in genetic variability is not straightforward, as they can either act to constrain gene flow and promote genetic structuring of populations, or promote gene flow and enhance the genetic connectivity between populations of a species.

1.6 Hybridization in the marine environment

Hybridization between species has been detected fairly often in the marine environment (Dibattista et al. 2012). Detecting and understanding hybrids is important in marine conservation because it can play a role in evolution, but can also contribute to the extinction or generation of new species, therefore having implications for the management of species (Allendorf et al. 2001). Hybridization has been observed in marine species such as pufferfish (Takahashi et al. 2017), corals (van Oppen et al. 2001, Willis et al. 2006), mussels (Riginos and Cunningham 2005), rays (Walter et al. 2014), teleosts (Schwenke 2012) and barnacles (Tsang et al. 2007). In crustaceans, it has been achieved artificially in two tiger prawn species (Benzie et al. 1995) and in Pacific white shrimp (Misamore and Browdy 1997). Hybridization in marine lobsters is less documented and a breeding experiment study between *Homarus americanus* and *Homarus gammarus* revealed that these lobsters preferred their own species to mate with (van der Meeren et al. 2008). In *P. homarus*, morphological evidence of intermediates between *P. h. homarus* and *P. h. rubellus* were identified from northern KwaZulu-Natal (Berry 1974b). Preliminary molecular evidence for hybridization between *P. h. homarus* and *P. h. rubellus* was only detected in a single lobster and no specimen was retained for morphological verification (Lavery et al. 2014).

1.7 Towards a seascape genetics approach

Seascape genetics is a spin-off of landscape genetics (Manel et al. 2003). This method utilizes oceanography, geography and ecology in conjunction with population genetics to understand

marine population genetic connectivity (Selkoe et al. 2008, 2016). The marine environment is characterized by complex, unstable factors such as currents, variability in sea surface temperature (SST), salinity and nutrient availability (Riginos and Liggins 2013). Biophysical ocean circulation models can be used to integrate biological information into physical oceanographic models, with the potential to predict the direction, magnitude and spatial scale of larval dispersal (Werner et al. 2007, Carr et al. 2008). These models can be used to understand how ocean currents and the life history of an organism can affect the genetic structure and connectivity among populations (Riginos and Liggins 2013).

Several studies have applied biophysical ocean circulation models alongside models of genetic connectivity, and then compared it to empirical population genetics data in order to elucidate vicariance processes. Coupling genetics with fine-scale physical oceanography was initially demonstrated with mussels (*Mytilus edulis*) along the southern coast of England (Gilg and Hilbish 2008). Other notable examples include investigation of patterns of dispersal in corals (Galindo et al. 2006, Baums et al. 2006), and the snail *Nerita atramentosa* in the southern Australian ocean; the latter revealed that genetic connectivity is mainly determined by on-shelf current flow (Teske et al. 2015). In the whelk *Kelletia kelletti* in the USA and Mexico, Euclidean distance was insufficient to predict genetic structure, and more likely resulted from the complex geography and ocean circulation in the region (White et al. 2010). A study on *Panulirus ornatus* spiny lobsters in the southeast Asian archipelago showed that oceanography may be responsible for the genetic connectivity of populations (Dao et al. 2015). Some studies have modelled other marine environmental variables to show their effects on patterns of genetic structure and population connectivity. Examples include a study on sea urchins in southeast Australia and northern New Zealand, where SST, coastal topography and benthic habitat distribution were used to explain genetic structure (Banks et al. 2007). SST, chlorophyll concentrations and water turbidity were used as environmental predictors of genetic structure in the short-beaked common dolphin (Amaral et al. 2012). These approaches have so far been utilized in few African marine taxa; examples include the African mud prawn, *Upogebia africana* (Teske et al. 2008), the crown crab *Hymenosoma orbiculare* (Teske et al. 2014) and the Cape hakes – *Merluccius paradoxus* and *M. capensis* (Henriques et al. 2016).

Going forward, using an integrative, multimarker approach such as seascape genetics to gain deeper insight into the mechanisms and processes that affect or drive genetic diversity in

spiny lobsters will be valuable. Seascape genetics has the potential to inform marine reserve design based on genetic connectivity of marine populations and the oceanographic and ecological factors that shape them (Selkoe et al. 2016, Amaral et al. 2012). It could also be used to understand the effects of climate change on oceanographic features and ecological systems, which potentially impact on genetic diversity (Selkoe et al. 2016).

1.8 Aims and objectives of the dissertation

Whereas lobster fisheries contribute to the livelihoods of coastal communities and to the economy through commercial fisheries, long-term sustainability can only be attempted through implementing well-informed management strategies. Resources for management of a species are often allocated on the basis of their potential economic value, or on their perceived vulnerability to exploitation or other risks. Endangered species are prioritized, but sometimes a subspecies or a population of a non-threatened species could be at risk. This makes it important to distinguish between species, subspecies or populations so that more specific management strategies can be devised in the place of blanket strategies that cover several taxa or a large geographical area, and that may be difficult to justify or enforce.

The aims and objectives of this PhD study were to determine the genetic diversity and stock structure of the three subspecies of the scalloped spiny lobster *P. homarus* in the Western Indian Ocean. Mitochondrial and nuclear intron DNA markers were used to investigate evolution, phylogenetic relationships and divergence times between the three subspecies. Mitochondrial markers were also used to investigate the demographic history and past migration rates of populations of the subspecies. A suite of microsatellite loci were used to infer recent population genetic structuring and genetic stock boundaries, and the contemporary patterns of gene flow. The definition of genetic stock units will have important implications for the development of fisheries management strategies. The utility of the seascape genetic approach was tested using coupled biophysical oceanographic models to understand the influence of oceanographic and ecological features on larval dispersal pathways and genetic connectivity. Ultimately, this study adds to the growing body of knowledge which aims to understand and predict how species adapt and cope with climate

change, by examining the effects of climate, ecology and geography on the genetic variation of species.

1.9 Dissertation overview

Chapter 2: A molecular phylogeny of the spiny lobster *Panulirus homarus* highlights a separately evolving lineage from the Southwestern Indian Ocean.

In this chapter, the phylogeny of the *P. homarus* subspecies complex was resolved using classical phylogenetic (maximum likelihood and Bayesian inference) and coalescent-based methods. Two mitochondrial (COI and part of the hypervariable control region) and two nuclear (β–tubulin and the internal transcribed spacer 1) genes were used to test the hypothesis that each subspecies may represent a separately evolving lineage. A concatenated multimarker dataset and fossil data were used to infer the divergence times between the subspecies in order to better understand the evolution of this subspecies complex.

Chapter 3: Comparative population genetics and phylogeography of the *P. homarus* subspecies complex in the Southwest Indian Ocean.

A combination of mitochondrial DNA and 21 microsatellite markers were used to examine the taxonomy, fine-scale population genetic structure and phylogeography of the three *P. homarus* subspecies, and to further investigate the population structure of the red megasculpta *P. h. rubellus* form from the Southwest Indian Ocean. The assumption that *P. h. rubellus* may be a separate species was tested at all markers. Clustering analyses were used to check for population structure. The demographic history of each of the subspecies was also investigated. Historical and contemporary gene flow analysis was conducted to check for connectivity between the populations of each of the subspecies and to investigate if the Mozambique Channel acts as a barrier to gene flow between African shelf and Madagascan *P. h. rubellus*. The influence of the prevailing ocean currents on genetic structure and connectivity is discussed.

Chapter 4: Linking population genetic, environmental and spatial variation using a seascape genetics approach

This chapter explored the use of Lagrangian particle tracking and coupled-biophysical ocean models to simulate larval dispersal of *P. homarus* in the Western Indian Ocean, to test the hypothesis that ocean currents are responsible for the genetic diversity between the subspecies. Spatial genetic clustering based on microsatellite data was used to pinpoint the locations of spatial genetic discontinuities. The effects of environmental data, such as SST, chlorophyll-a and turbidity, and spatial data such as latitude and longitude, along with larval recruitment at each sampling site measured using the particle tracking tool, on genetic differentiation between the subspecies, was tested.

1.10 References

Abdullah, M., F. Alimuddin, M. Muththalib, A. J. Salama, and H. Imai. 2014. Genetic isolation among the Northwestern, Southwestern and central-Eastern Indian ocean populations of the pronghorn spiny lobster *Panulirus penicillatus*. *International Journal of Molecular Sciences* **15**: 9242-9254.

Allendorf, F. W., R. F. Leary, P. Spruell, and J. K. Wenburg. 2001. The problems with hybrids: setting conservation guidelines. *Trends in Ecology & Evolution* **16**: 613-622.

Allendorf, F. W., and G. Luikart. 2013. Conservation and the genetics of populations. Second Edition. Blackwell Publishing. Malden, MA.

Al-Marzouqi, A., A. Al-Nahdi, N. Jayabalan, and J. Groeneveld. 2007. An assessment of the spiny lobster *Panulirus homarus* fishery in Oman — another decline in the Western Indian Ocean? *Western Indian Ocean Journal of Marine Science* **6**: 159-174.

Amaral, A. R., L. B. Beheregaray, K. Bilgmann, D. Boutov, L. Freitas, K. M. Robertson, M. Sequeira, K. A. Stockin, M. M. Coelho, and L. M. Möller. 2012. Seascape genetics of a globally distributed, highly mobile marine mammal: The short-beaked common dolphin (genus *Delphinus*). *PLoS ONE* **7**. DOI: 10.1371/journal.pone.0031482.

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific Phylogeography: The mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* **18**: 489-522.

Banks, S. C., M. P. Piggott, J. E. Williamson, U. Bové, N. J. Holbrook, and L. B. Beheregaray. 2007. Oceanic variability and coastal topography shape genetic structure in a long-dispersing sea urchin. *Ecology* **88**: 3055-3064.

Baums, I. B., C. B. Paris, and L. M. Chérubin. 2006. A bio-oceanographic filter to larval dispersal in a reef-building coral. *Limnology and Oceanography* **51**: 1969-1981.

Ben-Horin, T., M. Iacchei, K. A. Selkoe, T. T. Mai, and R. J. Toonen. 2009. Characterization of eight polymorphic microsatellite loci for the California spiny lobster, *Panulirus interruptus* and cross-amplification in other achelate lobsters. *Conservation Genetics*

Resources **1**: 193-197.

Benzie, J. A. H., M. Kenway, E. Ballment, S. Frusher, and L. Trott. 1995. Interspecific hybridization of the tiger prawns *Penaeus monodon* and *Penaeus esculentus*. *Aquaculture* **133**: 103-111.

Berry, P. F. 1970. Mating behaviour, oviposition and fertilization in the spiny lobster *Panulirus homarus* (Linnaeus). *South African Association for Marine Biological Research, Investigational Report* **24**: 3-16.

Berry, P. F. 1971a. The biology of the spiny lobster *Panulirus homarus* (Linnaeus) off the east coast of southern Africa. *South African Association for Marine Biological Research, Investigational Report* **28**: 3-75.

Berry, P. F. 1971b. The spiny lobsters (Palinuridae) of the coast of southern Africa: distribution and ecological notes. *South African Association for Marine Biological Research, Investigational Report* **27**: 4-23.

Berry, P. F. 1974a. Palinurid and scyllarid lobster larvae of the Natal coast, South Africa. *South African Association for Marine Biological Research, Investigational Report* **34**: 3-44.

Berry, P. F. 1974b. A revision of the *Panulirus homarus*-group of spiny lobsters (Decapoda, Palinuridae). *Crustaceana* **27**: 31-42.

Brierley, A. S., and M. J. Kingsford. 2009. Impacts of climate change on marine organisms and ecosystems. *Current Biology* **19**: 602-614.

Carr, S. D., X. J. Capet, J. C. McWilliams, J. T. Pennington, and F. P. Chavez. 2008. The influence of diel vertical migration on zooplankton transport and recruitment in an upwelling region: Estimates from a coupled behavioral-physical model. *Fisheries Oceanography* **17**: 1-15.

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

Chan, T. Y. 2010. A checklist of the world's marine lobsters (Custracea: Decapoda: Astacidae, Glypheidea, Achelata, Polychelida). *Raffles Bulletin of Zoology* **23**: 153-181.

Chunco, A. J. 2014. Hybridization in a warmer world. *Ecology and Evolution* **4**: 2019-2031.

Cobb, J. S. 1997. Oceanic processes affecting lobster larvae: report from a workshop. *Marine and Freshwater Research* **48**: 771-775.

Cockcroft, A., M. Butler and A. MacDiarmid. 2013. *Panulirus homarus*. The IUCN red list of threatened species 2013: e.T170062A6703197.

Costanza, R. 1999. The ecological, economic, and social importance of the oceans. *Ecological Economics* **31**: 199-213.

Cowen, R. K., C. B. Paris, and A. Srinivasan. 2006. Scaling of connectivity in marine opulations. *Science* **311**: 522-527.

Creer, S., A. Malhotra, R. S. Thorpe, and C. E. Pook. 2005. Targeting optimal introns for phylogenetic analyses in non-model taxa: experimental results in Asian pitvipers. *Cladistics* **21**: 390-395.

Dao, H. T., C. Smith-Keune, E. Wolanski, C. M. Jones, D. R. Jerry, and N. Suzuki. 2015. Oceanographic currents and local ecological knowledge indicate, and genetics does not refute, a contemporary pattern of larval dispersal for the ornate spiny lobster, *Panulirus ornatus* in the South-East Asian Archipelago. *PLoS ONE* **10**. DOI: 10.1371/journal.pone.0124568.

Dao, H. T., E. V. Todd, and D. R. Jerry. 2013. Characterization of polymorphic microsatellite loci for the spiny lobster *Panulirus* spp. and their utility to be applied to other *Panulirus* lobsters. *Conservation Genetics Resources* **5**: 43-46.

Delghandi, M., S. Goddard, D. R. Jerry, H. T. Dao, H. Afzal, and S. S. Al-Jardani. 2015. Isolation, characterization, and multiplexing of novel microsatellite markers for the tropical scalloped spiny lobster (*Panulirus homarus*). *Genetics and Molecular Research* **14**: 19066-19070.

Dibattista, J. D., L. A. Rocha, M. T. Craig, K. A. Feldheim, and B. W. Bowen. 2012. Phylogeography of two closely related Indo-Pacific butterflyfishes reveals divergent evolutionary histories and discordant results from mtDNA and microsatellites. *Journal of Heredity* **103**: 617-629.

Dingle, H. 1990. The evolution of life histories. Population Biology. Springer, Berlin,

Heidelberg. Pages 267-289.

Diniz, F. M., N. Maclean, M. Ogawa, I. H. A Cintra, and P. Bentzen. 2005. The hypervariable domain of the mitochondrial control region in Atlantic spiny lobsters and its potential as a marker for investigating phylogeographic structuring. *Marine Biotechnology* **7**: 462-473.

Diniz, F. M., N. Maclean, I. G. Paterson, and P. Bentzen. 2004. Polymorphic tetranucleotide microsatellite markers in the Caribbean spiny lobster, *Panulirus argus*. *Molecular Ecology Notes* **4**: 327-329.

Duffy, J. E., and J. J. Stachowicz. 2006. Why biodiversity is important to oceanography: Potential roles of genetic, species, and trophic diversity in pelagic ecosystem processes. *Marine Ecology Progress Series* **311**: 179-189.

Excoffier, L., and G. Heckel. 2006. Computer programs for population genetics data analysis: a survival guide. *Nature Reviews Genetics* **7**: 745-758.

FAO, 2017. FAO Publications Catalogue 2017. Food and Agriculture Organization of the United Nations, Rome, Italy.

Farhadi, A., H. Farahmand, M. A. Nematollahi, A. G. Jeffs, and S. D. Lavery. 2013. Mitochondrial DNA population structure of the scalloped lobster *Panulirus homarus* (Linnaeus 1758) from the West Indian Ocean. *ICES Journal of Marine Science* **70**: 1491-1498.

Fielding, P. J., W. D. Robertson, A. H. Dye, B. Q. Tomalin, R. P. van der Elst, L. E. Beckley, B. Q. Mann, S. Birnie, M. H. Schleyer, T. A. Lasiak. 1994. Transkei coastal fisheries resources. *South African Association for Marine Biological Research, Special Publication, Report* no. **3**: 1-167.

Fielding, P. J., and B. Q. Mann. 1999. The Somalia inshore lobster resources: A survey of the lobster fishery of the North Eastern Region (Puntland) between Foar and Eyl during November 1988. IUCN, Nairobi: 1-35.

Flatt, T., and A. Heyland. 2011. Mechanisms of life history evolution: the genetics and physiology of life history traits and trade-offs. Oxford University Press, Oxford. 506 pages.

Fogarty, M. J., and L. W. Botsford. 2007. Population connectivity and spatial management of marine fisheries. *Oceanography* **20**: 112-123.

Futuyma, D. J. 1995. Science on Trial: The Case for Evolution. Sinauer Associates, Sunderland, MA. 251 pages.

Galindo, H. M., D. B. Olson, and S. R. Palumbi. 2006. Seascape Genetics: A coupled oceanographic-genetic model predicts population structure of Caribbean corals. *Current Biology* **16**: 1622-1626.

García-Rodríguez, F. J., and R. Perez-Enriquez. 2006. Genetic differentiation of the California spiny lobster *Panulirus interruptus* (Randall, 1840) along the west coast of the Baja California Peninsula, Mexico. *Marine Biology* **148**: 621-629.

García-Rodríguez, F. J., and R. Perez-Enriquez. 2008. Lack of genetic differentiation of blue spiny lobster *Panulirus inflatus* along the Pacific coast of Mexico inferred from mtDNA sequences. *Marine Ecology Progress Series* **361**: 203-212.

García Molinos, J., M. T. Burrows, and E. S. Poloczanska. 2017. Ocean currents modify the coupling between climate change and biogeographical shifts. *Scientific Reports* **7**: 1332.

George, R. W. 1964. Variation and possible geographic populations in the Indo-West Pacific scalloped crayfish *Panulirus homarus*. *CSA specialists meeting on crustaceans* **11**: 1-5.

George, R. W., and A. R. Main. 1967. The evolution of spiny lobsters (Palinuridae): A study of evolution in the marine environment. *Evolution* **21**: 803-820.

George, R. W. 2005. Evolution of life cycles, including migration, in spiny lobsters (Palinuridae). *New Zealand Journal of Marine and Freshwater Research* **39**: 503-514.

George, R. W. 2006. Tethys origin and subsequent radiation of the spiny lobster (Palinuridae). *Crustaceana* **79**: 397-422.

Gilg, M. R., and T. J. Hilbish. 2008. The geography of marine larval dispersal : coupling genetics with fine-scale physical oceanography. *Ecology* **84**: 2989-2998.

Grantham, B. A., G. L. Eckert, and A. L. Shanks. 2003. Dispersal potential of marine invertebrates in diverse habitats. *Ecological Applications* **13**: 108-116.

Gregory, T. R. 2009. Understanding natural selection: essential concepts and common

misconceptions. *Evolution: Education and Outreach* **2**: 156-175.

Griffiths, C. L., T. B. Robinson, L. Lange, and A. Mead. 2010. Marine biodiversity in South Africa: an evaluation of current states of knowledge. *PLoS ONE* **5**. DOI: 10.1371/journal.pone.0012008.

Harley, C. D. G., A. Randall Hughes, K. M. Hultgren, B. G. Miner, C. J. B. Sorte, C. S. Thornber, L. F. Rodriguez, L. Tomanek, and S. L. Williams. 2006. The impacts of climate change in coastal marine systems. *Ecology Letters* **9**: 228-241.

Hazkani-Covo, E., R. M. Zeller, W. Martin, R. Viola, and A. van der Kuyl. 2010. Molecular poltergeists: Mitochondrial DNA copies (numts) in sequenced nuclear genomes. *PLoS Genetics* **6**:e1000834.

Hellberg, M. E. 2009. Gene flow and isolation among populations of marine animals. *Annual Review of Ecology, Evolution, and Systematics* **40**: 291-310.

Henriques, R. S., S. von der Heyden, M. R. Lipinski, N. du Toit, P. Kainge, P. Bloomer and C. A. Matthee. 2016. Spatio-temporal genetic structure and the effects of long-term fishing in two partially sympatric offshore demersal fishes. *Molecular Ecology* **25**: 5843-5861.

Heydorn, A. E. F. 1969. Notes on the biology of *Panulirus homarus* and on length/weight relationships of *Jasus lalandii*. *Division of Sea Fisheries, Investigational Report* **69**.

Hoegh-Guldberg, O., and J. F. Bruno. 2010. The impact of climate change on the world's marine ecosystems. *Science* **328**: 1523-1528.

Holderegger, R., and H. H. Wagner. 2008. Landscape genetics. *BioScience* **58**: 199.

Holthuis, L. B. 1991. FAO Species Catalogue. Marine lobsters of the world. An annotated and illustrated catalogue of species of interest to fisheries known to date. *FAO Fisheries Synopsis* No. 125. Rome, Food and Agricultural Organization of the United Nations. Page 292.

Houde, E. D., and C. E. Zastrow. 1993. Ecosystem- and taxon-especific dynamic and energetics properties of larval fish assemblages. *Bulletin of Marine Science* **53**: 290-335.

Hughes, T. P., A. H. Baird, D. R. Bellwood, M. Card, S. R. Connolly, C. Folke, R. Grosberg,

O. Hoegh-Guldberg, J. B. C. Jackson, J. Kleypas, J. M. Lough, P. Marshall, M. Nyström, S. R. Palumbi, J. M. Pandolfi, B. Rosen, and J. Roughgarden. 2003. Climate change, human impacts, and the resilience of coral reefs. *Science* **301**: 929-933.

Jayakody, D. 1989. Size at onset of sexual maturity and onset of spawning in female *Panulirus homarus* (Crustacea: Decapoda: Palinuridae) in Sri Lanka. *Marine Ecology Progress Series* **57**: 83-87.

Kaiser, M. J., M. J. Attrill, S. Jennings, D. N. Thomas, D. K. A. Barnes, A. S. Brierley, J. G. Hiddink, H. Kaartokallio, N. V. C. Polunin, and D. G. Raffaelli. 2011. Marine ecology: processes, systems, and impacts. Oxford University Press, Oxford. 528 pages.

Kelly, R. P., and S. R. Palumbi. 2010. Genetic structure among 50 species of the northeastern pacific rocky intertidal community. *PLoS ONE* **5**. DOI: 10.1371/journal.pone.0008594.

Kemp, J., and P. Britz. 2008. The effect of temperature on the growth, survival and food consumption of the east coast rock lobster *Panulirus homarus rubellus*. *Aquaculture* **280**: 227-231.

Kennington, W. J., E. Levy, O. Berry, D. M. Groth, A. M. Waite, M. S. Johnson, and R. Melville-Smith. 2010. Characterization of 18 polymorphic microsatellite loci for the western rock lobster *Panulirus cygnus*. *Conservation Genetics* **2**: 389-391.

Knowlton, N. 1993. Sibling species in the sea. *Annual Review of Ecology and Systematics* **24**: 189-216.

Knowlton, N. 2000. Molecular genetic analyses of species boundaries in the sea. *Hydrobiologia* **420**: 73-90.

Kulmiye, A. J., K. M. Mavuti, and J. C. Groeneveld. 2006. Size at onset of maturity of spiny lobsters *Panulirus homarus homarus* at Mambrui, Kenya. *African Journal of Marine Science* **28**: 51-55.

Lavery, S. D., A. Farhadi, H. Farahmand, T. Y. Chan, A. Azhdehakoshpour, V. Thakur, and A. G. Jeffs. 2014. Evolutionary divergence of geographic subspecies within the scalloped spiny lobster *Panulirus homarus* (Linnaeus 1758). *PLoS ONE* **9**. DOI: 10.1371/journal.pone.0097247.

Leese, F., and C. Held. 2011. Analyzing intraspecific genetic variation: A practical guide

using mitochondrial DNA and microsatellites. *In: Phylogeography and Population Genetics in Crustacea, Crustacean Issues* **19**: 3–30. Held, C., S. Koenemann, C. D. Schubart (Eds). CRC Press, Boca Raton.

MacArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. Princeton University Press, USA. 216 pages.

Manel, S., M. K. Schwartz, G. Luikart, and P. Taberlet. 2003. Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology and Evolution* **18**: 189-197.

Matthee, C. A., G. Eick, S. Willows-Munro, C. Montgelard, A. T. Pardini, and T. J. Robinson. 2007. Indel evolution of mammalian introns and the utility of non-coding markers in eutherian phylogenetics. *Molecular Phylogenetics and Evolution* **42**: 827-837.

McWilliams, J. P., I. M. Côté, J. A. Gill, W. J. Sutherland, and A. R. Watkinson. 2005. Accelerating impacts of temperature-induced coral bleaching in the Caribbean. *Ecology* **86**: 2055-2060.

Misamore, M., and C. L. Browdy. 1997. Evaluating hybridization potential between *Penaeus setiferus* and *Penaeus vannamei* through natural mating, artificial insemination and in vitro fertilization. *Aquaculture* **150**: 1–10.

Mohan, R. 1997. Size structure and reproductive variation of the spiny lobster *Panulirus homarus* over a relatively small geographic range along the Dhofar coast in the Sultanate of Oman. *Marine and Freshwater Research* **48**: 1085-1091.

Montooth, K. L., D. N. Abt, J. W. Hofmann and D. M. Rand. 2009. Comparative genomics of *Drosophila* mtDNA: Novel features of conservation and changes across functional domains and lineages. *Journal of Molecular Evolution* **69**: 94-114.

Moulton, J. 1957. Sound production in the spiny lobster *Panulirus argus* (Latreille). *Biological Bulletin* **113**: 286-295.

Naro-Maciel, E., B. Reid, K. E. Holmes, D. R. Brumbaugh, M. Martin, and R. DeSalle. 2011. Mitochondrial DNA sequence variation in spiny lobsters: population expansion, panmixia, and divergence. *Marine Biology* **158**: 2027-2041.

Neiman, M. and D. R. Taylor. 2009. The causes of mutation accumulation in mitochondrial genomes. *Proceedings of the Royal Society Biological Sciences* **276**: 1201-1209.

Palero, F., J. Lopes, P. Abelló, E. Macpherson, M. Pascual, and M. A. Beaumont. 2009. Rapid radiation in spiny lobsters (*Palinurus* spp) as revealed by classic and ABC methods using mtDNA and microsatellite data. *BMC Evolutionary biology* **9**: 263.

Palsbøll, P. J., M. Bérubé, and F. W. Allendorf. 2007. Identification of management units using population genetic data. *Trends in Ecology and Evolution* **22**: 11-16.

Patwardhan, A., S. Ray, and A. Roy. 2014. Molecular markers in phylogenetic studies - a review. *Journal of Phylogenetics & Evolutionary Biology* **2**: 1-9.

Pechenik, J. A. 1990. Delayed metamorphosis by larvae of benthic marine invertebrates: Does it occur? Is there a price to pay? *Ophelia* **32**: 63-94.

Phillips, B. F., and J. D. Booth. 1994. Early Life History of Spiny Lobster. *Crustaceana* **66**: 271-294.

Phillips, B. F., J. S. Cobb, A. Jeffs, and P. McWilliam. 2006. Larval and postlarval ecology. *In: Lobsters: Biology, Management, Aquaculture and Fisheries*. Blackwell Publishing, Oxford. Pages 231-262.

Phillips, B. F. 2013. Lobsters : Biology, Management, Aquaculture and Fisheries. Wiley-Blackwell.

Pink, R. C., K. Wicks, D. P. Caley, E. K. Punch, L. Jacobs, and D. R. F. Carter. 2011. Pseudogenes: pseudo-functional or key regulators in health and disease? *RNA* **17**: 792-798.

Pitcher, C. R. 1993. Spiny Lobster. *In: Nearshore marine resources of the South Pacific*. Hill, L., and Wright, A. (Eds). Forum Fisheries Agent, Honiara. Pages 539-607.

Pollock, D. E. 1990. Palaeoceanography and speciation in the spiny lobster genus *Jasus*. *Bulletin of Marine Science* **46**: 387-405.

Pollock, D. E., and R. Melville-Smith. 1993. Decapod life histories and reproductive dynamics in relation to oceanography off southern Africa. *South African Journal of Marine Science* **13**: 205-212.

Radhakrishnan, E. V., V. D. Deshmukh, M. K. Manisseri, M. Rajamani, J. K. Kizhakudan, and R. Thangaraja. 2005. Status of the major lobster fisheries in India. *New Zealand Journal of Marine and Freshwater Research* **39**: 723-732.

Reddy, M. M., A. H. H. MacDonald, J. C. Groeneveld, and M. H. Schleyer. 2014. Phylogeography of the scalloped spiny-lobster *Panulirus homarus rubellus* in the Southwest Indian Ocean. *Journal of Crustacean Biology* **34**: 773-781.

Reznick, D., M. J. Bryant, and F. Bashey. 2002. r- and K- selection revisited: The role of population regulation in life-history evolution. *Ecology* **83**: 1509-1520.

Rhymer, J. M., and D. Simberloff. 1996. Extinction by hybridization and introgression. *Annual Review of Ecology and Systematics* **27**: 83-109.

Ridley, M. 2004. Evolution. Third Edition. Oxford University Press, Oxford. 778 pages.

Riginos, C., and C. W. Cunningham. 2005. Local adaptation and species segregation in two mussel (*Mytilus edulis* x *Mytilus trossulus*) hybrid zones. *Molecular Ecology* **14**: 381-400.

Riginos, C., K. E. Douglas, Y. Jin, D. F. Shanahan, and E. A. Treml. 2011. Effects of geography and life history traits on genetic differentiation in benthic marine fishes. *Ecography* **34**: 566-575.

Riginos, C., and L. Liggins. 2013. Seascape Genetics: populations, individuals, and genes marooned and adrift. *Geography Compass* **7**:197-216.

Rocliffe, S., S. Peabody, M. Samoilys, and J. P. Hawkins. 2014. Towards a network of locally managed marine areas (LMMAs) in the Western Indian Ocean. *PLoS ONE* **9**. DOI: 10.1371/journal.pone.0103000.

Sabatini G, S. Salley, J. B. Ramanamanjato. 2007. A review of the spiny lobster fishery in the Tolagnaro (Fort-Dauphin) region. *In: Tolagnaro (Madagascar): Biodiversity, ecology and conservation of littoral ecosystems in southeastern Madagascar, Tolagnaro.* Ganzhorn J. U, Goodman S. M, Vincelette M. (Eds). Washington DC, USA: Smithsonian Institution. Pages 299–308.

Scheltema, R. S. 1971. Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. *The*

Biological Bulletin **140**: 284-322.

Schwenke, P. 2012. History and extent of introgressive hybridization in Puget Sound rockfishes (*Sebastes auriculatus*, *S. caurinus*, and *S. maliger*). MSc Thesis, University of Washington.

Sekiguchi, H., and N. Inoue. 2002. Recent advances in larval recruitment processes of Scyllarid and Palinurid Lobsters in Japanese waters. *Journal of Oceanography* **58**: 747-757.

Selkoe, K. A., and R. J. Toonen. 2006. Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecology Letters* **9**: 615-629.

Selkoe, K. A., C. M. Henzler, and S. D. Gaines. 2008. Seascape genetics and the spatial ecology of marine populations. *Fish and Fisheries* **9**: 363-377.

Selkoe, K. A, C. D'Aloia, E. Crandall, M. Iacchei, L. Liggins, J. Puritz, S. von der Heyden, and R. Toonen. 2016. A decade of seascape genetics: Contributions to basic and applied marine connectivity. *Marine Ecology Progress Series* **554**: 1-19.

Senevirathna, J. D. M., D. H. N. Munasinghe, and P. B. Mather. 2016. Assessment of genetic structure in wild populations of *Panulirus homarus* (Linnaeus, 1758) across the south coast of Sri Lanka inferred from mitochondrial DNA Sequences. *International Journal of Marine Science* **6**: 1-9.

Shaffer, H. B., M. Gidiş, E. McCartney-Melstad, K. M. Neal, H. M. Oyamaguchi, M. Tellez, and E. M. Toffelmier. 2014. Conservation genetics and genomics of amphibians and reptiles. *Annual Review of Animal Biosciences* **3**: 113-138.

Smale, M. J. 1978. Migration, growth and feeding in the Natal rock lobster *Panulirus homarus* (Linnaeus). *South African Association for Marine Biological Research, Investigational Report* **47**: 1-56.

Snell-Rood, E., R. Cothran, A. Espeset, P. Jeyasingh, S. Hobbie, and N. I. Morehouse. 2015. Life-history evolution in the anthropocene: effects of increasing nutrients on traits and trade-offs. *Evolutionary applications* **8**: 635-649.

Spencer, P., M. Canino, J. Dicosimo, M. Dorn, A. J. Gharrett, D. Hanselman, and K. Palof. 2010. Guidelines for determination of spatial management units for exploited

populations in Alaskan groundfish fishery management plans.

Stearns, S. C. 1977. The evolution of life history traits: a critique of the theory and a review of the data. *Annual Review of Ecology, Evolution, and Systematics* **8**: 145-171.

Stearns, S. C. 1992. The evolution of life histories. Oxford University Press, Oxford. 249 pages.

Stearns, S. C., and R. F. Hoekstra. 2000. Evolution, an introduction. Second Edition. Oxford University Press, Oxford. 600 pages.

Steyn, E., P. J. Fielding, and M. H. Schleyer. 2008. An assessment of the artisanal fishery of east coast rock lobster, *Panulirus homarus* (Linnaeus) in Transkei. *African Journal of Marine Science* **30**: 497-506.

Steyn, E., and M. Schleyer. 2011. Movement patterns of the East Coast rock lobster *Panulirus homarus rubellus* on the coast of KwaZulu-Natal, South Africa. *New Zealand Journal of Marine and Freshwater Research* **45**: 85-101.

Strathmann, R. R. 1985. Feeding and Nonfeeding Larval Development and Life-History Evolution in Marine Invertebrates. *Annual Review of Ecology and Systematics* **16**: 339-361.

Sweijd, N., R. Bowie, B. Evans, and A. Lopata. 2000. Molecular genetics and the management and conservation of marine organisms. *Hydrobiologia* **420**: 153-164.

Takahashi, H., A. Toyoda, T. Yamazaki, S. Narita, T. Mashiko, and Y. Yamazaki. 2017. Asymmetric hybridization and introgression between sibling species of the pufferfish Takifugu that have undergone explosive speciation. *Marine Biology* **164**: 90.

Teske, P. R., I. Papadopoulos, B. K. Newman, P. C. Dworschak. C. D. McQuaid and N. P. Barker. 2008. Oceanic dispersal barriers, adaptation and larval retention: an interdisciplinary assessment of potential factors maintaining a phylogeographic break between sister lineages of an African prawn. *BMC Evolutionary Biology* **8**: 341.

Teske, P. R., S. von der Heyden, C. D. McQuaid, and N. P. Barker. 2011. A review of marine phylogeography in southern Africa. *South African Journal of Science* **107**: 1-11.

Teske, P. R., I. Papadopoulos, N. P. Barker, C. D. McQuaid, and L. B. Beheregaray. 2014.

Mitonuclear discordance in genetic structure across the Atlantic/Indian Ocean biogeographical transition zone. *Journal of Biogeography* **41**: 392-401.

Teske, P. R., J. Sandoval-Castillo, E. van Sebille, J. Waters, and L. B. Beheregaray. 2015. On-shelf larval retention limits population connectivity in a coastal broadcast spawner. *Marine Ecology Progress Series* **532**: 1-12.

Tsang, L. M., B. K. K. Chan, K. Y. Ma, C.-H. Hsu, and K. H. Chu. 2007. Lack of mtDNA and morphological differentiation between two acorn barnacles *Tetraclita japonica* and *T. formosana* differing in parietes colours and geographical distribution. *Marine Biology* **151**: 147-155.

Tsang, L. M., T. Y. Chan, M. K. Cheung, and K. H. Chu. 2009. Molecular evidence for the Southern Hemisphere origin and deep-sea diversification of spiny lobsters (Crustacea: Decapoda: Palinuridae). *Molecular Phylogenetics and Evolution* **51**: 304-311.

van der Elst, R., B. Everett, N. Jiddawi, G. Mwatha, P. S. Afonso, and D. Boulle. 2005. Fish, fishers and fisheries of the Western Indian Ocean: their diversity and status. A preliminary assessment. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **363**: 263-284.

van der Meeren, G. I., A. Chandrapavan, and T. Breithaupt. 2008. Sexual and aggressive interactions in a mixed species group of lobsters *Homarus gammarus* and *H. americanus*. *Aquatic Biology* **2**: 191-200.

van Oppen, M. J. H., B. J. McDonald, B. Willis, and D. J. Miller. 2001. The evolutionary history of the coral genus *Acropora* (Scleractinia, Cnidaria) based on a mitochondrial and a nuclear marker: reticulation, incomplete lineage sorting, or morphological convergence? *Molecular Biology and Evolution* **18**: 1315-1329.

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

Walter, R. P., S. T. Kessel, N. Alhasan, A. T. Fisk, D. D. Heath, T. Chekchak, R. Klaus, M. Younis, G. Hill, B. Jones, C. D. Braun, M. L. Berumen, J. D. DiBattista, M. A. Priest, and N. E. Hussey. 2014. First record of living *Manta alfredi* × *Manta birostris* hybrid. *Marine Biodiversity* **44**: 1-2.

Waples, R. S. 1998. Separating the wheat from the chaff: patterns of genetic differentiation in high gene flow species. *Journal of Heredity* **89**: 438-450.

Webb, C. O., D. D. Ackerly, M. a. McPeek, and M. J. Donoghue. 2002. Phylogenies and community ecology. *Annual Review of Ecology and Systematics* **33**: 475-505.

Weersing, K., and R. J. Toonen. 2009. Population genetics, larval dispersal, and connectivity in marine systems. *Marine Ecology Progress Series* **393**: 1-12.

Werner, F. E., R. C. Cowen, and C. B. Paris. 2007. Coupled biological and physical models: present capabilities and necessary developments for future studies of population connectivity. *Oceanography* **20**: 54-69.

White, C., K. A. Selkoe, J. Watson, D. A., Siegel, D. C. Zacherl, and R. J. Toonen. 2010. Ocean currents help explain population genetic structure. *Proceedings of the Royal Society B Biological sciences* **277**: 1685-1694.

Willis, B. L., M. J. H. van Oppen, D. J. Miller, S. V. Vollmer, and D. J. Ayre. 2006. The role of hybridization in the evolution of reef corals. *Annual Review of Ecology, Evolution, and Systematics* **37**: 489-517.

Willows-Munro, S., T. J. Robinson, and C. A. Matthee. 2005. Utility of nuclear DNA intron markers at lower taxonomic levels: Phylogenetic resolution among nine *Tragelaphus spp*. *Molecular Phylogenetics and Evolution* **35**: 624-636.

Winkler, K. A., B. Pamminger-Lahnsteiner, J. Wanzenböck, and S. Weiss. 2011. Hybridization and restricted gene flow between native and introduced stocks of Alpine whitefish (*Coregonus* sp.) across multiple environments. *Molecular Ecology* **20**: 456-472.

Zhang, D. X., and G. M. Hewitt. 1996. Nuclear integrations-challenges for mitochondrial DNA markers. *Trends in Ecology & Evolution* **11**: 247-251.

Zhang, D. X., and G. M. Hewitt. 2003. Nuclear DNA analyses in genetic studies of populations: practice, problems and prospects. *Molecular Ecology* **12**: 563-584.

Chapter Two: A molecular phylogeny of the spiny lobster *Panulirus homarus* highlights a separately evolving lineage from the southwest Indian Ocean

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Abstract

Accurate species description in the marine environment is critical for estimating biodiversity and identifying genetically distinct stocks. Analysis of molecular data can potentially improve species delimitations, because they are easily generated and independent, and yield consistent results with high statistical power. We used classical phylogenetic (maximum likelihood and Bayesian inference) and coalescent-based methods (divergence dating with fossil calibrations and coalescent-based species delimitation) to resolve the phylogeny of the spiny lobster *Panulirus homarus* subspecies complex in the Indo-West Pacific. Analyses of mitochondrial data and combined nuclear and mitochondrial data recovered *P. h. homarus* and *P. h. rubellus* as separately evolving lineages, while the nuclear data trees were unresolved. Divergence dating analysis also identified *P. h. homarus* and *P. h. rubellus* as two distinct clades which diverged from a common ancestor during the Oligocene, approximately 26 million years ago. Species delimitation using coalescent-based methods corroborated these findings. A long pelagic larval life stage and the influence of ocean currents on post-larval settlement patterns suggest that a parapatric mode of speciation drives evolution in this subspecies complex. In combination, the results indicate that *P. h. rubellus* from the southwest Indian Ocean is a separately evolving lineage and possibly a separate species.

2.1 Introduction

What constitutes a species or subspecies? In light of conflicting hypotheses regarding species concepts, this is a difficult question to answer. Whereas all species concepts accept that a species is a separately evolving metapopulation lineage (Agapow et al. 2004, de Queiroz 2007), secondary criteria differ. For instance, the biological concept states that there must be reproductive isolation from other lineages (Mayr 1942), while the phylogenetic concept proposes that a lineage must be monophyletic to qualify as a species (Cracraft 1983). Furthermore, all the secondary characteristics that define lineage diversification don't necessarily occur at the same time or linearly (de Queiroz 2007), and as a result, organisms might be classified as a subspecies when they are in fact a recently diverged species (Parkin and Knox 2010). Subspecies are valuable to the studies of biodiversity and evolution, as they reflect the earliest stages of speciation (Johnsen et al. 2006).

The advent of molecular data has made it possible to test traditional subspecies delineations (Barrowclough 1980, Ball and Avise 1992, Burbrink et al. 2000, Phillimore and Owens 2006, Morin et al. 2010). Statistical power and rigor of methods and algorithms used for the molecular delimitation of species are constantly improving and yielding consistent results (de Queiroz and Gatesy 2007, Rannala and Yang 2013). In addition to classical multi-locus phylogenetic methods, coalescent-based species delimitations using molecular data have been applied successfully in many studies (Leache and Fujita 2010, Burbrink et al. 2011, Setiadi et al. 2011, Zhang et al. 2011), and are useful for identifying species that have recently diverged or are in the process of divergence (Knowles and Carstens 2007). Using coalescent theory (Kingman 1982, Hudson 1991) and applying the general lineage concept (de Queiroz 2007), probabilities for allele sorting under alternative hypotheses can be calculated. The shared ancestral polymorphisms detected using the genetic data and coalescent methods can enable species detection, or a lineage split, at the early stage of divergence, before monophyly (Knowles and Carstens 2007).

Marine organisms such as spiny lobsters (Palinuridae) are good models for the study of speciation and the validity of subspecies because of their high dispersal capabilities (Palumbi 1994). Spiny lobsters have high fecundity and long-lived phyllosoma larvae that drift in the water column for several months, with the potential to disperse over long distances (summarized by George 2005). This in turn promotes large populations, large geographic ranges and enables high levels of gene flow. Although there may be no apparent barriers to gene

flow, intrinsic factors such as microevolutionary, population-genetic/coalescent and genealogical processes as well as more macro-evolutionary processes operating at the phylogeographic and higher levels, can give rise to genetic breaks – such as at subspecies level – where they outweigh the extrinsic factors facilitating gene flow (Irwin 2002).

The earliest lineages of lobsters from all infraorders originated approximately 360 Ma ago (million years ago), during the late Devonian period in the Paleozoic era (Schram and Dixon 2004, Bracken-Grissom et al. 2014). The Achelata infraorder diverged into the spiny- (Palinuridae) and slipper lobster (Scyllaridae) families around 250 Ma ago (George 2006, Tsang et al. 2009, Bracken-Grissom et al. 2014). These authors propose that, approximately 230 Ma ago, the Palinuridae diverged into stridulating (sound-producing) Stridentes (*Linuparus, Justitia, Nupalirus, Palinustus, Puerulus, Palibythus, Palinurus* and *Panulirus*) and non-stridulating Silentes groups (*Projasus, Jasus, Sagmariasus* and *Palinurellus*). Within the Stridentes, the shallow warm-water *Panulirus* genus is probably the most recently evolved (George and Main 1967, Pollock 1992, George 1997, 2006). This is supported by a molecular phylogenetic study on the genus (Ptacek et al. 2001) and another study using fossil calibrated data in conjunction with molecular DNA markers, which showed that *Panulirus* emerged around 160 Ma ago (Bracken-Grissom et al. 2014). A conflicting hypothesis by Tsang et al. (2009), based on protein-coding molecular data, suggests that *Panulirus* is basal in the Stridentes group.

The scalloped spiny lobster *Panulirus homarus* comprises three economically important subspecies in the Indo-West Pacific region, extending northwards from southeast Africa and Madagascar, along the coast of the western Indian Ocean to the Arabian Sea and India in the north, and along the western rim of the Pacific, to Indonesia, Japan and Australia (Holthuis 1991b). The three subspecies are phenotypically distinguishable and their geographical ranges differ. The nominotypical *P. homarus homarus* has small squamae on the abdominal segments (microsculpta), is dark green in color, and occurs throughout the Indo-West Pacific (Berry 1971, Holthuis 1991, Lavery et al. 2014). *P. h. megasculptus* has large squamae (megasculpta), is olive green with yellow lateral markings, and appears to be restricted to the northern Arabian Sea (Berry 1974b, Holthuis 1991). *P. h. rubellus* is the red megasculpta form, which occurs in the southwest Indian Ocean, along the coasts of eastern South Africa, Mozambique and southern Madagascar (Berry 1974b, Holthuis 1991).

Three molecular studies have been done on *P. homarus* and its subspecies. Nuclear copies of mitochondrial DNA (numts or pseudogenes) COI data showed that there is significant genetic partitioning between *P. h. rubellus* from southeast Madagascar and those from the African shelf, which suggests the Mozambique Channel as a barrier to larval dispersal (Reddy et al. 2014). *P. homarus* samples from Tanzania and the Arabian Sea belonged to different stocks, likely because of the effects of local currents on larval dispersal (Farhadi et al. 2013). Using the genetic markers COI, control region, 18S rDNA and the ITS-1 intron, Lavery et al. (2014) found little genetic differentiation between the *P. h. homarus* and *P. h. megasculptus* sub-species, which indicates that *P. h. megasculptus* should not be considered a separate subspecies. *P. h. rubellus* was the most divergent subspecies, but a single observation of hybridization between *P. h. homarus* and *P. h. rubellus* suggested that interbreeding may occur.

Multilocus genetic data from mitochondrial (COI and Hypervariable Control Region) and nuclear (ITS-1 intron and β-tubulin) markers, and both classical phylogenetic (Bayesian inference and maximum likelihood) and coalescent-based methods were used to resolve the phylogeny of the *P. homarus* subspecies complex. Fossil data was used to infer divergence times between the *P. homarus* subspecies. This study extends the work done by Lavery et al. (2014) on *P. homarus* by analyzing a concatenated multi-marker dataset, and using additional coalescent-based methods and fossil data to better understand the evolution of the subspecies complex.

2.2 Materials and Methods

2.2.1 Sample collection

Panulirus homarus specimens were collected from five sites along the east coast of South Africa (Tinley Manor, Blood Reef, Scottburgh, Mdumbi and Port St Johns), three sites in Mozambique (Chidenguele, Xai Xai and Zavora) and one site in Madagascar (Fort Dauphin). Additional samples were sourced from four sites in Oman (Al Ashkharah, Dhalkoot, Duqm and Mirbat), and one site each in Yemen and Kenya (Figure 2.1). All specimens were identified to subspecies level based on phenotypic and geographic information.

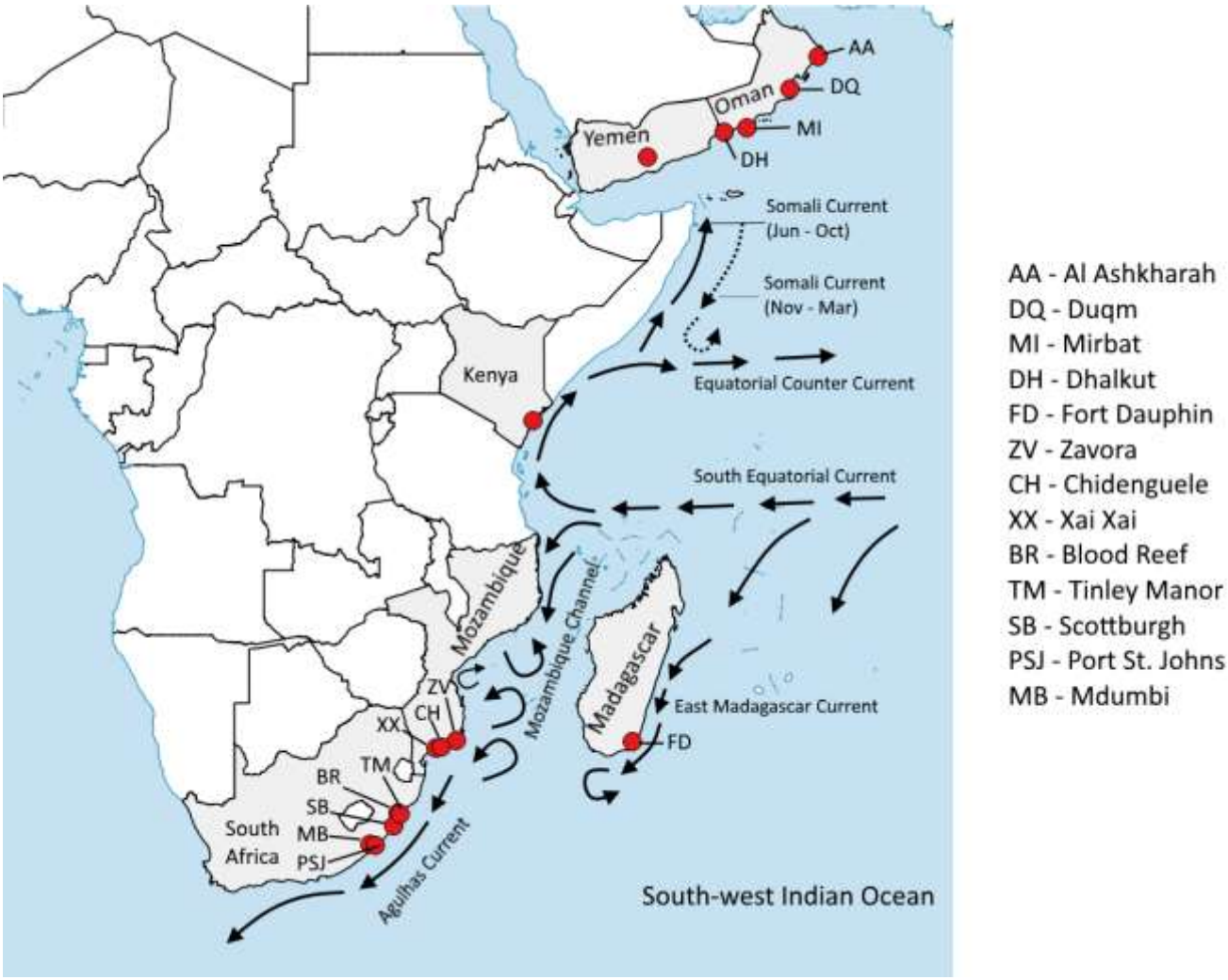


Figure 2.1 Sampling sites of the *Panulirus homarus* subspecies. The main ocean currents and eddy systems are depicted (adapted from Lutjeharms 2006).

2.2.2 DNA extraction, PCR amplification and Sequencing

DNA was extracted from pereiopod tissue using the Zymo ZR Tissue and Insect DNA kit (Inqaba, Zymo), as per the manufacturers protocol which was modified slightly to replace the bead bashing process with the addition of 15 µl of Proteinase K and a 3-hour incubation at 56 °C during the lysis step.

Molecular markers used in the study included mitochondrial COI (LCO-Ph 5’ - TCGGAGCATGAGCTGGGATAGT – 3’ and HCO-Ph 5’ – ACTTCTGGGTTGTCGAGGACTC – 3’; Lavery et al. 2014) and Control Region (CR1 5’ -

GCA AAG AAT ATA GCA AGA ATC AA – 3' and CR2 5' - GCA AAC CTT TTT ATC AGG CAT C – 3'; Diniz et al. 2005). Nuclear markers included ITS-1 (ITSF 5' – CACACCGCCCGTCGCTACTA – 3' and ITSR 5' – ATTTAGCTGCGGTCTTCATC – 3'; Chu et al. 2001) and β-tubulin (BTF2 5' – ATGTTYGAYGCHAAGAAYATGATGGC – 3' and BTR2 5' – TCCATGCCYTCNCCVGTGTACCAGTG – 3'; Jennings and Etter 2011). Amplification reactions were 25 µl in total and contained 2 µl of 10X PCR reaction buffer (Super-Therm®, Industricord), 2 µM $MgCl_2$, 0.2 µM of dNTP mix, 0.2 µM of each 10 µM primer and 0.2 µl of 1 U Taq polymerase (Super-Therm®, Industricord).

The thermal cycling program for all markers consisted of initial denaturation at 95 °C for 10 minutes, followed by 35 cycles of 95 °C for 30 seconds, annealing temperatures of 50 °C (COI), 57.4 °C (ITS-1 and CR) and 54 °C (β-tubulin) for 30 seconds, and 72 °C for 45 seconds. The final extension step was carried out at 72 °C for 10 minutes. All PCR reactions were run with a positive and negative control.

PCR clean-up and sequencing reactions were performed at the Central Analytical Facilities (CAF) at Stellenbosch University. Chromatograms were assembled and checked manually using BioEdit v. 7.2.5 (Hall 1999) and FinchTV v. 1.4.0 (www.geospiza.com). Multiple sequence alignment was done using the online version of MAFFT (Katoh et al. 2002) and then refined manually. The Gblocks server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html, v. 0.91b) was used to assess the confidence of the final alignments. The strict parameter of not allowing many contiguous non-conserved positions was chosen, but gap positions were allowed within final blocks. Additionally, the GUIDANCE2 server (http:// http://guidance.tau.ac.il/) was used to calculate confidence scores for each alignment. The COI sequences were checked for stop codons by using the NCBI ORF Finder (Wheeler et al. 2003) and translation to protein to ensure that a pseudogene was not being amplified. Nuclear data were phased using Seqphase (Flot 2009) and PHASE v. 2.1 (Stephens et al. 2001) to investigate the occurrence of hybridization between the sub-species.

In addition to *P. homarus* individuals, DNA was also extracted from eight other lobster species; *Jasus paulensis*, *Jasus lalandii*, *Palinurus gilchristi*, *Palinurus delagoae*, *Panulirus longipes*, *Panulirus versicolor*, *Scyllarides elisabethae* and *Scyllarides squammosus*. The four

markers were also amplified in these individuals for use as outgroup taxa and fossil calibration points for divergence dating analysis. All sequences used in this study are listed with their accession numbers in Table S2.1.

2.2.3 Phylogenetic analyses

To infer the phylogeny of *P. homarus*, a maximum likelihood (ML) approach implemented in Garli v. 2.0 (Zwickl 2006), and Bayesian inference (BI) approach implemented in MrBayes v. 3.2.6 (Ronquist and Huelsenbeck 2003, Huelsenbeck and Ronquist 2005), were used. The best models of nucleotide substitution for each gene were selected using jModeltest v. 2.0 (Darriba et al. 2012) and the corrected Akaike information (AICc) criterion (Table 2.1). Each gene was analysed separately and then combined by genomic location; mitochondrial (COI + CR) and nuclear (β-tubulin + ITS-1), and then concatenated into a single dataset (COI + CR + β-tubulin + ITS-1) in SequenceMatrix v. 1.8 (Vaidya et al. 2011). PartitionFinder v. 1.1.1 (Lanfear et al. 2012) was used to find the best partitioning strategy and model for each codon position in COI and for each partition in the concatenated datasets (mitochondrial, nuclear and the four genes concatenated).

Table 2.1 Sequence alignment characteristics and best models for nucleotide sequence evolution for datasets used in the analyses.

Marker	Sites	N	Variable	Parsimony Informative	Model
COI	565	79	165	95	cp01: TIMef + G , cp02: F81 + I , cp03: GTR + G
CR	541	55	184	142	TVM + I + G
β-tubulin	264	54	98	75	TVMef + I + G
ITS-1	437	61	231	145	TIMef + I + G
Mitochondrial	1106	55	314	214	*
Nuclear	701	47	280	198	*
Concatenated	1807	54	650	425	*

The Garli search was performed using two independent runs with two search replicates each. Nodal support was assessed by a 1000 bootstrap replicates (Felsenstein 1985). The number of generations run in the BI analysis was 20 000 000 for COI, CR and β-tubulin, and 50 000 000 for ITS-1, the combined mitochondrial, combined nuclear and the four genes concatenated. Two independent runs with four parallel MCMC chains were performed for each of the datasets. Trees were sampled every 1000 generation. The number of trees to be discarded as

burn-in and Effective Sample Size (ESS) values to check for MCMC convergence was assessed using Tracer v. 1.6 (Rambaut and Drummond 2007). ESS values that were greater than 200 indicated that there was chain convergence, and that the analysis was run long enough to obtain valid estimates of the parameters. Bootstrap values and posterior probabilities were mapped on to the most likely tree for each gene, the combined mitochondrial, combined nuclear and all four genes concatenated. The analyses for each of the genes was also performed excluding the outgroups and using the midpoint rooting method to see if the choice of outgroups had any effects on bootstrap and Bayesian posterior probability support of ingroups. Genetic distances (p-distance) were calculated in MEGA v. 6.0 (Tamura et al. 2013).

2.2.4 Molecular divergence dating

Divergence dates were estimated using a reduced four-gene concatenated dataset which contained 14 *P. h. homarus* individuals (Kenya: 7 and Mozambique: 6), 30 *P. h. rubellus* individuals (South Africa: 16, Mozambique: 11 and Madagascar: 3) and 9 *P. h. megasculptus* individuals (Oman: 7 and Yemen: 2). All taxa had sequence data for at least three markers. Analysis was performed using an uncorrelated Bayesian relaxed molecular clock approach in BEAST2 2.4.0 (Bouckaert et al. 2014). In order to introduce fossil calibration points, slipper lobsters from the family *Scyllarides* (*S. elisabethae* and *S. squammosus*), spiny lobsters from the *Jasus* (*J. paulensis* and *J. lalandii*) and *Palinurus* genera (*P. gilchristi*) and two other *Panulirus* species (*P. longipes* and *P. versicolor*) were added to the dataset. The fossil calibration points used, along with the offset and standard deviations, are given in Table 2.2. The substitution models chosen were the same as those used in the ML and BI analyses. The Yule speciation model was chosen as the tree prior, because it is appropriate for describing the relationships between individuals from different species (Aldous 2001). Divergence dates were estimated using an uncorrelated relaxed lognormal Bayesian molecular clock. The analysis consisted of two independent Markov Chain Monte Carlo (MCMC) analyses. The chains ran for 70 000 000 generations and trees were sampled every 10 000 generations. Tracer was used to check that the ESS values were greater than 200, confirming good mixing and convergence of the chains. The two runs were combined using LogCombiner v. 2.4.0 and the trees were summarized using TreeAnnotator v. 2.4.0 (included with the BEAST package). A maximum clade credibility consensus tree with mean node heights and posterior

probabilities greater than 0.5 was obtained using TreeAnnotator v. 2.4.0. A geological timescale tree was plotted using the packages strap (Bell and Lloyd 2014), coda (Plummer et al. 2006), phyloch (Heibl 2008) and phytools (Revell 2012) in the R statistical package v. 3.1.2 (R Development Core Team, 2008; http://www.R-project.org).

Table 2.2 Fossil calibration points used for the divergence dating analysis.

Fossil	Node	Timescale (Ma ago)	Offset	Standard Deviation	Reference
Yunnanopalinura schrami	Achelata/ root	241-247	241	0.9	Feldmann et al. 2012
Archaeopalinurus	Palinuridae	210-221	210	0.7	Pinna 1974
Panulirus destombesi	Panulirus	99-112	99	0.8	Garassino and Teruzzi 1993
Jasus flemingi	Jasus	5.3-23.8	5.3	0.98	Glaessner 1960
Scyllarides bolcensis	Scyllarides	33.7-54.8	33.7	1	De Angeli and Garassino 2008

2.2.5 Molecular species delimitation

The reversible-jump Bayesian Markov Chain Monte Carlo (rjMCMC) algorithm implemented in BP&P v. 3.2 (Bayesian Phylogenetics and Phylogeography; Yang and Rannala 2010, Rannala and Yang 2013, Yang 2015) was used to analyse phylogenetic data from the four loci to generate speciation probabilities based on the multispecies coalescent model (MSC). This model takes into account the coalescent processes in the ancestral and the modern species and the resulting gene-species tree conflicts (Degnan and Rosenberg 2009). The reduced four-gene concatenated dataset was used, and the maximum clade probability tree from BEAST2 was used as the initial guide tree.

The prior settings were as follows: (1) θ = G (2, 10) and τ_0 = G (2, 10) for large ancestral population size and deep divergence; (2) θ = G (2, 2000) and τ_0 = G (2, 2000) for small ancestral populations and shallow divergence; (3) θ = G (2, 10) and τ_0 = G (2, 2000) accounting for large ancestral populations and shallow divergence and (4) θ = G (2, 2000) and τ_0 = G (2, 10) for small ancestral population size and deep divergence. Algorithm 1 (species delimitation using a fixed guide tree) was used. In this model the rjMCMC algorithm jumps between various species delimitation models compatible with the guide tree supplied (Yang and Rannala 2010, Rannala and Yang 2013). The analysis was run twice to confirm stability between runs, each run consisted of 1 000 000 steps, sampling every 100 generations

and 800 trees were discarded as burnin. Tracer was used to confirm convergence of the chains.

2.3 Results

Amplification was successful for all four markers used in this study, and for the taxa listed in Table S2.1. The alignment for each locus received a confidence score of > 0.98 using the GUIDANCE2 server. The final sequence alignments generated for each marker included (Table 2.1): COI, 565 bp (165 variable sites); CR, 541 bp (184 variable sites); β-tubulin, 264 bp (98 variable sites) and ITS-1, 437 bp (231 variable sites). The nuclear marker ITS-1 exhibited the most variability (53% variable characters), followed by the nuclear marker β-tubulin (37% variable sites) and mitochondrial CR (34% variable sites) and COI (29% variable sites).

The best-fit model for nucleotide sequence evolution for each of the datasets is shown in Table 2.1. For the combined datasets, PartitionFinder found the same models as jModeltest for each of the partitions. The ML and BI analyses of the independent datasets, datasets combined by genomic location and the four-gene concatenated dataset, recovered similar topologies with Bayesian posterior probability (BPP) support often being higher than ML bootstrap support. Independent analysis of the four markers revealed no significant conflict (ML bootstrap > 50%, BPP > 0.5, Supplementary Figs. S2.1 – S2.8). There was also no conflict between the combined mitochondrial and combined nuclear trees (Figs. S2.9 – S2.12).

The mtDNA markers, analysed separately, with outgroup rooting, resulted in two distinct groupings supported by low ML bootstrap support and high BPP support. One group consisted of *P. h. rubellus* individuals (COI, ML bootstrap: < 50%, BPP: 0.95; CR, ML bootstrap: < 50%, BPP: 0.73) and the other of *P. h. homarus* and *P. h. megasculptus* individuals (COI, ML bootstrap: < 50%, BPP: 0.94; CR, ML bootstrap: < 50%, BPP: 0.85). The analysis of the combined mtDNA datasets also recovered the two groupings, *P. h. rubellus* (ML bootstrap: < 50%, BPP: 0.83) and *P. h. homarus* and *P. h. megasculptus* (ML bootstrap: < 50%, BPP: 0.94). The midpoint-rooted individual gene trees for COI and CR, and the combined mtDNA dataset resulted in better ML bootstrap support for *P. h. rubellus*

as a separately evolving group (COI, ML bootstrap: 60%, BPP: 0.95; CR, ML bootstrap: 85%, BPP: 1.0; Combined, ML bootstrap: 74%, BPP: 1.0), and strong support for the *P. h. homarus* and *P. h. megasculptus* grouping in the COI data (ML bootstrap: 98%, BPP: 0.9) but weak support in CR data (ML bootstrap: < 50%, BPP: <0.5) and the combined mtDNA dataset (ML bootstrap: < 50%, BPP: < 0.5). The nuclear DNA gene trees (separate and combined, analysed with and excluding outgroups) were largely unresolved, possibly due to incomplete lineage sorting, slow mutation rates and insufficient informative variation (McCracken and Sorenson 2005). The four-gene concatenated analysis of all the data, however, resulted in two monophyletic lineages, one containing *P. h. rubellus* individuals (ML bootstrap: 74%, BPP: 0.99, Fig. 2.2) and the other *P. h. homarus* and *P. h. megasculptus* individuals (ML bootstrap: 61%, BPP: 0.99, Fig. 2.2).

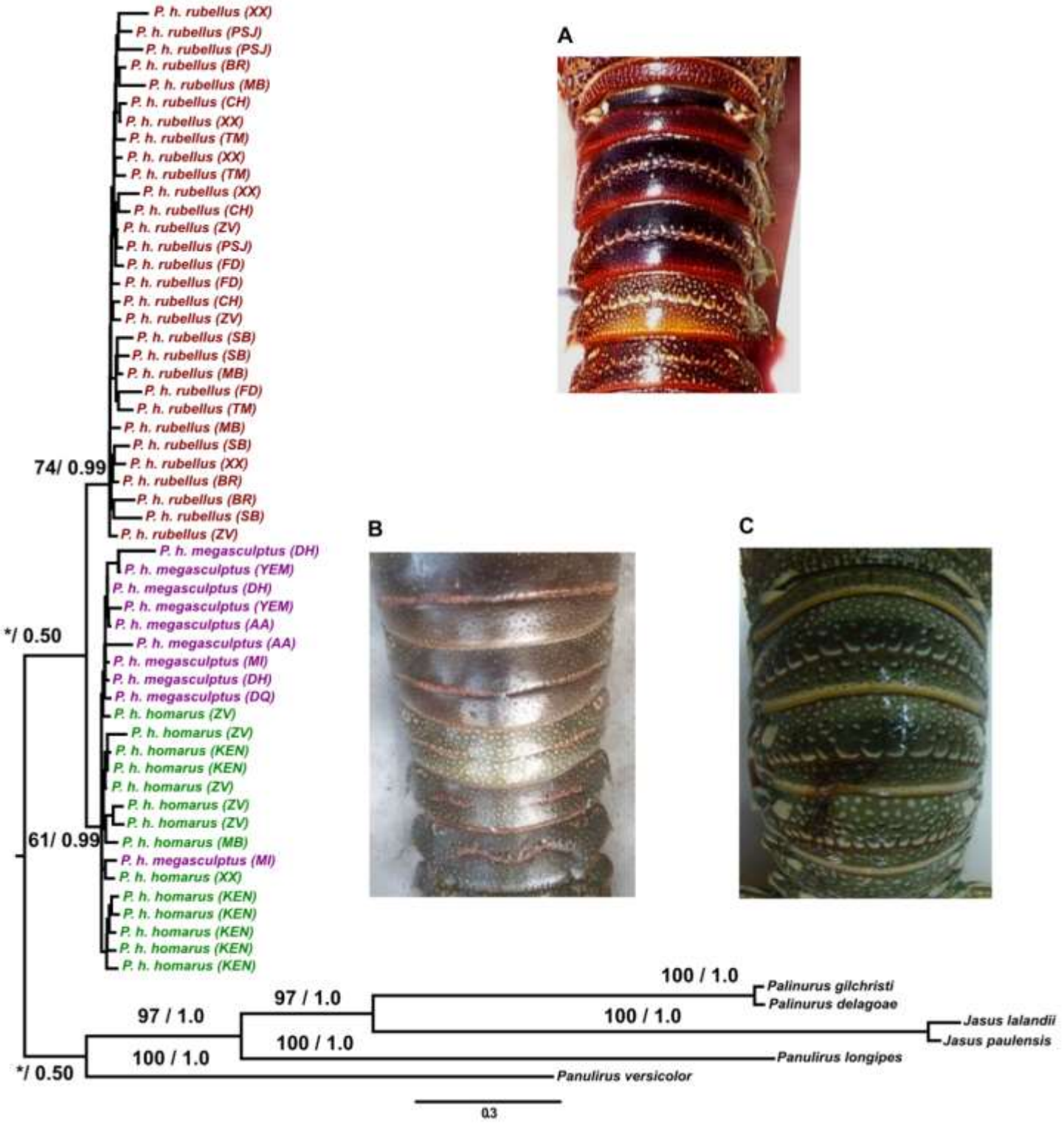


Figure 2.2 Four gene concatenated Maximum likelihood tree inferred from the concatenated (COI + CR + β-tubulin + ITS-1) data. Maximum likelihood bootstrap support values and Bayesian posterior probabilities are indicated on the nodes. Each colour represents a different subspecies. Photograph representatives of each subspecies are shown: (A) *Panulirus homarus rubellus*, (B) *Panulirus homarus homarus* and (C) *Panulirus homarus megasculptus*. * Indicates that there was no bootstrap support. The scale bar indicates the number of nucleotide substitutions per site.

Uncorrected mean pairwise genetic distances between subspecies and between outgroups were calculated for each marker. Genetic distances calculated for COI and CR were greater between subspecies and between outgroups than distances among subspecies and among certain outgroups. Interestingly in the β-tubulin gene, the pairwise distance between *P. h. homarus* and *P. h. megasculptus* (8.6%, Table S.2.4) was slightly larger than the distance between *P. h. rubellus* and *P. h. megasculptus* (8.3%, Table S.2.4). This contrasts with the mean pairwise distances between the three subspecies for the mitochondrial genes which was 4.8% for COI and 25.8% for CR between *P. h. rubellus* and *P. h. homarus* + *P. h. megasculptus*; and 1.6% between *P. h. homarus* and *P. h. megasculptus* for COI and 3.5% for CR (Table S.2.2 and S.2.3). The genetic distances along with their standard error estimates are included in supplementary Tables S.2.2 – S.2.5.

The maximum clade probability tree generated using BEAST2 with divergence times based on fossil calibration points is congruent with the four-gene concatenated phylogeny inferred using ML and BI methods. *P. h. homarus* and *P. h. rubellus* were recovered as two distinct monophyletic groups with high posterior probability of 1.0 (Fig. 2.3). *P. h. megasculptus* clustered with *P. h. homarus* individuals. *P. h. rubellus* and *P. h. homarus* last shared a common ancestor during the Oligocene, approximately 26 Ma ago (95% HPD 23.6 – 29.5, Table 2.3). The divergence times of the other species used as outgroups are consistent with those found by other studies (Palero et al. 2009a, Tourinho et al. 2012, Bracken-Grissom et al. 2014).

Table 2.3 Lobster divergence dates estimated using fossil calibrated nodes. Labels refer to Figure 2.3.

Label	Node	Mean	95% HPD (Ma ago)
A	Achelata	242	241.0 - 244.0
B	Palinuridae	215	210.3 - 226.3
C	*Palinurus*	184	164.3 - 204.7
D	*Panulirus*	160	139.5 - 185.1
E	*Panulirus*	111	93.2 - 132.1
F	*S. elisabethae*	98	76.9 - 119
G	*P. h. homarus subspecies complex*	26	23.6 - 29.5
H	*P. h. rubellus*	12.7	9.8 - 15.7
I	*P. h. homarus*	11.9	9.8 - 14.9
J	*P. delagoae & P. gilchristi*	5.6	3.5 - 7.8
K	*J. lalandii & J. paulensis*	11.7	8.5 - 15.1
L	*S. squammosus*	4.2	2.6 - 5.7

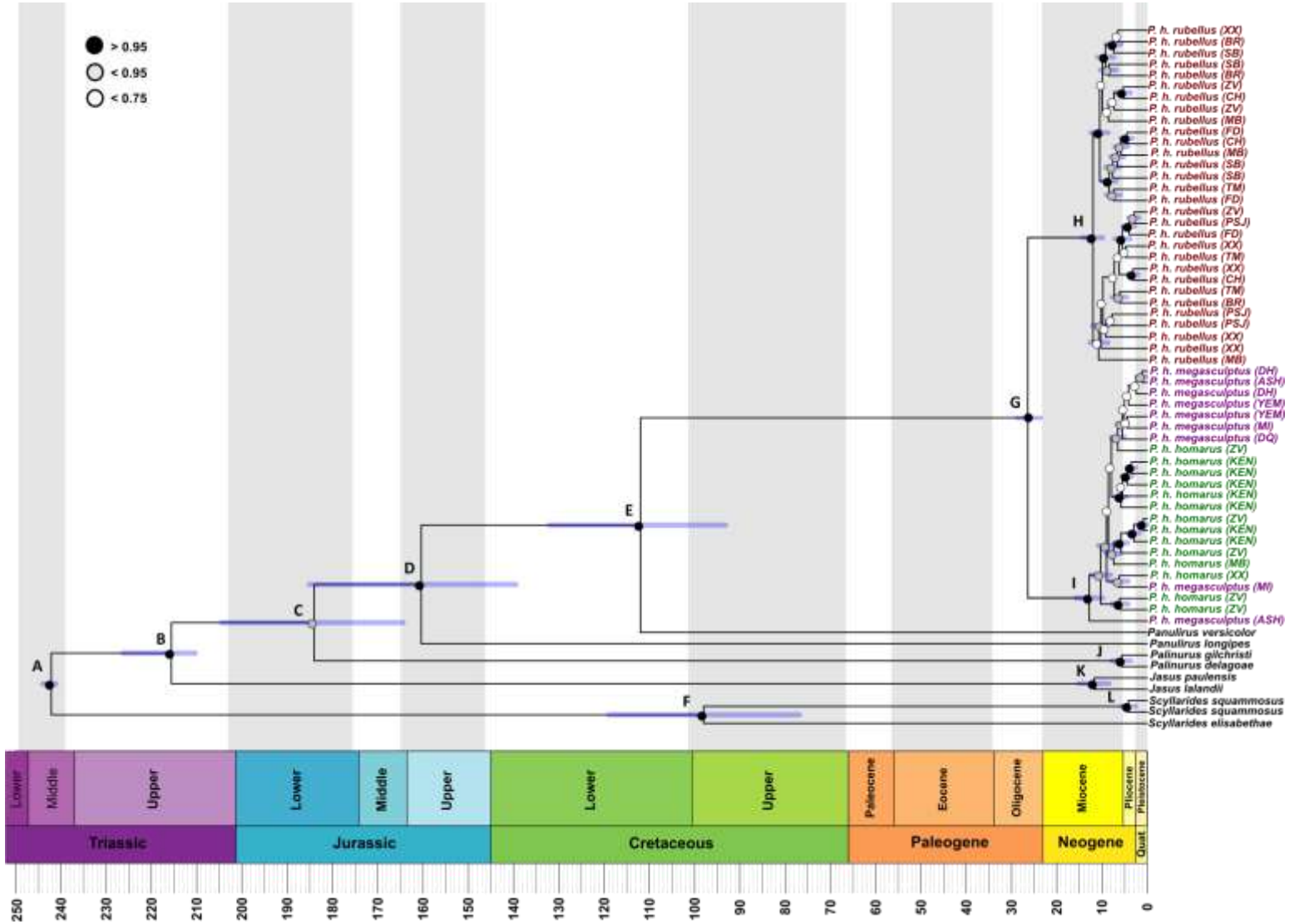


Figure 2.3 BEAST2 maximum clade credibility tree inferred from the concatenated dataset analysis with fossil calibrated nodes. Coloured circles on the nodes indicate Bayesian posterior probability support. Letters on the nodes correspond to Table 2.3. Shaded bars indicate the 95% highest posterior density (HPD) credibility intervals which are listed in Table 2.3.

For the species delimitation analysis using BP & P, the choice of prior distributions seemed to influence species delimitation. Here, the BPP values indicate the probability that the taxa at a particular node is a separate species. Prior combinations 1 and 4 resulted in high posterior probability support for *P. h. rubellus* as a separate species (BPP = 1.0, Fig. 2.4). Prior combination 3 yielded moderate support (BPP = 0.86, Fig. 2.4) while the prior combination specifying a deep divergence and small ancestral population size resulted in no support for *P. h. rubellus* as a separate species. The prior combination 4 supported the distinction of *P. h. homarus* and *P. h. megasculptus* (BPP = 1.0, Fig. 2.4). BP & P consistently delimited *P. longipes and P. versicolor* as distinct species (BPP > 0.90, Fig. 2.4), except for prior combination 2 (BPP = 0.13) and confirmed that the two *S. squammosus* individuals were not separate species (BPP < 0.50, Fig. 2.4). Only prior combination 3 did not support *S. elisabethae* as being a distinct species (BPP = 0.66, Fig 2.4.). Interestingly, there was low support for the separation of *J. paulensis* and *J. lalandii* (BPP = 0.2 & 0.5, Fig. 2.4) under prior combinations 1 and 3. There was also low support for the separation of *P. delagoae* and *P. gilchristi* (BPP = 0.06, 0.5 & 0.28, Fig. 2.4) under prior combinations 1, 3 and 4.

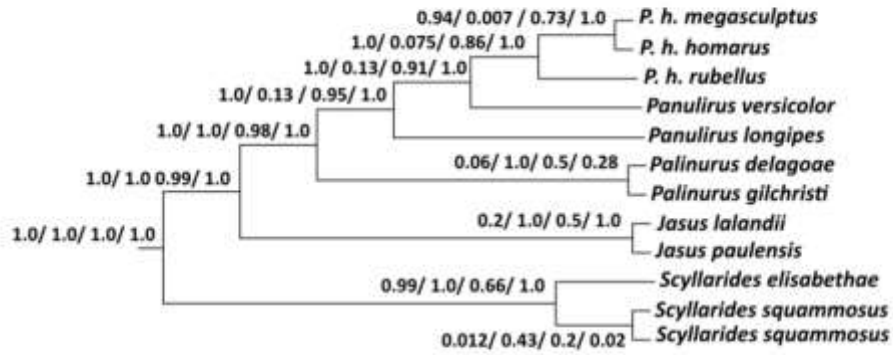


Figure 2.4 BP & P majority rule consensus tree obtained using the BEAST2 guide tree and rjMCMC algorithm one (species delimitation using a fixed guide tree) showing Bayesian posterior probability values supporting the separation of species for each of the different prior combinations.

2.4 Discussion

This study incorporated evidence from molecular (mtDNA and ncDNA), morphology and fossil information to explore the phylogeny of the *P. homarus* subspecies complex throughout the Indo-West Pacific. An important question addressed was whether the *P. h. rubellus* subspecies, occurring along the southeast African coast and Madagascar, was an independently evolving lineage. The results from this study, using more individuals from a wider geographic range, and additional analyses, corroborate the findings of Lavery et al. (2014). They recognized *P. h. rubellus* as being a distinct lineage. In addition, genetic differences found in this study between *P. h. homarus* and the Arabian Sea *P. h. megasculptus* was not substantial enough to warrant the subspecies classification and we suggest that these taxa represent a single morphologically polymorphic lineage. It is suggested that *P. h. rubellus* be elevated to species level, and named *Panulirus rubellus*, or the African spiny lobster, under the universal species concept (de Queiroz 2007). According to this concept, the only defining property of the species is being a separately evolving metapopulation lineage, and the other species concepts are treated as secondary defining characteristics (de Queiroz 2007). The *P. h. rubellus* lineage fulfils the secondary defining criteria for the morphological species concept (Nelson and Platnick 1981, Mishler 1985) as its morphology appears distinct from the other two subspecies. The *P. h. rubellus* lineage forms a distinct genetic cluster or a monophyletic group, fulfilling the secondary characteristics of the genotypic (Mallet 1995) and phylogenetic (Cracraft 1983) species concepts.

At the individual gene level, nuclear gene trees did not support the separation of the subspecies as the trees were unresolved (Supplementary Figs 2.3 and 2.4). When all four genes were analyzed in combination, phylogenetic signal increased along with maximum likelihood bootstrap and BPP support (Fig. 2.2). Incongruence between phylogenies produced using only single gene datasets is a challenge in molecular phylogenetics. To circumvent this issue, many studies now use combined genetic data instead of relying on single gene trees to represent the species tree. Studies have demonstrated that analyses using several genes concatenated can reveal character support for relationships in the overall tree from data sets which on their own do not support the relationships. Concatenated trees can increase

discriminatory power and phylogenetic signal (Olmstead and Sweere 1994, Gatesy et al. 1999, Willows-Munro et al. 2005, de Queiroz and Gatesy 2006).

The uncorrected pairwise distance for COI was 4.8% between *P. h. rubellus* and *P. h. homarus* + *P. h. megasculptus* in this study, compared to the 9% estimate of Lavery et al. (2014). The estimate here was, however, based on a much larger sample size (44 sequences) than in the previous study (7 sequences), which could explain the difference. The pairwise distance for CR in this study of 25.8% between *P. h. rubellus* and *P. h. homarus* + *P. h. megasculptus* was comparable to the 30% of Lavery et al. (2014), thus providing further confidence in the results. For the nuclear markers, pairwise distances between the subspecies were as expected, higher than what was obtained for the COI marker, 8.3% for β-tubulin and 7.7% for ITS-1 because these markers are highly variable intron regions.

The question then arises of how *P. h. rubellus* and *P. h. homarus* diverged, from both life-history and oceanographic perspectives. *P. homarus* has a long planktonic larval phase which may drift in ocean currents for many months (Berry 1974a), during which they can be dispersed over long distances. Phyllosoma larvae can also swim actively to position themselves in the water column, or facilitate dispersal or return them to coastal settlement areas (Phillips et al. 2006). In spite of high dispersal potential of larvae, potentially facilitating larval mixing and genetic connectivity (Siegel et al. 2003), dispersal patterns can be constrained by larval retention in semi-permanent gyres or current systems, which, in turn, are affected by climate change (Pollock 1993, Cowen and Sponaugle 2009).

Based on the signal from the genetic analysis, it is speculated that *P. h. rubellus* larvae are constrained to the southern part of the Southwest Indian Ocean when they become trapped within inshore ocean gyres of the Mozambique Channel and over the southeast African shelf (Reddy et al., 2014). Larvae that stray further offshore, and become entrained in the Agulhas Current will be swept southwestwards and lost. A similar scenario was proposed for another spiny lobster species (*Palinurus gilchristi*) in the same region, in which larvae retained over the shelf and the Agulhas Bank, between the Agulhas Current and the coast, would remain viable, whereas those caught up in the Current would be lost (Groeneveld and Branch 2002, Tolley et al. 2005a). In southern Australia, larvae of a coastal broadcast spawner that remain on the continental shelf (where currents are erratic and often shoreward), returned to the coast in much larger numbers than those entrained in shelf-edge boundary currents (Teske et al.

2015). It is proposed that similar source and sink mechanisms act to constrain *P. h. rubellus* to coastal areas in the Southwest Indian Ocean. Adult *P. h. homarus* occur sympatrically along the southeast African coast, at a low rate, possibly because of larval spill-over from further north in the Mozambique Channel, facilitated by surface drift resulting from monsoon winds (Pollock 1993).

New species may arise if larval retention mechanisms persist, separating species geographically, and in time leading to reproductive isolation (Pollock 1995). In the present study, there was no evidence of hybridization between *P. h. homarus* and *P. h. rubellus*. The occurrence of hybrids between *P. h. homarus* and *P. h. rubellus* was first reported by Berry (1974b), in a boundary area where both subspecies occurred (southern Mozambique), and where the frequency of *P. h. homarus* increases and that of *P. h. rubellus* tapers off. This region could be a contact zone where *P. h. homarus* and *P. h. rubellus* individuals may interbreed, after secondary contact. The single case of hybridization found by Lavery et al. (2014) also highlights that mating can occur between them, but given the highly significant genetic differentiation between the two subspecies, it occurs at a low rate. van der Meeren et al. (2008) also showed that while mating is possible between clawed lobsters *Homarus americanus* and *Homarus gammarus*, the preference is towards conspecifics.

Allopatric speciation, or complete isolation between *P. h. homarus* and *P. h. rubellus* may not occur due to the dynamic nature of the ocean and few barriers to dispersal (Lessios et al. 1998, Waples 1998). Rather, parapatric speciation (partial isolation) may be responsible for the genetic distinctiveness between them (Rocha and Bowen 2008). The patterns in the genetic data correspond with the model of parapatric speciation (Wu and Ting 2004). For example, mitochondrial COI is under strong selection and thus segregates first, whilst the other markers that are not under such selective pressure move between incipient species until complete separation is achieved (Wu and Ting 2004). The factors that promote parapatric speciation are characteristic of the *P. homarus* subspecies complex, as they have a relatively wide geographic range, temporal and spatial differences in their ecological conditions and there is a reduction of effective migration rates between the subspecies because of local ocean currents, eddies and gyres (Coyne and Orr 2004). The influence of oceanographic features and ecological factors such as sea temperature, salinity and turbidity on the distribution differences, speciation and genetic diversity between *P. h. homarus* and *P. h. rubellus* warrant further investigation.

The timing of the emergence of *Panulirus* in the early Mesozoic with divergence dating tree using the multilocus dataset and fossil calibrations, is consistent with morphological evidence within the genus (George and Main 1967). Lavery et al. (2014) used a divergence rate of 1% for COI, which suggested an estimated divergence of 9 Ma ago for *P. h. rubellus*. Estimates in this study using fossil calibrated nodes and four loci demonstrate that *P. h. rubellus* might have radiated between 10 and 16 Ma ago, and last shared a common ancestor with the *P. h. homarus* + *P. h. megasculptus* group approximately 26 Ma ago. George (2006) suggests that the fragmentation of the Tethys Sea is responsible for the radiation of *Panulirus*. Studies have shown that the final closure of the Tethys seaway, 14 Ma ago, during the Middle Miocene, had a significant impact on global ocean circulation (Hamon et al. 2013). Other investigations using marine isotopic data indicated that heat was transported from the northern Indian Ocean to the southern Ocean by a warm, saline water mass known as the Tethyan Indian Saline Water mass, and then ended due to the Tethys Sea closure (Woodruff and Savin 1989, Flower and Kennett 1994, Ramsay et al. 1998). Modelling studies show that during this time, the closure involved changes in salinity and temperature in the Indian Ocean, leading to changes in latitudinal density gradient (Hamon et al. 2013). These oceanographic factors could have had an impact on the formation and speciation of *P. homarus* given that they arose around this period.

The BP & P posterior probability results of the species delimitation analysis were dependent on choosing appropriate prior combinations, as observed in other studies (McKay et al. 2013, Zhang et al. 2011). It is proposed that the most biologically relevant prior combination would be a small ancestral population size and shallow divergence (prior combination 3) because recently evolved lobsters such as the *P. homarus* subspecies and *P. versicolor* have lower levels of sequence divergence and shorter branch lengths between species, than between more ancestral species such as *P. longipes* (Ptacek et al. 2001). This result provides further evidence that the taxonomy of *P. h. rubellus* should be reviewed.

To conclude, classical multilocus phylogenetic, coalescent-based, and divergence time estimation with fossil calibration methods were used to resolve the *P. homarus* subspecies complex. The lack of haplotypes shared between *P. h. homarus* and *P. h. rubellus,* and their distinct groupings on the four-gene concatenated phylogeny suggest that they are genetically distinct lineages. *P. h. rubellus* exhibits reciprocal monophyly because the clade consists only

of *P. h. rubellus* individuals and no *P. h. rubellus* individuals are present in the *P. h. homarus* + *P. h. megasculptus* clade. The observed genetic differentiation could be attributed to local larval retention mechanisms and ocean currents affecting dispersal capability of their long-lived phyllosoma stage. Based on the morphological (Berry 1971) and distribution (Holthuis 1991) differences and the results from the present study using a concatenated dataset of four genes - which strongly support the findings of Lavery et al. (2014) - that the taxonomic status of *P. h. rubellus* as a subspecies of *P. homarus* should be re-evaluated. It should be acknowledged as a separately evolving lineage and a new species, *Panulirus rubellus*, from the southwest Indian Ocean

2.5 References

Agapow P. M., O. R. P. Bininda-Emonds, K. A. Crandall, J. L. Gittleman, G. M. Mace, J. C. Marshall, and A. Purvis. 2004. The impact of species concept on biodiversity studies. *Quarterly Review of Biology* **79**: 161-179.

Aldous D. J. 2001. Stochastic models and descriptive statistics for phylogenetic trees, from Yule to today. *Statistical Science* **16**: 23-34.

Ball R. M., and J. C. Avise. 1992. Mitochondrial DNA phylogeographic differentiation among avian populations and the evolutionary significance of subspecies. *Auk* **109**: 626-636.

Barrowclough G. F. 1980. Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. *Auk* **97**: 655-668.

Bell M. A., and G. T. Lloyd. 2014. Strap: an R package for plotting phylogenies against stratigraphy and assessing their stratigraphic congruence. *Palaeontology* **58**: 379-389.

Berry P. F. 1971. The biology of the spiny lobster *Panulirus homarus* (Linnaeus) off the east coast of southern Africa. *South African Association for Marine Biological Research, Investigational Report* **28**: 3-75.

Berry P. F. 1974a. Palinurid and scyllarid lobster larvae of the Natal Coast, South Africa. *South African Association for Marine Biological Research, Investigational Report* **34**: 3-44.

Berry P. F. 1974b. A revision of the *Panulirus homarus*-group of spiny lobsters (Decapoda, Palinuridae). *Crustaceana* **27**: 31-42.

Bouckaert R., J. Heled, D. Kuhnert, T. Vaughan, C-H. Wu, D. Xie, M. A. Suchard, A. Rambaut, and A. J. Drummond. 2014. BEAST 2: a software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **10**: e1003537.

Bracken-Grissom H. D., S. T. Ahyong, R. D. Wilkinson, R.M Feldmann, C. E. Schweitzer, J. W. Breinholt, M. Bendall, F. Palero, T. Y. Chan, D. L. Felder DL et al. 2014. The emergence of lobsters: phylogenetic relationships, morphological evolution and divergence time comparisons of an ancient group (Decapoda: Achelata, Astacidea,

Glypheidea, Polychelida). *Systematic Biology* **63**: 457-479.

Burbrink F. T., R. Lawson, and J. B. Slowinski. 2000. Mitochondrial DNA phylogeography of the polytypic North American rat snake (*Elaphe obsoleta*): a critique of the subspecies concept. *Evolution* **54**: 2107-2118.

Burbrink F. T., H. Yao, M. Ingrasci, R. W. J. Bryson, T. J. Guiher, and S. Ruane. 2011. Speciation at the Mogollon Rim in the Arizona mountain kingsnake (*Lampropeltis pyromelana*). *Molecular Phylogenetics and Evolution* **60**: 445-454.

Chu K. H., C. P. Li, and H. Y. Ho. 2001. The first internal transcribed spacer (ITS-1) of ribosomal DNA as a molecular marker for phylogenetic and population analyses in crustacea. *Marine Biotechnology* **3**: 355-361.

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

Coyne J. A., and H. A. Orr. 2004. Speciation. Sunderland: Sinauer Associates, Inc. 400 pages.

Cracraft J. 1983. Species concepts and speciation analysis. *Current Ornithology* **1**: 159-187.

Darriba D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.

De Angeli A., and A. Garassino. 2008. *Pseudosquilla lessinea* n. sp. (Crustacea, Stomatopoda, Pseudosquillidae) and *Scyllarides bolcensis* n. sp. (Crustacea, Decapoda, Scyllaridae) from the lower Eocene (Ypresian) of Monte Postale (Altissimo, Vincenza, NE Italy. *Atti della Societa italiana di Scienze naturali e del Museo civico di Storia naturale in Milano* **149**: 167-178.

de Queiroz A., and J. Gatesy. 2007. The supermatrix approach to systematics. *Trends in Ecology & Evolution* **22**: 34-41.

de Queiroz K. 2007. Species concepts and species delimitation. *Systematic Biology* **56**: 879-886.

Degnan J. H., and N. A. Rosenberg. 2009. Gene tree discordance, phylogenetic inference and the multispecies coalescent. *Trends in Ecology & Evolution* **24**: 332-340.

Diniz F. M., N. Maclean, M. Ogawa, I. H. A. Cintra, and P. Bentzen. 2005. The hypervariable domain of the mitochondrial control region in Atlantic spiny lobsters and its potential as a marker for investigating phylogeographic structuring. *Marine Biotechnology* **7**: 462-473.

Farhadi A., H. Farahmand, M. A. Nematollahi, A. G. Jeffs, and S.D. Lavery. 2013. Mitochondrial DNA population structure of the scalloped lobster *Panulirus homarus* (Linnaeus 1758) from the West Indian Ocean. *ICES Journal of Marine Science* **70**: 1491-1498.

Feldmann R. M., C. E. Schweitzer, S. Hu, Q. Zhang, C. Zhou, T. Xie, J. Huang, and W. Wen. 2012. Macrurous Decapoda from the Luoping biota (Middle Triassic) of China. *Journal of Paleontology* **86**: 425-441.

Flot J-F. 2009. SEQPHASE: a web tool for interconverting PHASE input/output files and FASTA sequence alignments. *Molecular Ecology Resources* **10**: 162-166.

Flower B. P., and J. P. Kennett. 1994. The middle Miocene climatic transition: east Antarctic ice sheet development, deep ocean circulation and global carbon cycling. *Palaeogeography Palaeoclimatology Palaeoecology* **108**: 537-555.

Garassino A., and G. Teruzzi. 1993. A new decapod crustacean assemblage from the Upper Triassic of Lombardy (N. Italy). *Paleontologia Lombarda, (nuova serie)* **1**: 1-27.

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

George R. W., and A. R. Main. 1967. The evolution of spiny lobsters (Palinuridae): a study of evolution in the marine environment. *Evolution* **21**: 803-820.

George R. W. 1997. Tectonic plate movements and the evolution of *Jasus* and *Panulirus* spiny lobsters (Palinuridae). *Marine and Freshwater Research* **48**: 1121-1130.

George R. W. 2005. Evolution of life cycles, including migration, in spiny lobsters (Palinuridae). *New Zealand Journal of Marine and Freshwater Research* **39**: 503-514.

George R. W. 2006. Tethys origin and subsequent radiation of the spiny lobster (Palinuridae). *Crustaceana* **79**: 397-422.

Glaessner M. F. 1960. The fossil decapod Crustacea of New Zealand and the evolution of the order Decapoda. *New Zealand Geological Survey Paleontological Bulletin* **31**: 3-63.

Groeneveld J. C., and G. M. Branch. 2002. Long-distance migration of South African deep-water rock lobster *Palinurus gilchristi*. *Marine Ecology Progress Series* **232**: 225-238.

Hall T. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**: 95-98.

Hamon N., P. Sepulchre, V. Lefebvre, and G. Ramstein. 2013. The role of eastern Tethys seaway closure in the Middle Miocene Climatic Transition (ca. 14 Ma) *Climate of the Past* **9**: 2687-2702.

Heibl C. 2008. PHYLOCH: R language tree plotting tools and interfaces to diverse phylogenetic software packages. http://www.christophheibl.de/Rpackages.html.

Holthuis L. B. 1991. FAO species catalogue. Marine lobsters of the world. An annotated and illustrated catalogue of species of interest to fisheries known to date. *FAO Fisheries Synopsis* No. 125. Rome: Food and Agricultural Organization of the United Nations. Page 292.

Hudson R. R. 1991. Gene genealogies and the coalescent process. *In: Oxford Surveys in Evolutionary Biology*. Futuyama D, Antonovics J (Eds). Oxford University Press. Pages 1-44.

Huelsenbeck J., and F. Ronquist. 2005. Bayesian analysis of molecular evolution using MrBayes. *In: Statistical Methods in Molecular Evolution*. Springer. Pages 183-226.

Irwin. D. E. 2002. Phylogeographic breaks without geographic barriers to gene flow. *Evolution* **56**: 2383-2394.

Jennings R. M., and R. J. Etter. 2011. Exon-primed, intron-crossing (EPIC) loci for five nuclear genes in deep-sea protobranch bivalves: primer design, PCR protocols and locus utility. *Molecular Ecology Resources* **11**: 1102-1112.

Johnsen A., S. Andersson, J. G. Fernandez, B. Kempenaers, V. Pavel, S. Questiau, M. Raess, E. Rindal, and J. T. Lifjeld. 2006. Molecular and phenotypic divergence in the bluethroat (*Luscinia svecica*) subspecies complex. *Molecular Ecology* **15**: 4033-4047.

Katoh K., K. Misawa, K. Kuma, and T. Miyata. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research* **30**: 3059-3066.

Kingman J. F. C. 1982. The coalescent. *Stochastic Processes and their Applications* **13**: 235-248.

Knowles L. L., and B. C. Carstens. 2007. Delimiting species without monophyletic gene trees. *Systematic Biology* **56**: 887-895.

Lanfear R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**: 1695-1701.

Lavery S. D., A. Farhadi, H. Farahmand, T. Y. Chan, A. Azhdehakoshpour, V. Thakur, and A. G. Jeffs. 2014. Evolutionary divergence of geographic subspecies within the scalloped spiny lobster *Panulirus homarus* (Linnaeus 1758) *PLoS ONE* **9**. DOI: 10.1371/journal.pone.0097247.

Leache A. D., and M. K. Fujita. 2010. Bayesian species delimitation in West African forest geckos (*Hemidactylus fasciatus*). *Proceedings of the Royal Society B: Biological Sciences* **277**: 3071-3077.

Lessios H. A., B. D. Kessing, and D. R. Robertson. 1998. Massive gene flow across the world's most potent marine biogeographic barrier. *Proceedings of the Royal Society B: Biological Sciences* **265**: 583-588.

Lutjeharms J. R. E. 2006. The Agulhas Current. Heidelberg: Springer. Page 329.

Mallet J. 1995. A species definition for the modern synthesis. *Trends in Ecology & Evolution* **10**: 294-299.

Mayr E. 1942. Systematics and the Origin of Species. Columbia University Press. 382 pages.

McCracken K. G., and M. D. Sorenson. 2005. Is homoplasy or lineage sorting the source of incongruent mtDNA and nuclear gene trees in the stiff-tailed ducks (*Nomonyx-Oxyura*)? *Systematic Biology* **54**: 35-55.

McKay B. D., H. L. Mays, Y. Wu, H. Li, C-T. Yao, I. Nishium, and F. Zou. 2013. An

empiracle comparison of character-based and coalescent-based approaches to species delimitation in a young avian complex. *Molecular Ecology* **22**: 4943-4957.

Mishler B. D. 1985. The morphological, developmental, and phylogenetic basis of species concepts in bryophytes. *Bryologist* **88**: 207-217.

Morin P. A., F. I. Archer, A. D. Foote, J. Vilstrup, E. E. Allen, P. Wade, J. Durban, K. Parsons, R. Pitman, L. Li et al. 2010. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. *Genome Research* **20**: 908-916.

Nelson G., and N. Platnick. 1981. Systematics and Biogeography: Cladistics and Vicariance. Columbia University Press. 567 pages.

Olmstead R. G., and J. A. Sweere. 1994. Combining data in phylogenetic systematics: an empirical approach using three molecular data sets in the *Solanaceae*. *Systematic Biology* **43**: 467-481.

Palero F., K. A. Crandall, P. Abelló, E. Macpherson, and M. Pascual. 2009. Phylogenetic relationships between spiny, slipper and coral lobsters (Crustacea, Decapoda, Achelata) *Molecular Phylogenetics and Evolution* **50**: 152-162.

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

Parkin D., and Knox A. 2010. The Status of Birds in Britain and Ireland. Bloomsbury Publishing. 440 pages.

Phillimore A. B., and I. P. F Owens. 2006. Are subspecies useful in evolutionary and conservation biology? *Proceedings of the Royal Society B: Biological Sciences* **273**: 1049-1053.

Phillips B. F., J. S. Cobb, A. Jeffs, and P. McWilliam. 2006. Larval and postlarval ecology. *In: Lobsters: Biology, Management, Aquaculture and Fisheries.* Blackwell Publishing, Oxford. Pages 231-262.

Pinna G. 1974. I crostacei della fauna triassica di Cene in Val Seriana (Bergamo). *Memorie della Societa italiana di Scienze naturali e del Museo civico di Storia naturale in Milano* **21**: 5-34.

Plummer M., N. Best, K. Cowles, and K. Vines. 2006. CODA: convergence diagnosis and output analysis for MCMC. *R News* **6**: 7-11.

Pollock D. E. 1992. Palaeoceanography and speciation in the spiny lobster genus *Panulirus* in the Indo-Pacific. *Bulletin of Marine Science* **51**: 135-146.

Pollock D. E. 1993. Speciation in spiny lobsters—clues to climatically-induced changes in ocean circulation patterns. *Bulletin of Marine Science* **53**: 937-944.

Pollock D. E. 1995. Evolution of life-history patterns in three genera of spiny lobsters. *Bulletin of Marine Science* **57**: 516-526.

Ptacek M. B., S. K. Sarver, M. J. Childress, and W. F. Herrnkind. 2001. Molecular phylogeny of the spiny lobster genus *Panulirus* (Decapoda: Palinuridae). *Marine and Freshwater Research* **52**: 1037-1047.

Rambaut A., and A. J. Drummond. 2007. Tracer. v. 1.6. http://tree.bio.ed.ac.uk/software/tracer/.

Ramsay T. S., C. W. Smart, and J. C. Zachos. 1998. A model of early to middle Miocene Deep Ocean circulation for the Atlantic and Indian Oceans. *Geological Society, London, Special Publications* **131**: 55-70.

Rannala B., and Z. Yang. 2013. Improved reversible jump algorithms for Bayesian species delimitation. *Genetics* **194**: 245-253.

R Development Core Team. 2008. R: A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. 3-900051-07-0

Reddy M. M., A. H. H. MacDonald, J. C. Groeneveld, and M. H. Schleyer. 2014. Phylogeography of the scalloped spiny-lobster *Panulirus homarus rubellus* in the Southwest Indian Ocean. *Journal of Crustacean Biology* **34**: 773-781.

Revell L. J. 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217-223.

Rocha L. A., and B. W. Bowen. 2008. Speciation in coral-reef fishes. *Journal of Fish Biology* **72**: 1101-1121.

Ronquist F., and J. P. Huelsenbeck. 2003. MrBAYES 3: Bayesian phylogenetic inference

under mixed models. *Bioinformatics* **19**: 1572-1574.

Schram F. R., and C. J. Dixon. 2004. Decapod phylogeny: addition of fossil evidence to a robust morphological cladistic data set. *Bulletin of the Mizunami Fossil Museum* **31**: 1-19.

Setiadi M. I., J. A. McGuire, R. M. Brown, M. Zubairi, D. T. Iskandar, N. Andayani, J. Supriatna, and B. J. Evans. 2011. Adaptive radiation and ecological opportunity in Sulawesi and Philippine fanged frog. *American Naturalist* **178**: 221-240.

Siegel D. A., B. P. Kinlan, B. Gaylord, and S. D. Gaines. 2003. Lagrangian descriptions of marine larval dispersion. *Marine Ecology Progress Series* **260**: 83-96.

Stephens M., N. J. Smith, and P. Donnelly. 2001. A new statistical method for haplotype reconstruction from population data. *American Journal of Human Genetics* **68**: 978-989.

Tamura K., G. Stecher, D. Peterson, A. Filipski, and S. Kumar. 2013. MEGA 6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725-2729.

Teske P. R., J. Sandoval-Castillo, E. van Sebille, J. Waters, and L. B. Beheregaray. 2015. On-shelf larval retention limits population connectivity in a coastal broadcast spawner. *Marine Ecology Progress Series* **532**: 1-12.

Tolley K. A., J. C. Groeneveld, K. Gopal, and C. A. Matthee. 2005. Mitochondrial DNA panmixia in spiny lobster *Palinurus gilchristi* suggests a population expansion. *Marine Ecology Progress Series* **297**: 225-231.

Tourinho J. L., A. M. Solé-Cava, and C. Lazoski. 2012. Cryptic species within the commercially most important lobster in the tropical Atlantic, the spiny lobster *Panulirus argus*. *Marine Biology* **159**: 1897-1906.

Tsang L. M., T. Y. Chan, M. K. Cheung, and K. H. Chu. 2009. Molecular evidence for the Southern Hemisphere origin and deep-sea diversification of spiny lobsters (Crustacea: Decapoda: Palinuridae). *Molecular Phylogenetics and Evolution* **51**: 304-311.

Vaidya G., D. J. Lohman, and R. Meier. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* **27**: 171-180.

van der Meeren G. I., A. Chandrapavan, and T. Breithaupt. 2008. Sexual and aggressive interactions in a mixed species group of lobsters *Homarus gammarus* and *H. americanus*. *Aquatic Biology* **2**: 191-200.

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

Wheeler D. L., D. M. Church, S. Federhen, A. E. Lash, T. L. Madden, J. U. Pontius, G. D. Schuler, L. M. Schriml, E. Sequeira, T. A. Tatusova, and L. Wagner. 2003. Database resources of the National Center for Biotechnology. *Nucleic Acids Research* **31**: 28-33.

Willows-Munro S., C. A. Matthee, and T. J. Robinson. 2005. Utility of nuclear DNA intron markers at lower taxonomic levels: phylogenetic resolution among the nine *Tragelaphus spp*. *Molecular Phylogenetics and Evolution* **35**: 624-636.

Woodruff F, and S. M. Savin. 1989. Miocene deepwater oceanography. *Paleoceanography* **4**: 87-140.

Wu C. I., and C. T. Ting. 2004. Genes and speciation. *Nature Reviews Genetics* **5**: 114-122.

Yang Z., and B. Rannala. 2010. Bayesian species delimitation using multilocus sequence data. *Proceedings of the National Academy of Sciences USA* **107**: 9264-9269.

Yang Z. 2015. A tutorial of BPP for species tree estimation and species delimitation. *Current Zoology* **61**: 854-865.

Zhang C., D. X. Zhang, Z. Yang. 2011. Evaluation of a bayesian coalescent method of species delimitation. *Systematic Biology* **60**: 747-761.

Zwickl D. J. 2006. Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion. Ph.D. dissertation, The University of Texas at Austin.

2.6 Supplementary information

Figure S2.1 Maximum likelihood tree for *P. homarus* using COI, with midpoint rooting and no outgroups. Maximum likelihood bootstrap support values and Bayesian posterior probabilities are indicated on the nodes. Each colour represents the different subspecies and the two lineages are highlighted. The scale bar indicates the number of nucleotide substitutions per site.

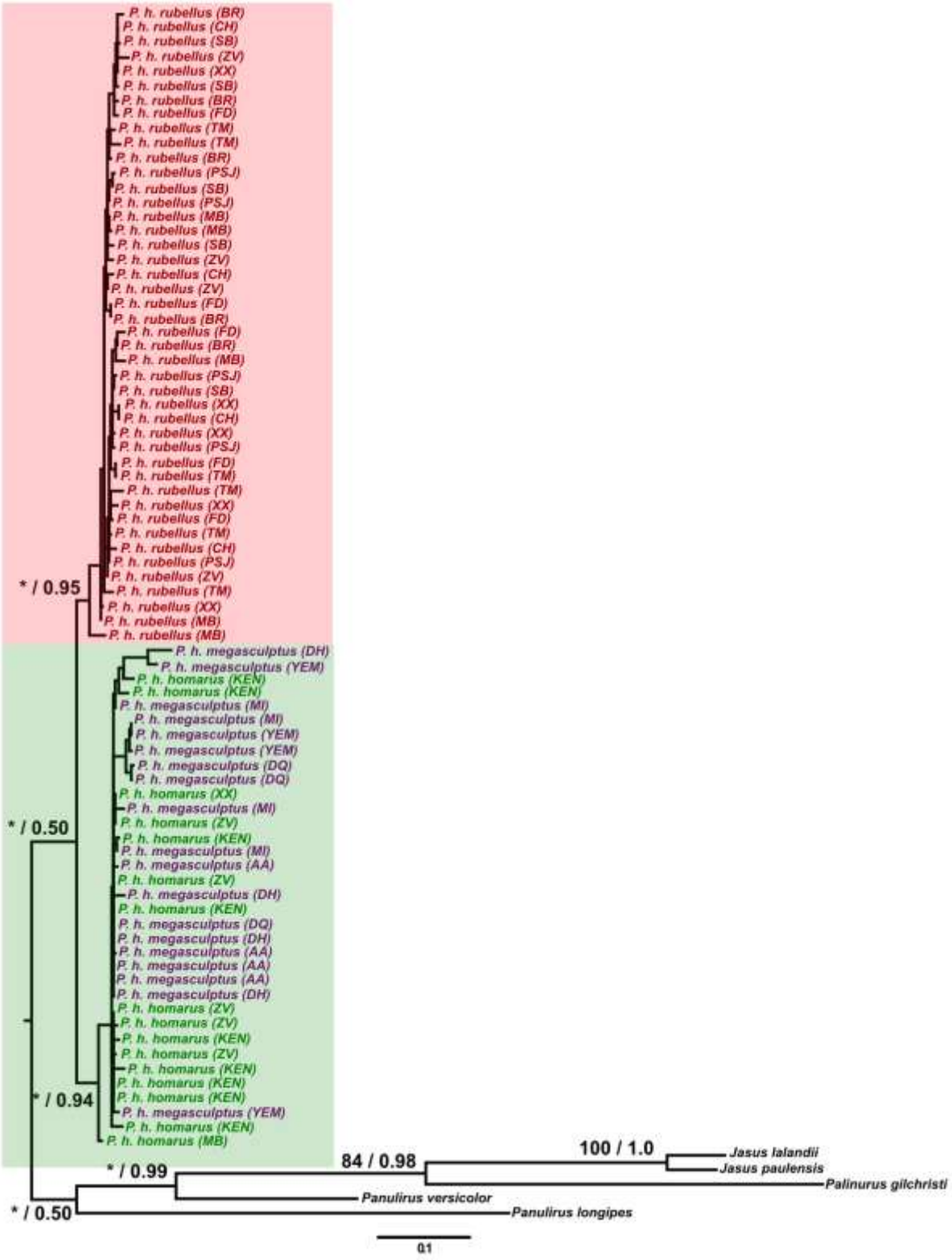


Figure S2.2 Maximum likelihood tree for *P. homarus* using COI, with outgroups. Maximum likelihood bootstrap support values and Bayesian posterior probabilities are indicated on the nodes. * indicates no bootstrap or BPP support. Each colour represents the different subspecies and the two lineages are highlighted. The scale bar indicates the number of nucleotide substitutions per site.

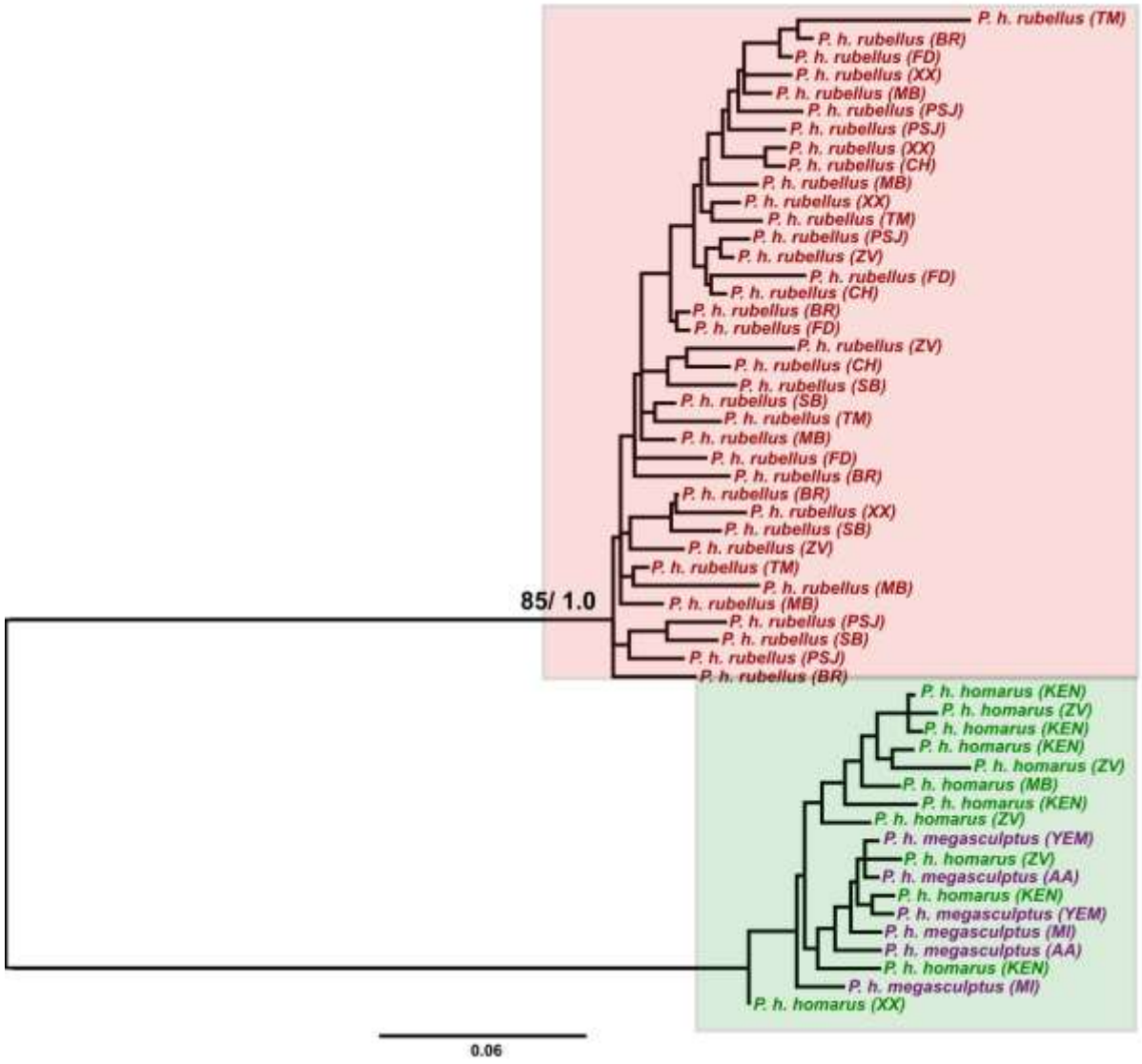


Figure S2.3 Maximum likelihood tree for *P. homarus* using CR, with midpoint rooting and no outgroups. Maximum likelihood bootstrap support values and Bayesian posterior probabilities are indicated on the nodes. Each colour represents the different subspecies and the two lineages are highlighted. The scale bar indicates the number of nucleotide substitutions per site.

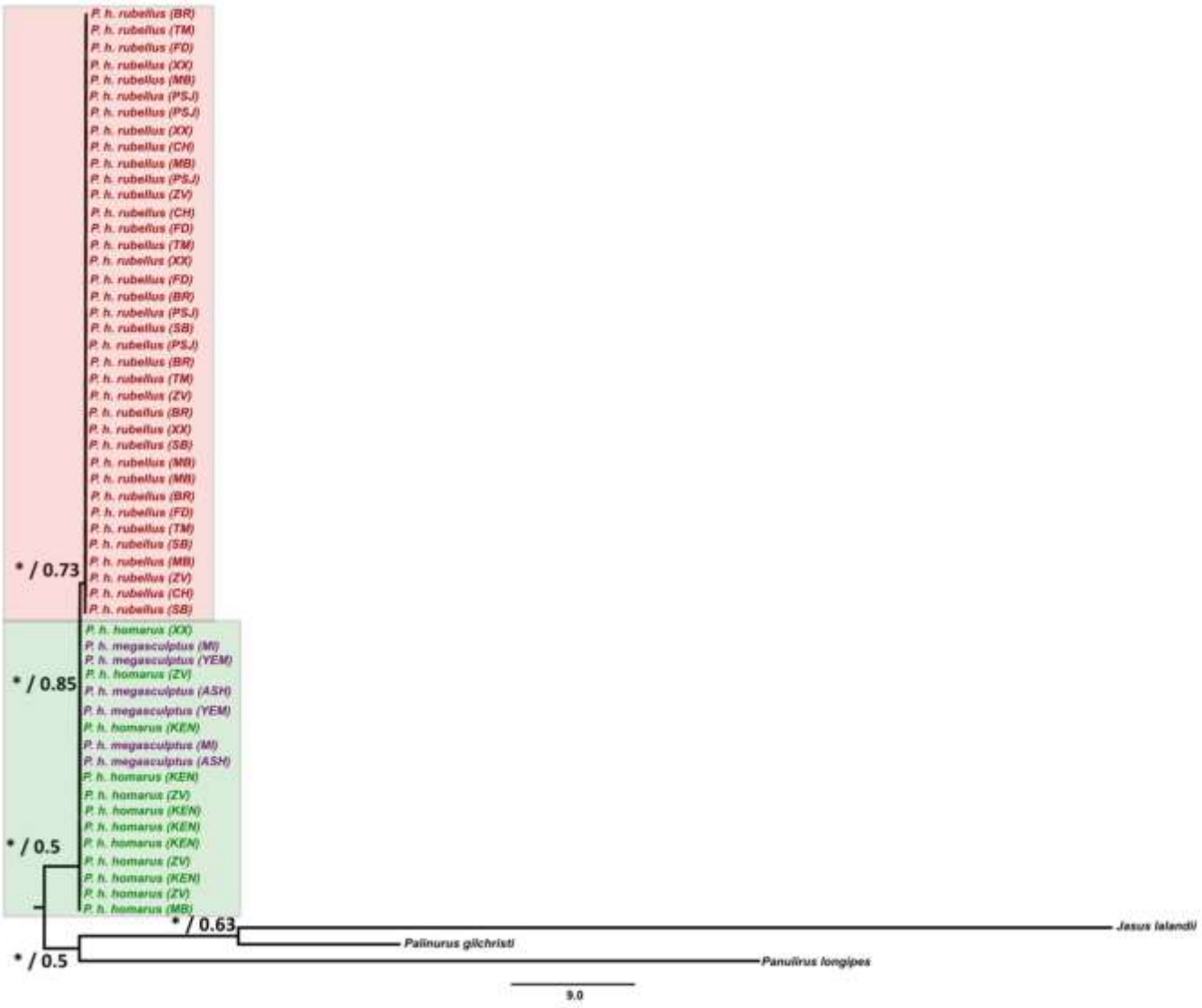


Figure S2.4 Maximum likelihood tree for *P. homarus* using CR, with outgroups. Maximum likelihood bootstrap support values and Bayesian posterior probabilities are indicated on the nodes. * indicates no bootstrap or BPP support. Each colour represents the different subspecies and the two lineages are highlighted. The scale bar indicates the number of nucleotide substitutions per site.

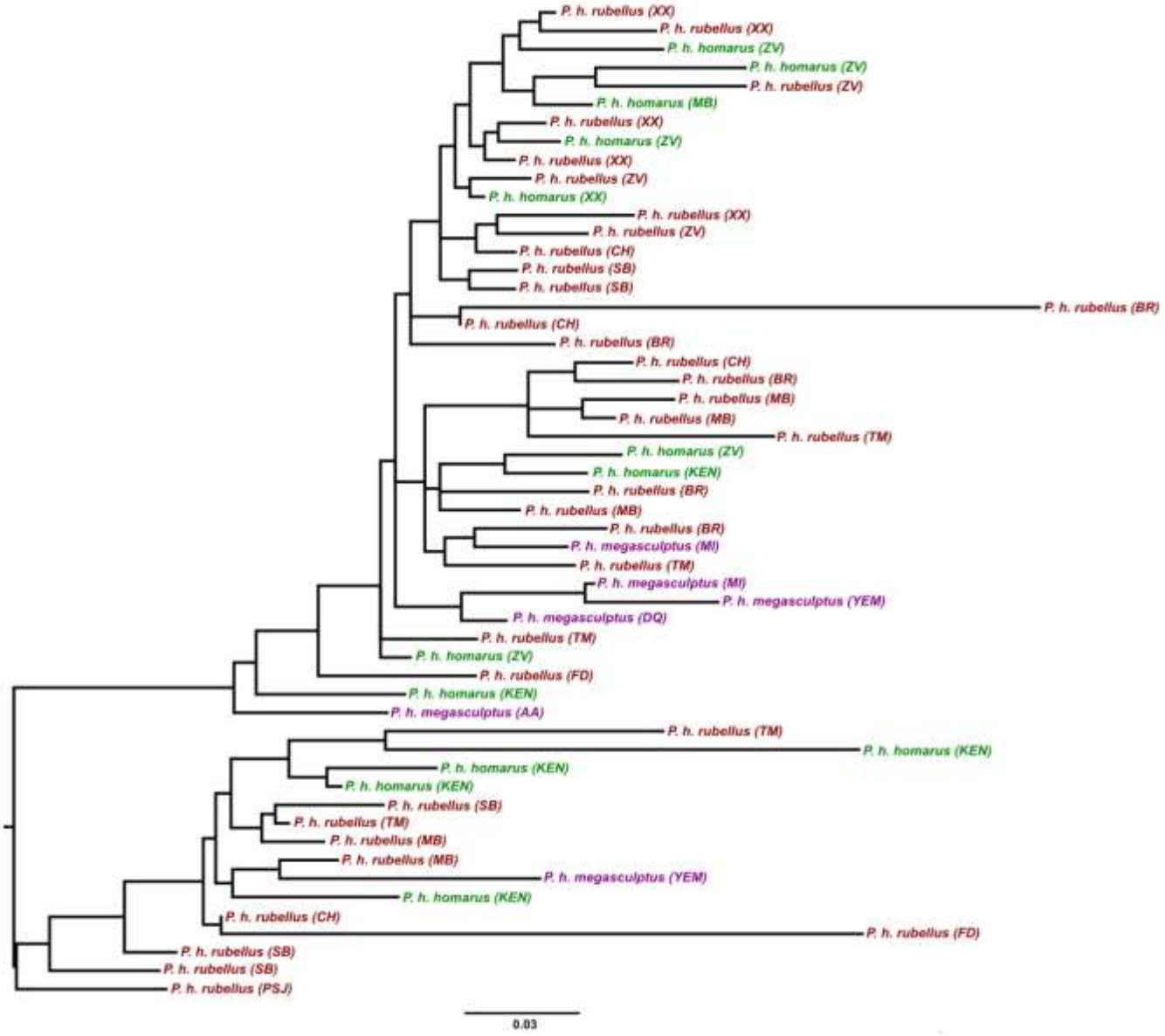


Figure S2.5 Maximum likelihood tree for *P. homarus* using β-tubulin, with midpoint rooting and no outgroups. Each colour represents the different subspecies. The scale bar indicates the number of nucleotide substitutions per site.

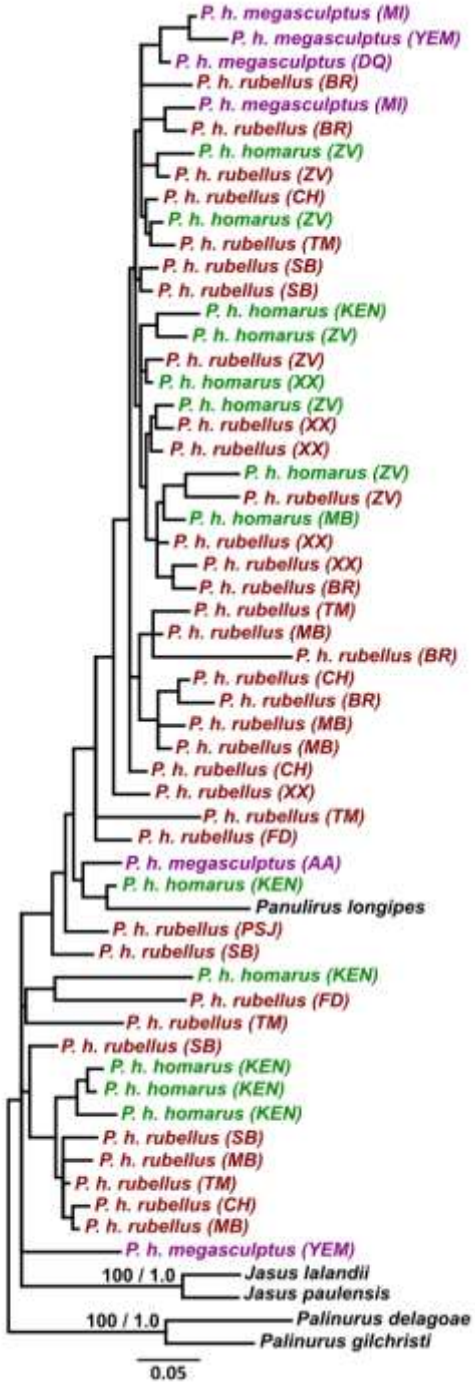


Figure S2.6 Maximum likelihood tree for *P. homarus* using β-tubulin, with outgroups. Each colour represents the different subspecies. Maximum likelihood bootstrap support values and Bayesian posterior probabilities are indicated on the nodes. The scale bar indicates the number of nucleotide substitutions per site.

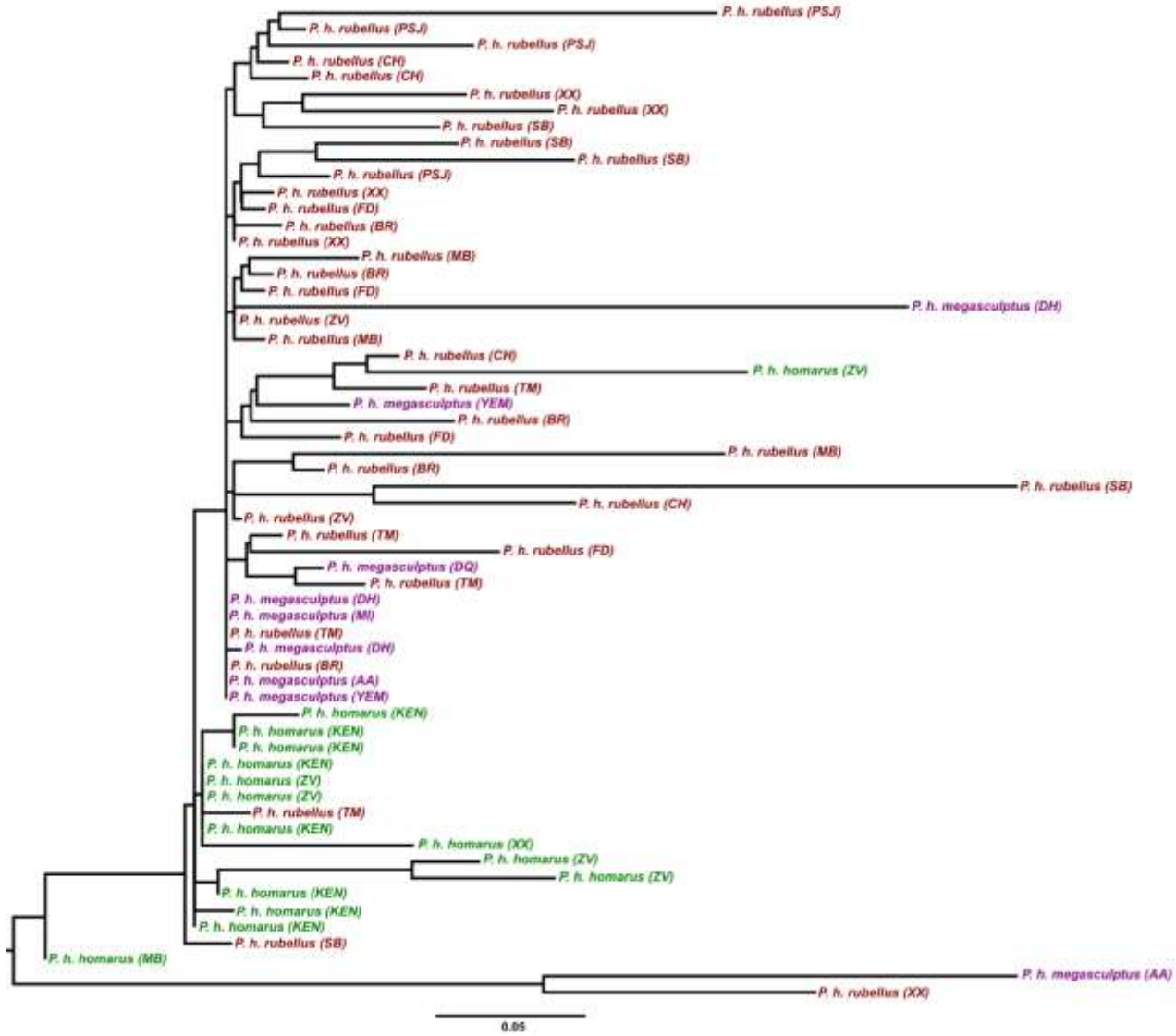


Figure S2.7 Maximum likelihood tree for *P. homarus* using ITS-1, with midpoint rooting and no outgroups. Each colour represents the different subspecies. The scale bar indicates the number of nucleotide substitutions per site.

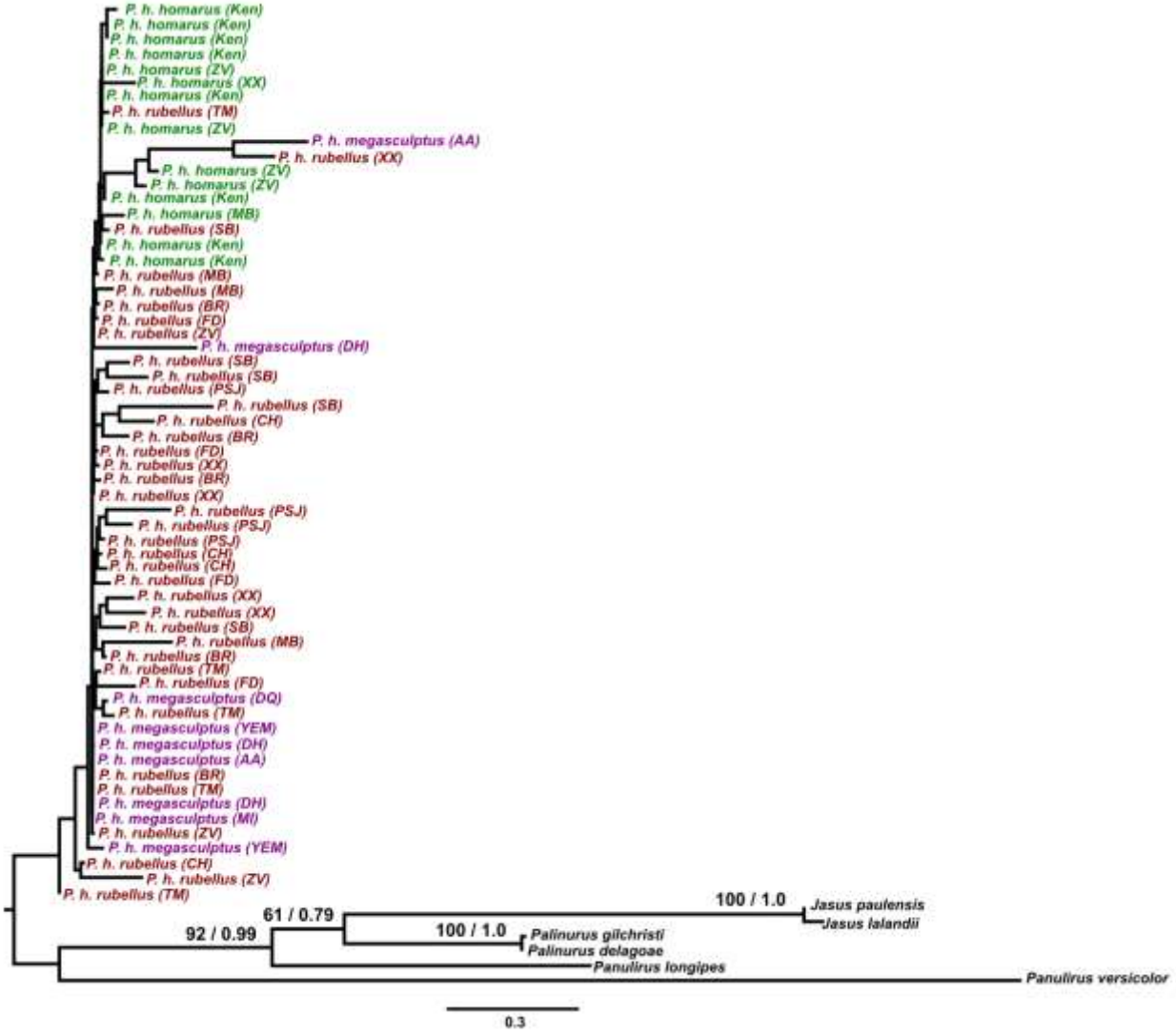


Figure S2.8 Maximum likelihood tree for *P. homarus* using ITS-1, with outgroups. Each colour represents the different subspecies. Maximum likelihood bootstrap support values and Bayesian posterior probabilities are indicated on the nodes. The scale bar indicates the number of nucleotide substitutions per site.

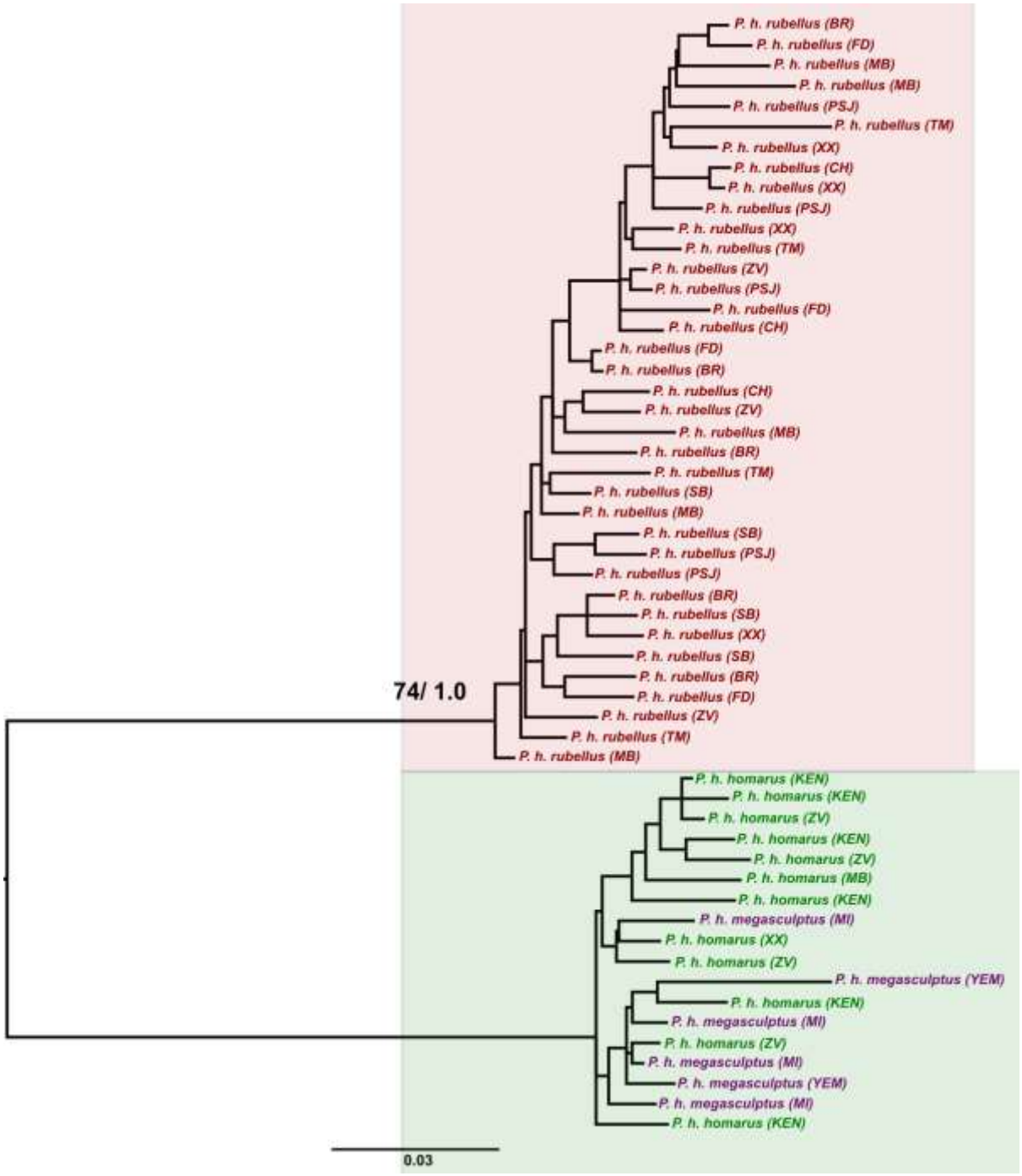


Figure S2.9 Maximum likelihood tree for *P. homarus* using the combined mitochondrial DNA dataset (COI+CR), with midpoint rooting and no outgroups. Maximum likelihood bootstrap support values and Bayesian posterior probabilities are indicated on the nodes. Each colour represents the different subspecies and the two lineages are highlighted. The scale bar indicates the number of nucleotide substitutions per site.

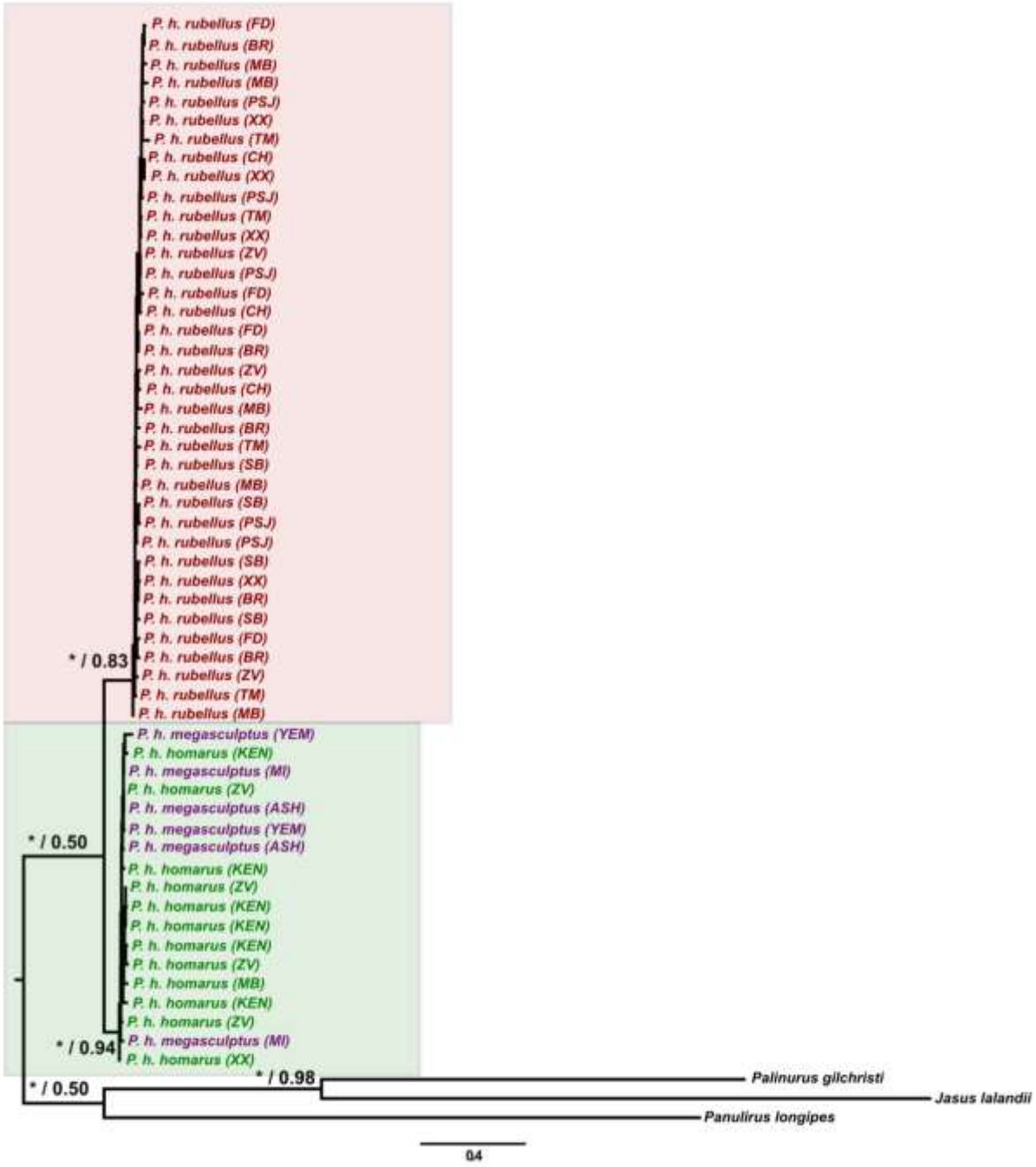


Figure S2.10 Maximum likelihood tree for *P. homarus* using a combined mitochondrial DNA dataset (COI+CR), with outgroups. Maximum likelihood bootstrap support values and Bayesian posterior probabilities are indicated on the nodes. * indicates no bootstrap or BPP support. Each colour represents the different subspecies and the two lineages are highlighted. The scale bar indicates the number of nucleotide substitutions per site.

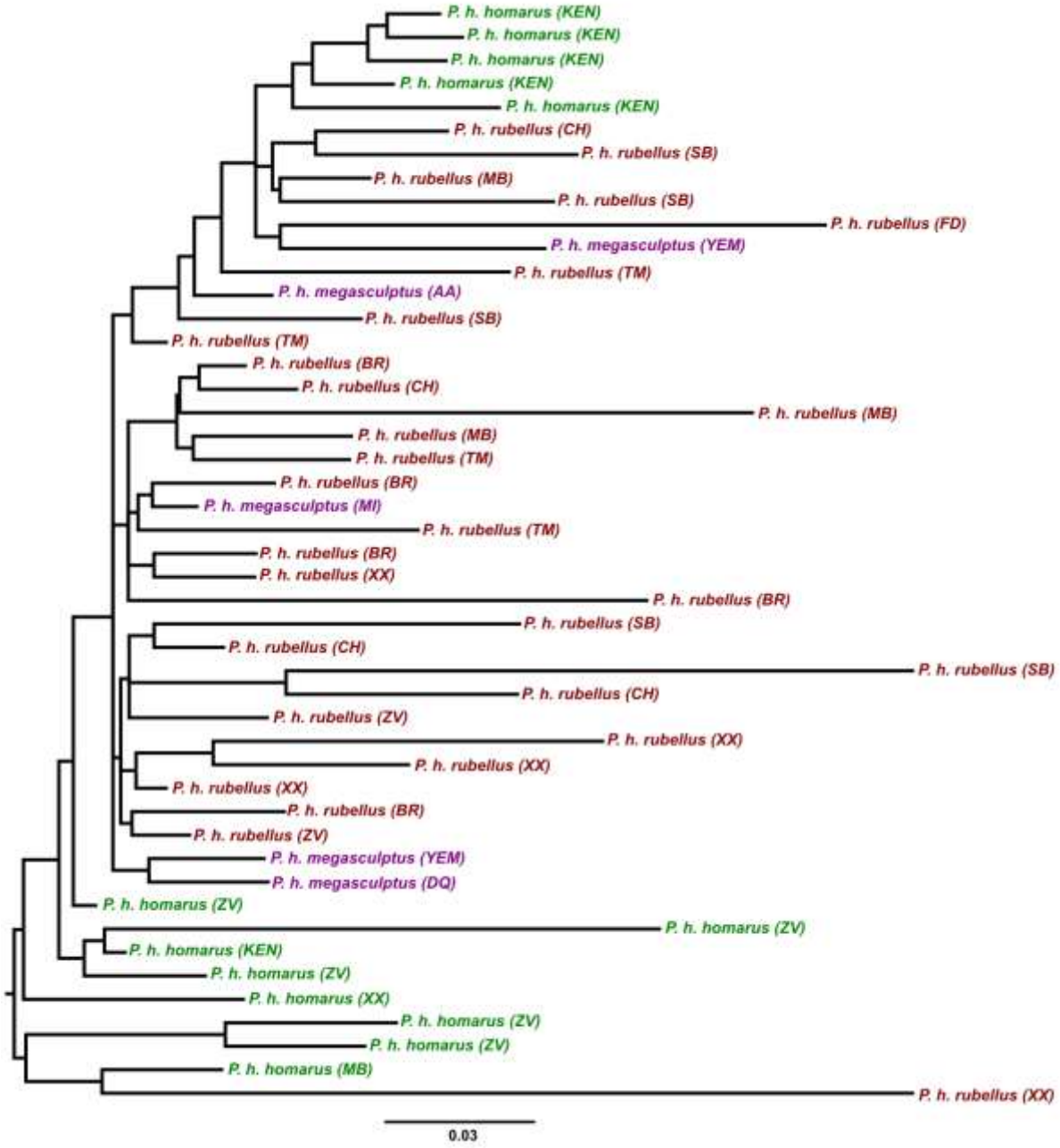


Figure S2.11 Maximum likelihood tree for *P. homarus* using the combined nuclear DNA dataset (β-tubulin + ITS-1), with midpoint rooting and no outgroups. Each colour represents the different subspecies. The scale bar indicates the number of nucleotide substitutions per site.

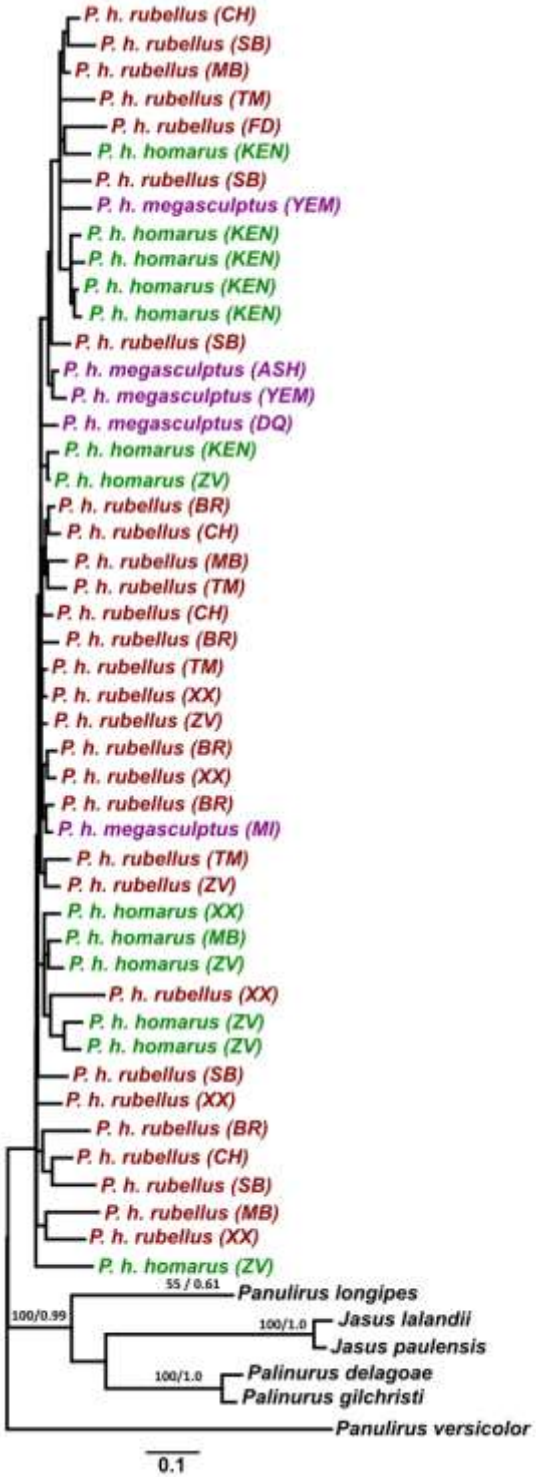


Figure S2.12 Maximum likelihood tree for *P. homarus* using the combined nuclear DNA dataset (β-tubulin + ITS-1), with outgroups. Maximum likelihood bootstrap support values and Bayesian posterior probabilities are indicated on the nodes. Each colour represents the different subspecies. The scale bar indicates the number of nucleotide substitutions per site.

Table S2.1 List of *P. homarus* subspecies and outgroup taxa used for phylogenetic analyses. * Indicates that the individual was not successfully sequenced using that marker.

Specimen ID	Identification	Location	COI	CR	BTUB	ITS-1	Reference
KB01	*P. h. homarus*	Kenya	**KX275311**	**KX349875**	**KX397099**	**KX349818**	This study
KB02	*P. h. homarus*	Kenya	**KX275312**	**KX349876**	**KX397100**	**KX349819**	This study
KB08	*P. h. homarus*	Kenya	**KX275313**	**KX349877**	**KX397101**	**KX349820**	This study
KB10	*P. h. homarus*	Kenya	**KX275314**	**KX349878**	**KX397102**	**KX349821**	This study
SB10	*P. h. rubellus*	Scottburgh, South Africa	**KX275316**	**KX349826**	**KX397046**	**KX349757**	This study
SB11	*P. h. rubellus*	Scottburgh, South Africa	**KX275317**	**KX349827**	**KX397047**	**KX349758**	This study
SB12	*P. h. rubellus*	Scottburgh, South Africa	**KX275318**	**KX349828**	**KX397048**	**KX349759**	This study
SB14	*P. h. rubellus*	Scottburgh, South Africa	KX275319	*	KX397049	KX349760	This study
SB16	*P. h. rubellus*	Scottburgh, South Africa	KX275320	KX349829	KX397050	KX349761	This study
FD3	*P. h. rubellus*	Fort Dauphin, Madagascar	**KX275321**	**KX349862**	**KX397085**	**KX349799**	This study
FD04	*P. h. rubellus*	Fort Dauphin, Madagascar	KX275322	*	*	KX349800	This study
FD7	*P. h. rubellus*	Fort Dauphin, Madagascar	**KX275323**	**KX349859**	**KX397086**	**KX349801**	This study
FD8	*P. h. rubellus*	Fort Dauphin, Madagascar	**KX275324**	**KX349860**	**KX397087**	**KX349802**	This study
BR1	*P. h. rubellus*	Blood Reef, South Africa	**KX275326**	**KX349841**	**KX397074**	**KX349781**	This study
BR3	*P. h. rubellus*	Blood Reef, South Africa	**KX275327**	**KX349842**	**KX397075**	**KX349782**	This study
BR4	*P. h. rubellus*	Blood Reef, South Africa	**KX275328**	**KX349843**	**KX397076**	**KX349783**	This study

Specimen ID	Identification	Location	COI	CR	BTUB	ITS-1	Reference
CH5	*P. h. rubellus*	Chidenguele, Mozambique	**KX275331**	**KX349825**	**KX397053**	**KX349775**	This study
CH7	*P. h. rubellus*	Chidenguele, Mozambique	KX275332	*	KX397054	KX349776	This study
CH2	*P. h. rubellus*	Chidenguele, Mozambique	**KX275333**	**KX349823**	**KX397051**	**KX349773**	This study
CH3	*P. h. rubellus*	Chidenguele, Mozambique	**KX275334**	**KX349824**	**KX397052**	**KX349774**	This study
MB2	*P. h. rubellus*	Mdumbi, South Africa	KX275335	KX349835	KX397079	KX349786	This study
MB4	*P. h. homarus*	Mdumbi, South Africa	KX275336	KX349836	KX397080	KX349787	This study
MB5	*P. h. rubellus*	Mdumbi, South Africa	KX275337	KX349837	KX397081	KX349788	This study
MB6	*P. h. rubellus*	Mdumbi, South Africa	**KX275338**	**KX349838**	**KX397082**	**KX349789**	This study
MB7	*P. h. rubellus*	Mdumbi, South Africa	**KX275339**	**KX349839**	**KX397083**	**KX349790**	This study
MB8	*P. h. rubellus*	Mdumbi, South Africa	**KX275340**	**KX349840**	**KX397084**	**KX349791**	This study
PSJ1	*P. h. rubellus*	Port St. Johns, South Africa	KX275341	KX349830	*	KX349777	This study
PSJ2	*P. h. rubellus*	Port St. Johns, South Africa	KX275342	KX349831	*	KX349778	This study
PSJ5	*P. h. rubellus*	Port St. Johns, South Africa	KX275343	KX349832	*	KX349779	This study
PSJ7	*P. h. rubellus*	Port St. Johns, South Africa	KX275344	KX349833	KX397073	*	This study
PSJ9	*P. h. rubellus*	Port St. Johns, South Africa	KX275345	KX349834	*	KX349780	This study
TM01	*P. h. rubellus*	Tinley Manor, South Africa	KX275347	*	*	KX349763	This study
TM2	*P. h. rubellus*	Tinley Manor, South Africa	**KX275348**	**KX349849**	**KX397061**	**KX349762**	This study
TM3	*P. h. rubellus*	Tinley Manor, South Africa	**KX275349**	**KX349850**	**KX397062**	**KX349764**	This study

Specimen ID	Identification	Location	COI	CR	BTUB	ITS-1	Reference
TM07	*P. h. rubellus*	Tinley Manor, South Africa	KX275350	*	KX397063	KX349765	This study
XX1	*P. h. rubellus*	Xai Xai, Mozambique	**KX275351**	**KX349844**	**KX397055**	**KX349767**	This study
XX4	*P. h. rubellus*	Xai Xai, Mozambique	**KX275352**	**KX349845**	**KX397056**	**KX349768**	This study
XX5	*P. h. rubellus*	Xai Xai, Mozambique	KX275353	*	KX397057	KX349769	This study
XX6	*P. h. homarus*	Xai Xai, Mozambique	**KX275354**	**KX349846**	**KX397058**	**KX349770**	This study
XX9	*P. h. rubellus*	Xai Xai, Mozambique	**KX275355**	**KX349847**	**KX397059**	**KX349771**	This study
XX10	*P. h. rubellus*	Xai Xai, Mozambique	**KX275356**	**KX349848**	**KX397060**	**KX349772**	This study
ZV01	*P. h. homarus*	Zavora, Mozambique	KX275357	*	KX397065	KX349792	This study
ZV02	*P. h. homarus*	Zavora, Mozambique	KX275358	*	*	*	This study
ZV03	*P. h. homarus*	Zavora, Mozambique	**KX275359**	**KX349852**	**KX397066**	**KX349793**	This study
ZV04	*P. h. homarus*	Zavora, Mozambique	**KX275360**	**KX349853**	**KX397067**	**KX349794**	This study
ZV05	*P. h. homarus*	Zavora, Mozambique	**KX275361**	**KX349854**	**KX397068**	**KX349795**	This study
ZV07	*P. h. rubellus*	Zavora, Mozambique	KX275362	KX349855	KX397069	*	This study
ZV10	*P. h. homarus*	Zavora, Mozambique	**KX275363**	**KX349856**	**KX397070**	**KX349796**	This study
ZV14	*P. h. rubellus*	Zavora, Mozambique	**KX275364**	**KX349857**	**KX397071**	**KX349797**	This study
ZV15	*P. h. rubellus*	Zavora, Mozambique	**KX275365**	**KX349858**	**KX397072**	**KX349798**	This study
OM3	*P. h. megasculptus*	Dhalkhut, Oman	KX275366	*	*	KX349810	This study
OM6	*P. h. megasculptus*	Dhalkhut, Oman	KX275367	*	*	KX349811	This study

Specimen ID	Identification	Location	COI	CR	BTUB	ITS-1	Reference
Ash08	*P. h. megasculptus*	Al Ashkharah, Oman	**KX275368**	**KX349864**	**KX397097**	**KX349814**	This study
Ash04	*P. h. megasculptus*	Al Ashkharah, Oman	**KX275369**	**KX349863**	*****	**KX349815**	This study
DQ08	*P. h. megasculptus*	Duqm, Oman	KX275370	*	*	*	This study
M01	*P. h. megasculptus*	Mirbat, Oman	**KX275371**	**KX349865**	**KX397095**	*****	This study
Yem02	*P. h. megasculptus*	Yemen	**KX275372**	**KX349867**	**KX397093**	**KX349816**	This study
Yem10	*P. h. megasculptus*	Yemen	**KX275373**	**KX349868**	**KX397094**	**KX349817**	This study
DQ07	*P. h. megasculptus*	Duqm, Oman	**KX275374**	*****	**KX397098**	**KX349812**	This study
M11	*P. h. megasculptus*	Mirbat, Oman	**KX275375**	**KX349866**	**KX397096**	**KX349813**	This study
Ken04	*P. h. homarus*	Kenya	**KX275376**	**KX349869**	**KX397089**	**KX349804**	This study
Ken07	*P. h. homarus*	Kenya	KX275377	*	*	KX349805	This study
Ken08	*P. h. homarus*	Kenya	KX275378	*	KX397090	KX349806	This study
Ken10	*P. h. homarus*	Kenya	KX275379	*	KX397092	KX349808	This study
Ken13	*P. h. homarus*	Kenya	KX275380	KX349871	*	KX349809	This study
J_lal	*Jasus lalandii*	uShaka Marine World, South Africa	**KX275382**	**KX349872**	**KX397037**	**KX349748**	This study
J_paul	*Jasus paulensis*	uShaka Marine World, South Africa	**KX275383**	*****	**KX397038**	**KX349749**	This study
P_gil	*Palinurus gilchristi*	uShaka Marine World, South Africa	**KX275384**	**KX349873**	**KX397040**	**KX349751**	This study

Specimen ID	Identification	Location	COI	CR	BTUB	ITS-1	Reference
P_long	*Panulirus longipes*	uShaka Marine World, South Africa	**KX275385**	**KX349874**	**KX397041**	**KX349752**	This study
P_versi	*Panulirus vesicolor*	uShaka Marine World, South Africa	**KX275386**	*****	**KX397042**	**KX349753**	This study
SE1	*Scyllarides elisabethae*	uShaka Marine World, South Africa	**KX275387**	*****	**KX397044**	**KX349755**	This study
SS1	*Scyllarides squammosus*	Durban, South Africa	**KX275388**	*****	**KX397045**	**KX349756**	This study
SS2	*Scyllarides squammosus*	Durban, South Africa	**KX275389**	*****	**KX397043**	**KX349754**	This study
P_dela	*Palinurus delagoae*	uShaka Marine World, South Africa	*	*	KX397039	KX349750	This study
Ash01	*P. h. megasculptus*	Al Ashkharah, Oman	KY860538	*	*	*	This study
Ash03	*P. h. megasculptus*	Al Ashkharah, Oman	KY860539	*	*	*	This study
DQ06	*P. h. megasculptus*	Duqm, Oman	KY860540	*	*	*	This study
M02	*P. h. megasculptus*	Mirbat, Oman	KY860541	*	*	*	This study
M04	*P. h. megasculptus*	Mirbat, Oman	KY860542	*	*	*	This study
OM01	*P. h. megasculptus*	Dhalkhut, Oman	KY860543	*	*	KY860557	This study
OM03	*P. h. megasculptus*	Dhalkhut, Oman	KY860544	*	*	*	This study
YEM04	*P. h. megasculptus*	Yemen	KY860545	*	*	*	This study

Specimen ID	Identification	Location	COI	CR	BTUB	ITS-1	Reference
YEM05	*P. h. megasculptus*	Yemen	KY860546	*	*	*	This study
FD10	*P. h. rubellus*	Fort Dauphin, Madagascar	KY860547	KY860552	*	KY860558	This study
BR02	*P. h. rubellus*	Blood Reef, South Africa	KY860548	KY860553	KY860562	KY860559	This study
BR05	*P. h. rubellus*	Blood Reef, South Africa	KY860549	KY860554	KY860560	KY860563	This study
TM04	*P. h. rubellus*	Tinley Manor, South Africa	KY860550	KY860555	KY860564	*	This study
TM06	*P. h. rubellus*	Tinley Manor, South Africa	KY860551	KY860556	KY860565	KY860561	This study

Table S2.2 Uncorrected pairwise distances for COI (below the diagonal) with standard error estimates (above the diagonal) between the *P. homarus* subspecies and outgroups.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1. *P. h. megasculptus*		0.002	0.009	0.021	0.020	0.019	0.019	0.015
2. *P. h. homarus*	0.016		0.009	0.021	0.020	0.019	0.019	0.016
3. *P. h. rubellus*	0.049	0.047		0.020	0.020	0.019	0.018	0.015
4. *J. lalandii*	0.190	0.191	0.180		0.011	0.020	0.021	0.020
5. *J. paulensis*	0.203	0.203	0.193	0.071		0.021	0.022	0.019
6. *P. gilchristi*	0.180	0.178	0.183	0.179	0.184		0.022	0.019
7. *P. longipes*	0.183	0.183	0.185	0.209	0.207	0.217		0.018
8. *P. versicolor*	0.132	0.133	0.136	0.191	0.191	0.189	0.184	

Table S2.3 Uncorrected pairwise distances for CR (below the diagonal) and standard error estimates (above the diagonal) between the *P. homarus* subspecies and outgroups.

	1	**2**	**3**	**4**	**5**	**6**
1. *P. h. megasculptus*		0.014	0.058	0.069	0.072	0.055
2. *P. h. homarus*	0.035		0.056	0.067	0.071	0.056
3. *P. h. rubellus*	0.258	0.258		0.067	0.061	0.054
4. *P. gilchristi*	0.480	0.488	0.545		0.061	0.067
5. *P. longipes*	0.462	0.455	0.442	0.614		0.059
6. *J. lalandii*	0.526	0.525	0.595	0.632	0.526	

Table S2.4 Uncorrected pairwise distances for β-tubulin (below the diagonal) and standard error estimates (above the diagonal) between the *P. homarus* subspecies and outgroups.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1. *P. h. megasculptus*		0.011	0.011	0.020	0.020	0.019	0.019	0.017	0.018
2. *P. h. homarus*	0.086		0.009	0.019	0.018	0.016	0.019	0.016	0.017
3. *P. h. rubellus*	0.083	0.081		0.019	0.018	0.018	0.019	0.016	0.018
4. *J. lalandii*	0.156	0.151	0.154		0.014	0.022	0.022	0.021	0.021
5. *J. paulensis*	0.143	0.144	0.143	0.065		0.022	0.023	0.021	0.022
6. *P. delagoae*	0.179	0.163	0.172	0.174	0.162		0.018	0.022	0.021
7. *P. gilchristi*	0.168	0.163	0.165	0.178	0.182	0.113		0.020	0.023
8. *P. versicolor*	0.131	0.112	0.120	0.158	0.154	0.162	0.134		0.020
9. *P. longipes*	0.128	0.132	0.135	0.166	0.150	0.194	0.182	0.130	

Table S2.5 Uncorrected pairwise distances for ITS-1 (below the diagonal) and standard error estimates (above the diagonal) between the *P. homarus* subspecies and outgroups.

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1. *P. h. megasculptus*		0.008	0.007	0.029	0.029	0.026	0.026	0.026	0.026
2. *P. h. homarus*	0.075		0.008	0.029	0.029	0.027	0.027	0.027	0.026
3. *P. h. rubellus*	0.077	0.068		0.029	0.029	0.026	0.026	0.026	0.026
4. *J. paulensis*	0.346	0.341	0.338		0.009	0.028	0.027	0.029	0.030
5. *J. lalandii*	0.345	0.337	0.337	0.028		0.028	0.028	0.030	0.029
6. *P. gilchristi*	0.296	0.287	0.291	0.280	0.298		0.003	0.027	0.030
7. *P. delagoae*	0.296	0.287	0.291	0.276	0.295	0.003		0.027	0.030
8. *P. longipes*	0.298	0.294	0.297	0.345	0.345	0.289	0.289		0.026
9. *P. versicolor*	0.361	0.354	0.353	0.435	0.438	0.429	0.429	0.382	

Chapter Three: Comparative population genetics and phylogeography of the *Panulirus homarus* subspecies complex in the southwest Indian Ocean

Abstract

Mitochondrial DNA (Cytochrome oxidase I and the hypervariable portion of the control region) and 21 microsatellite loci were used to describe the population genetic structure and phylogeography of three *Panulirus homarus* subspecies in the Western Indian Ocean. The phylogenetic study in Chapter 2 suggests that *P. h. rubellus* from southeast Africa and Madagascar is a distinct species. This is again strongly supported by the additional markers used in this chapter. In addition, the faster evolving microsatellite loci suggest that *P. h. homarus* (Indo-West Pacific) and *P. h. megasculptus* (Arabian Sea) represent recently diverged lineages. Seasonally reversing currents, linked to the monsoon climate, potentially act to retain *P. h. megasculptus* larvae in the Arabian Sea, thus giving rise to separately evolving lineages. Clustering analyses indicated that *P. h. rubellus* and *P. h. homarus* are sympatric in the Delagoa Bight region of southern Mozambique, but despite this contact, little conclusive evidence of hybridization between the subspecies was found. Isolation by distance alone could not explain the genetic differentiation between *P. h. homarus* and *P. h. rubellus*. Instead it is proposed that eddies in the Mozambique Channel retain larvae of *P. h. homarus*, thus restricting their dispersal further southwards. Analyses indicate a population decline and subsequent expansion during the Pleistocene, corresponding to a period of strengthening ocean currents, warming and sea level rise after the last glacial maximum. These factors presumably facilitated a southerly expansion of *P. h. rubellus*. Migration analyses showed that the *P. h. rubellus* gene pool, south of the contact zone at Delagoa Bight, is augmented from the population in southeast Madagascar, but that the latter receives very few immigrants. In this scenario, long-lived phyllosoma larvae are entrained in eddies formed by the East Madagascar Current off southern Madagascar, and then transported westwards across the southern part of the Mozambique Channel, to settle along the coast of southeast Africa.

3.1 Introduction

3.1.1 Taxonomy of *P. homarus*

In Chapter 2, multilocus genetic data from mitochondrial DNA (COI and hypervariable control region) and nuclear (ITS-1 intron and β-tubulin) sequence markers were used to resolve the phylogeny of the *Panulirus homarus* subspecies complex in the Western Indian Ocean (Singh et al. 2017). The results indicate that *P. homarus rubellus* from the southwest Indian Ocean (eastern South Africa, southern Mozambique and southeastern Madagascar) is a separately evolving lineage. It was recommended that its taxonomic status be re-evaluated, and that it should be acknowledged as a new species, *Panulirus rubellus*. Given previous reports of hybridization between *P. h. homarus* and *P. h. rubellus* in northern KwaZulu-Natal (Berry 1974a), it was suggested that the taxonomic status of *P. h. rubellus* be re-evaluated using faster evolving markers to test for recent gene flow among these two subspecies. Past studies have found limited genetic differentiation between the other two nominal subspecies, *P. h. homarus* from the Indo-West Pacific and *P. h. megasculptus* found in the Arabian Sea (Lavery et al. 2014, Singh et al. 2017), suggesting that they form a single lineage, despite morphological differences between them (Berry 1974a). The molecular clock approach implemented in Chapter 2 identified *P. h. rubellus* and *P. h. homarus* as two distinct clades, which diverged from a common ancestor during the Oligocene, approximately 26 million years ago.

In this chapter, the fine-scale genetic structuring within each subspecies of *P. homarus* is examined. The population genetic structure and demographic history of each of the *P. homarus* subspecies are clarified using fast evolving mitochondrial markers (COI and CR) and nuclear microsatellites. In particular, the population genetic structure of *P. h. rubellus* populations along the coasts of southeast Africa and southeast Madagascar are evaluated to provide information on stock structure and boundaries. Historical and contemporary gene flows are examined to ascertain whether the Mozambique Channel acts as a barrier to gene flow between African mainland and Madagascar populations of *P. h. rubellus*.

3.1.2 Genetic analyses and the stock concept in fisheries

Sustainable management of fisheries requires knowledge of stock structure and boundaries. This is often difficult, because there are several different definitions of the stock concept (Carvalho and Hauser 1994), and the geographic boundaries of fish stocks do not often coincide with clearly demarcated political boundaries. Stocks important for fisheries are replenished by recruitment and migration, sometimes from populations outside the boundaries of the fished areas. By better describing the genetic structure of species important for fisheries, and identifying populations that provide recruits or migrants, authorities can prevent overfishing and depletion of stocks that are isolated (Reiss et al. 2009, Lane et al. 2016). The traditional stock concept employed by fisheries management is defined as a group of harvested marine species fished in a specific area, but this stock concept ignores biological substructuring within species (Carvalho and Hauser 1994, Waples and Gaggiotti 2006, Reiss et al. 2009). By identifying individual stocks and the linkages between them, we can adjust the management of isolated stocks to prevent their depletion (Reiss et al. 2009, Lane et al. 2016). Incorporating the study of population genetics into fisheries management is therefore important, because it allows for the inference of effective population size, genetic diversity, parentage and the identification of cryptic species (von der Heyden 2009). Population genetic structure should thus be considered alongside traditional stock assessment methods in effective and sustainable fisheries management strategies (Laikre et al. 2005).

Molecular tools are increasingly used for the identification of genetically distinct stocks in fisheries management. Examples include commercially important species such as sardines and anchovies (Grant and Bowen 1998), Atlantic salmon (King et al. 2001), black bream (Farrington et al. 2000), Cape hake (von der Heyden et al. 2007a), and penaied prawns *Fenneropenaeus indicus* and *Metapenaeus monoceros* in the southwestern Indian Ocean (Mkare et al. 2014, Mkare et al. 2017). DNA-based identification has also proven to be useful in delimiting genetically distinct stocks of commercially important spiny lobsters such as *Panulirus marginatus* and *P. penicillatus* from Hawaii (Iacchei et al. 2014), *P. argus* in Mexico (Truelove et al. 2015), *Palinurus delagoae* from South Africa and Mozambique (Gopal et al. 2006) and *Jasus lalandii* in western South Africa (Matthee et al. 2007).

Although mitochondrial DNA markers are often the popular choice for marine population genetic studies, nuclear DNA microsatellite markers are increasingly being used. Both

marker types have advantages, which make them ideal for examining genetic differentiation at a range of taxonomic levels. Mitochondrial markers are haploid and sensitive to genetic drift and other demographic changes because they are maternally inherited and therefore have a smaller effective population size (Moritz 1994). Most lobster population genetic studies have relied mainly on the use of mitochondrial DNA markers such as Cytochrome oxidase I (COI; Senevirathna et al. 2016), the control region (CR; Tolley et al. 2005, Gopal et al. 2006), 16S ribosomal RNA (16S rRNA; Matthee et al. 2007), cytochrome *b* (cytb; Senevirathna et al. 2016) and 12S ribosomal RNA (12S rRNA; Kennington et al. 2013a), but recent studies have used a combination of mitochondrial and nuclear markers (Kennington et al. 2013a, Dao et al. 2015). Nuclear microsatellites are ideal for inferring fine-scale population genetic structure because of increased statistical power from including multiple independent loci that are codominant, hypervariable and highly polymorphic (Wright and Bentzen 1994, Bentzen et al. 1996).

3.1.3 Dispersal and genetic connectivity in spiny lobsters

Marine invertebrates with long pelagic larval phases have high dispersal capabilities, potentially resulting in high demographic and genetic connectivity (Avise 2000, Cowen et al. 2000). However, many studies using molecular genetic techniques have shown that despite this, some species exhibit clearly structured populations (Teske et al. 2014, Haye et al. 2014). Genetic structure in the marine environment can arise through isolation by distance or vicariance events and secondary contact, often related to semi-permanent oceanographic features, such as predominant currents, gyres and mesoscale eddies or fronts which can either aid or constrain dispersal. Historical events such as the rise and fall of sea levels during the Pleistocene may have resulted in the development of biogeographic breaks, affecting genetic connectivity between species or populations that occur in the littoral zone (Palumbi 1994).

Population genetic and phylogeographic studies of spiny lobsters with similar life history traits found contrasting results, ranging from panmixia in some species to structured populations in others. Populations of spiny lobsters such as *Jasus lalandii* (Matthee et al. 2007) on the south and west coasts of southern Africa, *P. homarus* in Sri Lanka (Senevirathna et al. 2016), *Palinurus gilchristi* on the southern coast of South Africa (Tolley et al. 2005), *P. cygnus* in Australia (Kennington et al. 2013a) and *P. ornatus* in the south-east Asian Archipelago (Dao et al. 2015), showed no evidence of genetically structured

populations and are considered panmictic. On the other hand, *J. edwardsii* from Australia (Morgan et al. 2013), *P. argus* in the Carribbean (Truelove et al. 2015), and *P. delagoae* in the southwestern Indian ocean (Gopal et al. 2006) demonstrated genetically structured populations. Genetic studies have led to the description of new species, such as *Palinurus barbarae* at Walters Shoals in the Western Indian Ocean (Groeneveld et al. 2006). Why ecologically similar species show such different genetic population structure is not well understood.

3.1.4 Hypotheses and aims

Panulirus homarus has a drifting larval phase of 4–6 months, during which larvae are dispersed by water movements (Berry 1974b). They then settle on the sea floor, to resume a benthic existence, usually with short distance nomadic movements (Steyn and Schleyer 2011). No long-distance benthic migrations have been identified in *P. homarus*, and hence the drifting larval phase is the only mode likely to facilitate dispersal and lead to gene flow.

A recent ocean-wide phylogeographic study of *P. homarus* across the Indo-West Pacific found that populations distributed on the west and east of the species range were quite distinct. These distinct lineages were recognized as 'subspecies' from South Africa and Mozambique in the west, and another peripheral lineage at the Marquesas Islands in the Central Pacific in the east (Farhadi et al. 2017). The study, based on mitochondrial CR and nuclear microsatellites, also exposed fine-scale population structure, particularly in the Indian Ocean. Reddy et al. (2014), based on COI-like nuclear copies (numts), suggested that the Madagascan populations of *P. h. rubellus* could be a genetically distinct from those on the African shelf, with the Mozambique channel acting as a barrier to gene flow. Neither Farhadi et al. (2017), nor Singh et al. (2017) could reproduce these results, based on independent analyses of COI sequences. In addition Reddy et al. (2014) did not comprehensively sample *P. h. homarus.* This is particularly problematic in this group because the distributions of *P. h. homarus* and *P. h. rubellus* overlap, and morphological intermediates between these two subspecies have been identified at the northern end of the *P. h. rubellus* distribution (Berry 1974a). A single specimen was identified as a possible hybrid between the two subspecies in the study by Lavery et al. (2014), but this specimen was unfortunately not retained for morphological analysis.

Therefore, the aims of Chapter 3 are: (i) to examine the level of genetic differentiation between and within the three subspecies using microsatellites (contemporary structure) and mitochondrial DNA (historical structure) markers; (ii) to determine if there is a contact zone and possible hybridization between *P. h. homarus* and *P. h. rubellus*; (iii) to further explore the historical and contemporary genetic structure and genetic diversity within *P. h. rubellus* along the African mainland coast and in southeast Madagascar. In particular, this study will test if the Mozambique Channel serves as a barrier to gene flow between African mainland *P. h. rubellus* and Madagascan *P. h. rubellus* populations.

3.2 Materials and methods

3.2.1 Sample collection and DNA extraction

Specimens of *P. h. rubellus* and *P. h. homarus* were collected from Tinley Manor, Blood Reef, Scottburgh, Mdumbi and Port St Johns along the east coast of South Africa, Chidenguele, Xai Xai and Zavora in Mozambique, and Fort Dauphin on the southeast coast of Madagascar. *P. h. megasculptus* samples were sourced from Oman and Yemen, and *P. h. homarus* samples from Kenya (for more details see sampling map in Chapter 2). DNA was extracted from pereiopod tissue using the Zymo gDNA Universal DNA extraction kit (Inqaba, Zymo), as per the manufacturers protocol.

3.2.2 Mitochondrial and nuclear microsatellite DNA amplification

The amplication of the mitochondrial markers used in this Chapter is outlined in detail in Chapter 2.

A panel of 21 microsatellite loci, consisting of eight loci (Orn4, Orn5, Orn11, Orn12, Orn16, Orn17, Orn21 and Orn32) described by Dao et al. (2013) and 13 loci (Pho G01, Pho G03, Pho G21, Pho G22, Pho G25, Pho G27, Pho G30, Pho G32, Pho G35, Pho G36, Pho G42, Pho G53 and Pho G58) described by Delghandi et al. (2015), was chosen for PCR amplification. Each of the forward primers were labelled with a fluorescent dye on the 5' end.

The microsatellite panel was partitioned into six multiplex reactions and a single reaction for Orn 17. The multiplexes were as follows, A: Orn 4, Orn 16 and Orn 21, B: Orn 5 and Orn 11, C: Orn 12 and Orn 32, E: G01, G32, G35, G36 and G53, F: G03, G21, G42, G58 and G: G22, G25, G27 and G30 (Table S3.1)

PCR's were carried out in 10 µl volume reactions, which contained 5 µl of 1X Kapa 2G Fast Multiplex Mastermix (Kapa Biosystems), 0.2 µM of each primer, and 1 µl of 10–50 ng of genomic DNA. For multiplexes A, B, C and Orn 17, cycling conditions were as follows: an initial denaturation at 95 °C for 5 minutes, followed by 28 cycles of denaturation at 95 °C for 30 seconds, annealing at 60 °C for 90 seconds, extension at 72 °C for 30 seconds, and a final extension step at 60 °C for 30 minutes. For multiplexes E, F and G, cycling conditions were: an initial denaturation step at 95 °C for 15 minutes, 32 cycles of denaturation at 94 °C for 30 seconds, annealing at 60 °C for 90 seconds, extension for 72 °C for 60 seconds and a final extension at 72 °C for 30 minutes.

Fragment analysis of amplified products was done at CAF at Stellenbosch University. GeneMarker v. 2.4.0 (Soft Genetics) was used to score the genotypes. To monitor the consistency in genotype scoring, 20% of the samples were reamplified and scored. Microchecker v.2.2.3 (van Oosterhout et al. 2004) was used to check for genotyping errors such as allelic dropout, stuttering and for the presence of null alleles. Microchecker revealed that several loci exhibited a general excess of homozygotes, which could indicate deviations from Hardy-Weinberg equilibrium (HWE) and null alleles. Null alleles are noted for having an impact on the estimation of population differentiation and therefore the Expectation Maximization Algorithm (EM) implemented in FreeNA (Chapuis and Estoup 2007) was used to estimate the null allele frequencies for each marker and population. Bootstrap resampling over loci was conducted with 100 000 replicates. The excluding null alleles (ENA) method was used to compare null allele corrected and uncorrected global and population F_{ST} values. The ENA-corrected and non-ENA-corrected F_{ST} values were then compared using paired t-tests to test for significant differences between the values.

3.2.3 Genetic diversity indices

Population genetic indices were calculated for each of the three subspecies and for *P. homarus* as a whole. Using microsatellite data, GenAlEx v.6.5 (Peakall and Smouse 2006) was used to compute the number of alleles per locus and population. Arlequin v. 3.5.2.2 (Excoffier and Lischer 2010) was used to calculate observed and expected heterozygosities and to test for departures from HWE and linkage equilibrium (10 000 replicates). The Bonferroni Correction was applied for multiple comparisons. The allelic richness per microsatellite loci was calculated using FSTAT v.2.9.3.2 (Goudet 1995). The polymorphic information content (PIC) for each locus was estimated using Cervus v.3.0.7 (Marshall et al. 1998, Slate et al. 2000, Kalinowski et al. 2007).

The number of haplotypes (H), haplotype (h) and nucleotide (π) diversities in the COI and CR data were determined using Arlequin. Median-joining haplotype networks were constructed to visualize the relationships among mitochondrial haplotypes and locations, using PopArt v.1.7 (http://popart.otago.ac.nz). Population genetic differentiation in the mitochondrial data was examined by calculating pairwise Φ_{ST} in Arlequin. Significance of pairwise values was tested with 10 000 permutations.

3.2.4 Population structure

Population genetic structure was investigated using an analysis of molecular variance (AMOVA) in Arlequin for the mitochondrial DNA and microsatellite markers. The groupings tested were Arabia (Oman and Yemen), Kenya, Mozambique (Zavora, Chidenguele and Xai Xai), Madagascar and South Africa (Blood Reef, Tinley Manor, Scottburgh, Port St Johns and Mdumbi) for the *P. homarus* dataset (including all three subspecies) and by location (South Africa, Madagascar and Mozambique) for the subset of *P. h. rubellus* individuals.

Bayesian genetic mixture analysis in BAPS v.6.0 (Corander et al. 2003) was performed using COI and CR data to describe the genetic structure among the three subspecies. A separate analysis was conducted using only *P. h. rubellus* mitochondrial data. This method groups samples into user-defined numbers of *K* clusters based on MCMC simulations. The optimal *K* is chosen by the number of clusters with the highest marginal log-likelihood. Analysis was carried out for 10 iterations of $K = 1–12$ for the *P. homarus* COI and CR datasets and $K = 1–9$

for the *P. h. rubellus* COI and CR datasets. Admixture analysis was then performed with 1000 iterations (*P. homarus*, 100 reference individuals with 10 iterations per individual; *P. h. rubellus*, 50 reference individuals with 10 iterations per individual).

To explore population structure in the microsatellite data, the multivariate method DAPC, implemented in the R-package (R Development Core Team 2011) adegenet v.2.0.0 (Jombart 2008) was used. This method does not make any assumptions about the underlying genetic model (HWE or linkage equilibrium). DAPC uncovers genetic structuring using coefficients of alleles in linear combinations that produce the largest between-group and smallest within-group variances. Sequential *K*-means clustering from $K = 1–12$ for the entire dataset, and $K = 1–9$ for the *P. h. rubellus* dataset, was run for 100 000 iterations and 1000 random starting centroids for each run of *K*, in order for the algorithm to converge. The optimal number of principal components was chosen using the optim.a.score function, which calculates an a-score that measures the difference between the observed discrimination and random discrimination. The Bayesian Information Criterion (BIC) was used to select the optimal value of *K*. DAPC was also used to highlight the most admixed individuals in the dataset, which were defined as having less than 90% probability of membership to a single cluster.

STRUCTURE v. 2.3.4 (Pritchard et al. 2000) uses a Bayesian clustering algorithm to infer population genetic structure and probabilistically assigns individuals to one population or more populations if there is admixture. For the *P. homarus* microsatellite dataset, *K* was set from 1–12 and for the subset of *P. h. rubellus*, *K* was set from 1–9. Each *K* was run for 10 iterations. The admixture model was chosen and run using both with locprior (using individuals sampling location as a prior) and without locprior. A total of 1 000 000 MCMC iterations were performed after a burnin period of 250 000 iterations. The Structure Harvester (Earl and von Holdt 2012) website (http://taylor0.biology.ucla.edu/structureHarvester/) and the Pophelper (Francis 2017) website (http://pophelper.com/) were used to identify the optimal number of genetic clusters (K) that best fit the data, using the Evanno method (Evanno et al. 2005). STRUCTURE plots were visualized using Pophelper.

The relationship between genetic and geographic distance was assessed using a Mantel test (Mantel 1967) for isolation-by-distance with the program IBDWS (Jensen et al. 2005, http://ibdws.sdsu.edu/~ibdws/). The genetic distances (F_{ST} / $(1 – F_{ST})$ for COI, CR and the nuclear microsatellite dataset were regressed against the geographic distances (with 1000

randomizations) between each population which was measured using Google Earth as the straight line distance between two points along the coast.

3.2.5 Contemporary and historical gene flow

Contemporary gene flow between populations of *P. h. homarus* + *P. h. megascultpus* grouped by country (Mozambique, Kenya, Oman and Yemen), and populations of *P. h. rubellus* grouped by country (Mozambique, Madagascar and South Africa), was estimated using BayesAss v.3 (Wilson and Rannala 2003) and the microsatellite dataset. This method uses Bayesian inference with MCMC simulations and multilocus genotypes to estimate posterior probabilities and recent immigration rates between populations. MCMC simulations were run for 10 million generations, sampling every 1000 generations, with a burnin of 250 000 and 10 independent replicates. Convergence for each run was assessed with Tracer.

Historical migration rates between populations of *P. h. homarus* + *P. h. megascultpus* grouped by country (Mozambique, Kenya, Oman and Yemen), and populations of *P. h. rubellus* grouped by country (Mozambique, Madagascar and South Africa) were investigated using Migrate-N v.3.6 (Beerli and Palczewski 2010) and the mitochondrial datasets. The prior boundaries used for the analyses were estimated from initial exploratory analyses by first evaluating a full migration model. The simple island model of migration was then tested for the *P. h. homarus* + *P. h. megasculptus* grouping and for the three *P. h. rubellus* locations. Slice sampling with a uniform prior distribution was chosen for θ (0–2.0, $\delta = 0.2$) and an exponential prior distribution was chosen for M (0–2000, mean = 200). Analyses were conducted using 50 million steps, sampling every 1000 generations and 25% of the run was discarded as burnin. Four heated chains were also used (1, 1.5, 3, 100 000). The number of migrants per generation was calculated using the formula $2N_m = \theta \times M$.

3.2.6 Demographic history

Inferences of the demographic history of *P. homarus* subspecies were based on mitochondrial data (COI and CR). Tajima's D (Tajima 1989) and Fu's Fs (Fu 1997) statistics were computed using Arlequin, for each locality, in order to test for selective neutrality or population expansion. Significance of these statistics was assessed with 10 000 permutations.

The deviation of populations from the null model of sudden expansion was tested using mismatch distributions (the observed distribution of pairwise differences between sequences compared with the expected distribution), goodness of fit tests such as sum of squares deviation (SSD) and Harpending's Raggedness Index (smoothness of the mismatch distribution, Harpending et al. 1993, Harpending 1994) were estimated in Arlequin. The demographic expansion parameter Tau (τ) was also calculated in Arlequin.

Bayesian skyline analyses which uses an MCMC coalescent method to assess historical demography were performed using BEAST2 v.2.4.0 (Bouckaert et al. 2014), for the *P. homarus* and *P. h. rubellus* datasets. A strict clock was employed and models of nucleotide substitution were chosen using PartitionFinder v.1.1.1 (Lanfear et al. 2012) for COI by codon position (cp1: TIMef + G, cp2: F81 + I, cp3: GTR +G) and jModeltest v.2.0 (Darriba et al. 2012) for CR (TVM + I + G) to estimate the nucleotide substitution rates. A mutation rate of 9×10^{-8} per year for COI and 5×10^{-7} per year for CR, calculated from Singh et al. (2017) was used. Two independent runs of 30 million and 10 million MCMC steps were run for COI and CR respectively. Tracer v.1.5 (Rambaut and Drummond 2007) was used to assess convergence of the run, estimate burnin and visualize the Bayesian skyline plots

3.3 Results

3.3.1 Genetic diversity

The *P. homarus* mitochondrial dataset consisted of 120 individuals amplified for COI and 75 for CR. The *P. h. rubellus* dataset consisted of 75 individuals for COI and 57 for CR. Haplotype and nucleotide diversities were very high at both mitochondrial markers, for each population and each subspecies. The lowest haplotype and nucleotide diversities were observed for *P. h. megasculptus* lobsters from Yemen (Table 3.1, H = 0.95, π = 0.016) using COI. The lowest diversity estimates using CR were observed in Madagascar (Table 3.2, H = 0.95, π = 0.03).

Table 3.1 Summary statistics for *P. homarus* COI data. N = total no. of individuals, H = total no. of haplotypes, Hd = haplotype diversity, Hsd = standard deviation of haplotype diversity, π = nucleotide diversity, πsd = standard deviation of nucleotide diversity, τ = tau, SSD = sum of squares deviation, PHH = *P. h. homarus*, PHM = *P. h. megasculptus*, PHR = *P. h. rubellus*. Abbreviated site names are as per Figure 2.1. Values in bold are statistically significant.

Site	**N**	**H**	**Hd**	**Hsd**	**π**	**πsd**	**Tajima's D**	**Fu's Fs**	**τ**	**SSD**	**Raggedness**
All	120	102	0.99	0.003	0.041	0.0200	-0.72	**-23.87**	4.59	0.0141 (0.258)	0.002 (0.732)
OM	22	17	0.96	0.029	0.014	0.0017	-1.59	**-5.37**	12.17	0.013(0.638)	0.034 (0.347)
YEM	7	6	0.95	0.096	0.016	0.009	0.04	-0.02	11.50	0.069 (0.229)	0.133 (0.320)
KEN	9	9	1	0.052	0.017	0.009	-1.32	-2.87	6.22	0.0097 (0.933)	0.0247 (0.935)
ZV	10	10	1	0.045	0.039	0.021	1.17	-1.63	36.38	0.054 (0.121)	0.102 (0.130)
CH	10	10	1	0.045	0.018	0.009	-0.82	-3.49	8.89	0.019 (0.453)	0.0355 (0.564)
XX	9	9	1	0.052	0.031	0.017	-1.19	-1.63	10.43	0.029 (0.404)	0.077 (0.269)
FD	9	8	0.97	0.064	0.019	0.011	0.07	-0.81	11.34	0.024 (0.498)	0.061 (0.379)
BR	8	8	1	0.063	0.020	0.012	-0.82	-1.90	8.43	0.013 (0.856)	0.0357 (0.856)
TM	8	8	1	0.063	0.019	0.011	-0.85	-2.10	8.79	0.037 (0.219)	0.112 (0.260)
SB	10	9	0.98	0.054	0.017	0.009	-0.73	-1.73	7.69	0.0124 (0.773)	0.0262 (0.830)
PSJ	9	9	1	0.052	0.018	0.010	-0.57	-2.78	5.44	0.0261 (0.371)	0.0478 (0.651)
MB	9	9	1	0.052	0.023	0.013	-0.99	-2.36	2.78	0.0313 (0.503)	0.089 (0.285)
PHH	16	15	0.99	0.025	0.020	0.011	**-1.86**	**-5.29**	2.97	0.008 (0.848)	0.012 (0.886)
PHM	29	21	0.96	0.024	0.015	0.008	**-1.55**	**-6.86**	11.82	0.0077 (0.733)	0.015 (0.689)
PHR	75	70	0.99	0.003	0.020	0.011	**-1.86**	**-24.38**	7.92	0.00046 (0.861)	0.0023 (0.895)

Table 3.2 Summary statistics for *P. homarus* CR data. N = total no. of individuals, H = total no. of haplotypes, Hd = haplotype diversity, Hsd = standard deviation of haplotype diversity, π = nucleotide diversity, πsd = standard deviation of nucleotide diversity, τ = tau, SSD = sum of squares deviation, PHH = *P. h. homarus*, PHM = *P. h. megasculptus*, PHR = *P. h. rubellus*. Abbreviated site names are as per Figure 2.1. Values in bold indicate statistical significance.

Site	N	H	Hd	Hsd	π	πsd	Tajima's D	Fu's Fs	τ	SSD	Raggedness
All	75	73	0.99	0.002	0.079	0.039	0.30	**-24.23**	15.43	0.014 (0.068)	0.001 (0.818)
OM	4	4	1	0.177	0.024	0.005	-0.47	-35.89	16.06	0.189 (0.111)	0.555 (0.372)
YEM	2	2	1	0.500	0.015	0.016	0	2.08	0	0	0
KEN	6	6	1	0.096	0.029	0.018	-0.480	-0.27	18.69	0.058 (0.346)	0.133 (0.548)
ZV	7	7	1	0.076	0.089	0.050	0.45	0.66	85.47	0.052 (0.205)	0.072 (0.852)
CH	8	8	1	0.063	0.038	0.022	-0.63	-0.85	21.91	0.028 (0.518)	0.053 (0.657)
XX	7	7	1	0.076	0.070	0.040	-0.82	0.38	24.19	0.061 (0.244)	0.068 (0.726)
FD	7	6	0.95	0.096	0.030	0.017	-0.14	0.99	16.53	0.031 (0.680)	0.048 (0.832)
BR	5	5	1	0.126	0.039	0.025	-0.89	0.61	17.98	0.043 (0.930)	0.1 (0.909)
TM	3	3	1	0.272	0.054	0.041	0	2.26	37.93	0.288 (0.149)	0.666 (0.688)
SB	8	7	0.96	0.077	0.038	0.022	-0.36	0.86	23.33	0.014 (0.860)	0.029 (0.957)
PSJ	8	8	1	0.063	0.044	0.025	-0.64	-0.65	20.58	0.018 (0.864)	0.036 (0.921)
MB	10	10	1	0.045	0.056	0.030	-1.15	-1.03	19.19	0.026 (0.464)	0.052 (0.409)
PHH	12	12	1	0.034	0.050	0.027	-1.23	-2.01	17.98	0.014 (0.532)	0.019 (0.818)
PHM	6	6	1	0.096	0.022	0.013	-0.68	-0.72	12.28	0.099 (0.178)	0.284 (0.094)
PHR	57	55	0.99	0.004	0.046	0.023	-1.32	**-24.11**	18.30	0.0013(0.561)	0.0019 (0.890)

The *P. homarus* microsatellite dataset included 271 individuals genotyped across 21 microsatellite loci. The *P. h. rubellus* data set included 175 individuals. Summary statistics are presented in Table 3.3 and Table S3.2. Microchecker did not detect evidence for scoring errors due to stuttering or large allelic dropout, but indicated that null alleles may be present at ten loci (Orn 11, Orn 12, Orn 16, G21, G25, G27, G30, G32, G53 and G58). Using the ENA method implemented in FreeNA, the ENA-corrected F_{ST} was compared to the non-ENA-corrected F_{ST} at each locus and population using t-tests. There was no significant difference found between ENA-corrected vs. non-ENA corrected F_{ST} values globally and for each population ($P > 0.05$) and all loci were therefore included in subsequent analyses.

High genetic variability was observed at most of the loci, but was low in Orn 21 (PIC = 0.072) and G27 (PIC = 0.185). The N_A per locus ranged from 2 (locus G42) to 14 (locus Orn 12). The expected heterozygosity was higher than the observed heterozygosity in Orn 4, Orn 11, Orn 12, Orn 16, G01, G21, G25, G27, G30, G32, G53 and G58. Following Bonferroni correction, Orn 5, Orn 12, Orn 16, Orn 17, Orn 32, G03, G21, G22, G27, G32, G36, G42 and

G53 deviated significantly from HWE. However, out of the 12 populations, Orn 5 deviated significantly from HWE in 5 populations, Orn 12 in 6, Orn 16 in 3, Orn 17 in 5, Orn 32 in 1, G21 in 5, G22 in 2, G32 in 6, G36 in 2, G42 in 9 and G53 in 1 population. This suggests that the Wahlund Effect, which is the increase in homozygosity when discrete subpopulations are pooled with different allele frequencies that do not interbreed as a single randomly mating unit, could be responsible for deviations from HWE. Therefore, all loci were retained for further analysis. At the population level, N_A and A_R was highest in Oman (N_A = 7.19, A_R = 6.07) and lowest in Zavora (N_A = 4.95, A_R = 4.8). At the subspecies level, Na and A_R were highest in *P. h. rubellus* (N_A = 8.6, A_R = 8.6).

Table 3.3 *P. homarus* summary statistics for microsatellite data by population. N = total no. of individuals, Na = mean no. of alleles, Ho = observed heterozygosity, He = expected heterozygosity, Ar = allelic richness, PHH = *P. h. homarus*, PHM = *P. h. megasculptus*, PHR = *P. h. rubellus*.

Population	**N**	**Na**	**Ho**	**He**	**Ar**
All	271	5.849	0.604	0.590	5.86
OM	29	7.190	0.644	0.615	6.070
YEM	24	6.333	0.605	0.610	5.690
KEN	22	5.238	0.603	0.564	4.780
ZV	17	4.952	0.573	0.555	4.800
CH	19	5.524	0.584	0.589	5.260
XX	22	6.048	0.621	0.614	5.460
FD	29	6.619	0.644	0.626	5.660
BR	19	5.476	0.616	0.577	5.240
TM	19	5.095	0.551	0.549	4.920
SB	30	6.048	0.588	0.594	5.110
PSJ	20	5.667	0.591	0.590	5.400
MB	21	6.000	0.631	0.597	5.540
PHH	43	6.571	0.585	0.578	6.550
PHM	53	7.762	0.627	0.621	7.690
PHR	175	8.667	0.607	0.613	8.630

3.3.2 The three *P. homarus* subspecies

The median-joining haplotype networks using COI (Figure 3.1A and B) and CR (Figure 3.2 A and B) revealed that *P. h. rubellus* was distinct from the other subspecies. Using COI, haplotypes were shared between *P. h. megasculptus* and *P. h. homarus*. Within *P. h. rubellus* COI haplotypes were shared between Mozambique and South Africa, and Madagascan

haplotypes were shared with South Africa. At the more variable CR, no haplotype was shared between locations or subspecies.

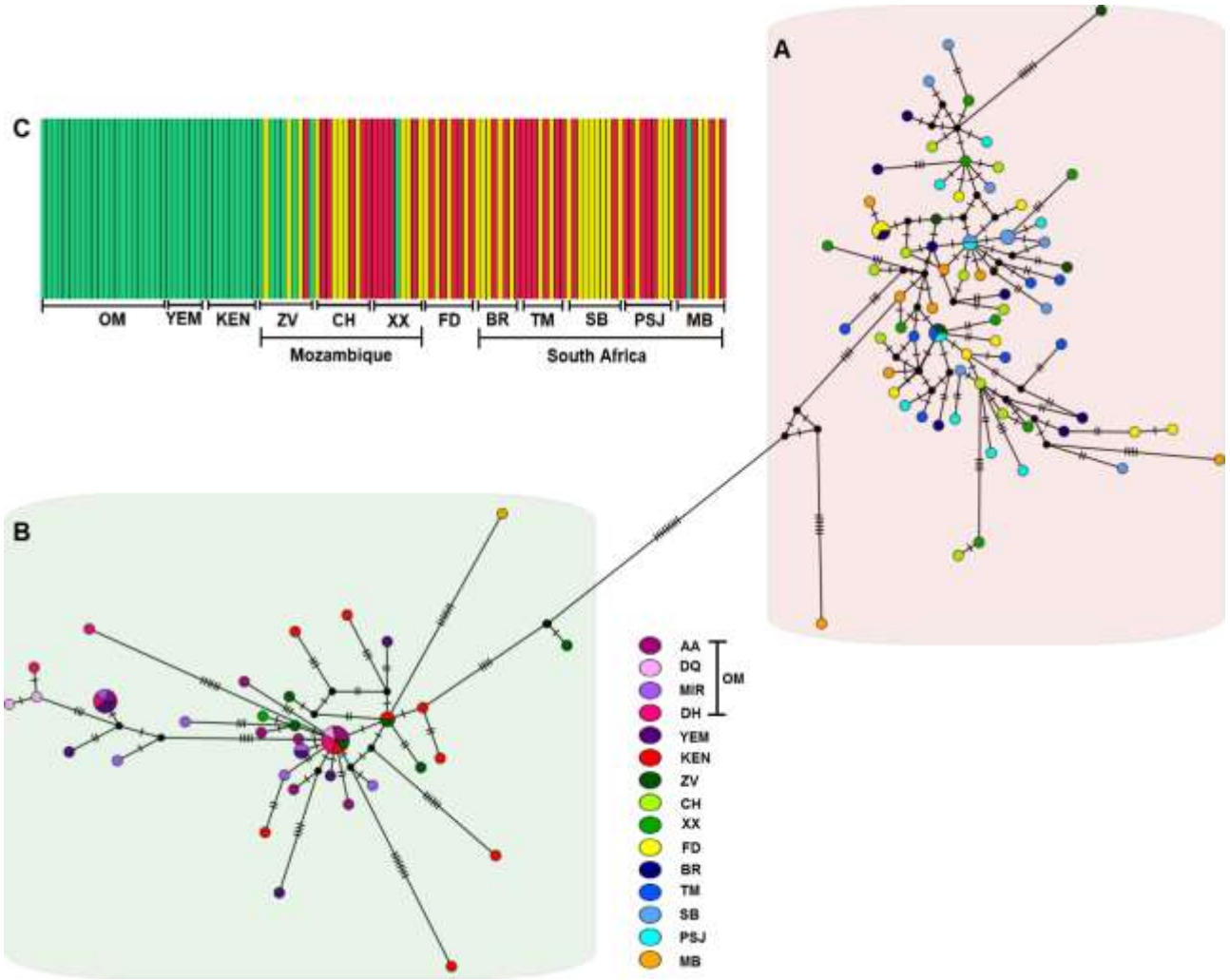


Figure 3.1 COI Median-joining haplotype network showing (A) the *P. h. rubellus* group and (B) the *P. h. homarus* + *P. h. megasculptus* group. (C) COI BAPS plot showing three genetic clusters, one corresponding to the *P. h. homarus* and *P. h. megasculptus* lineages, and two corresponding to the *P. h. rubellus* lineage. Abbreviated site names are as per Figure 2.1.

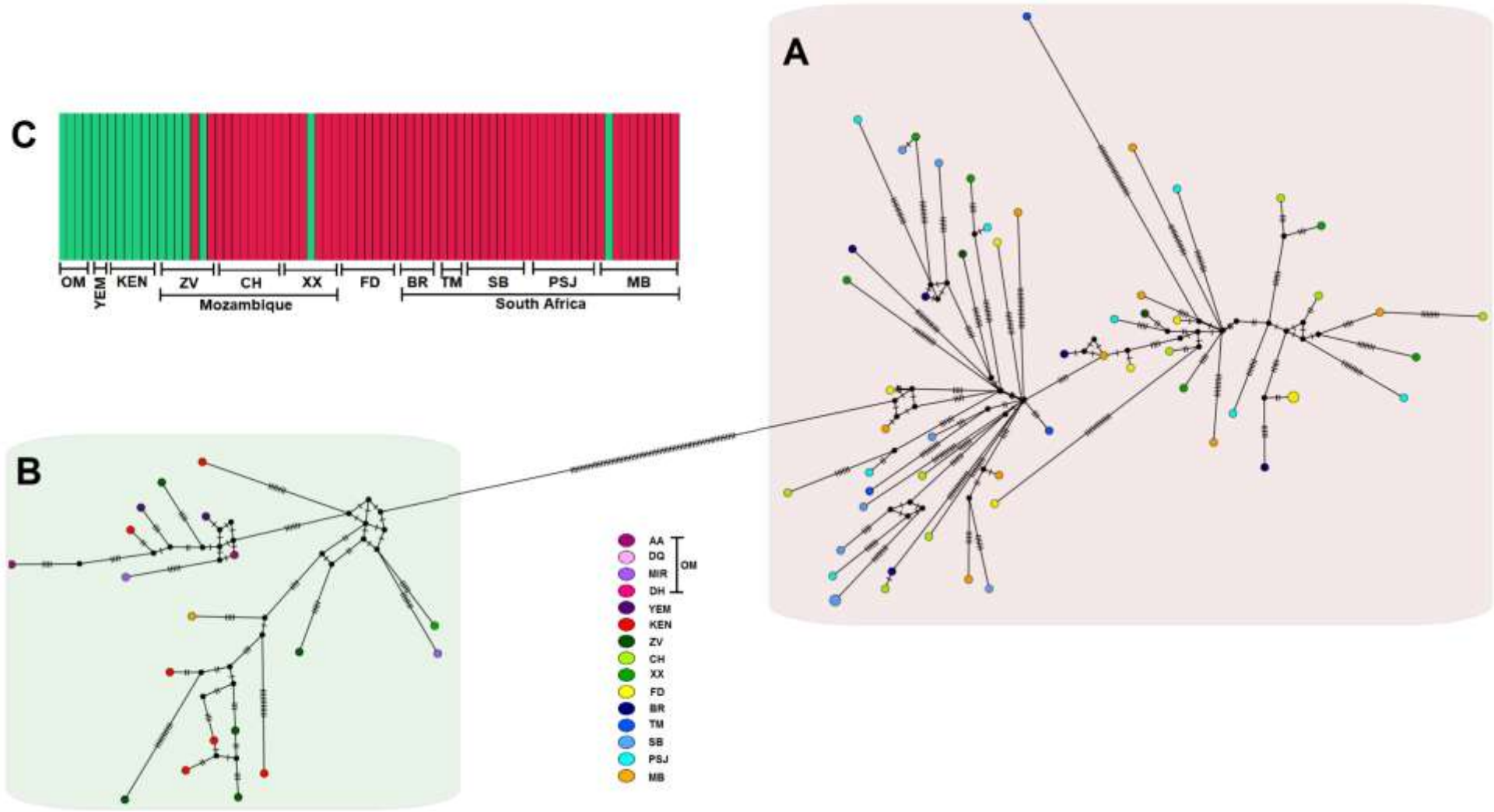


Figure 3.2 CR Median-joining haplotype network showing (A) the *P. h. rubellus* group and (B) the *P. h. homarus* + *P. h. megasculptus* group. (C) CR BAPS plot showing two genetic clusters, one corresponding to the *P. h. homarus* and *P. h. megasculptus* lineages, and one corresponding to the *P. h. rubellus* lineage. Abbreviated site names are as per Figure 2.1.

The BAPS analysis for COI with the *P. homarus* dataset indicated that there were three genetic clusters, one corresponding to *P. h. homarus* individuals from Kenya, Zavora, Xai Xai and Mdumbi, and *P. h. megasculptus* individuals from Yemen and Oman, and two clusters corresponding to *P. h. rubellus* individuals from Mozambique, Madagascar and South Africa (Figure 3.1 C). The CR BAPS analysis indicated that there were two clusters, one corresponding to *P. h. homarus* and *P. h. megasculptus* individuals, and one corresponding to *P. h. rubellus* individuals (Figure 3.2 C).

The DAPC analysis indicated that the optimal number of principle components to retain for the *P. homarus* dataset was 7 and the lowest BIC score indicated that the optimal number of clusters was K = 5 (Figure 3.3 A and B). Cluster 1 corresponds to *P. h. megacsulptus*, cluster 2, 3 and 5 correspond to *P. h. rubellus* and cluster 4 corresponds to *P. h. homarus*. A total of 85 out of 271 (31%) of individuals showed evidence of admixture (probability of belonging to a single cluster < 0.90).

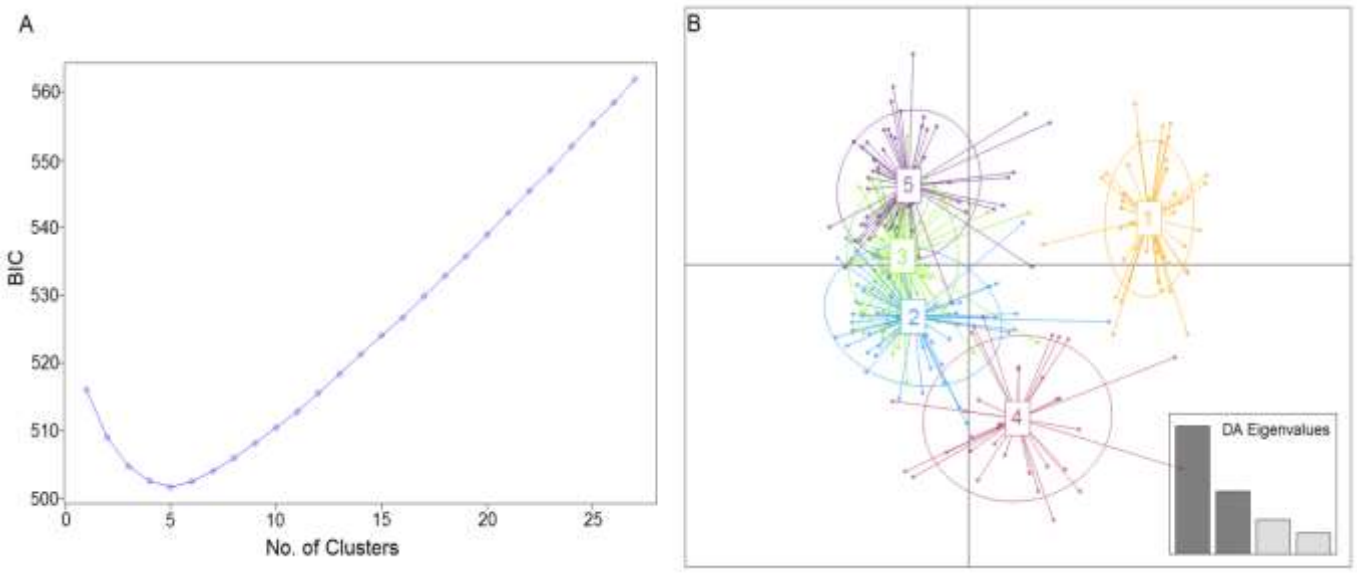


Figure 3.3 DAPC analysis of the *P. homarus* dataset using microsatellite data. (A) The lowest BIC score indicates that the optimal K = 5. (B) Scatterplot of individual genotypes from the DAPC analysis and the clusters they belong to.

STRUCTURE analysis indicated that the optimal K was 3 for the *P. homarus* subspecies dataset (Figure 3.4 A and B). Each of the subspecies could clearly be distinguished using the microsatellite data. When the locprior model was used along with the admixture model, the Scottburgh and Fort Dauphin *P. h. rubellus* populations showed a small degree of admixure with *P. h. homarus* and *P. h. megasculptus* respectively, relative to the other populations.

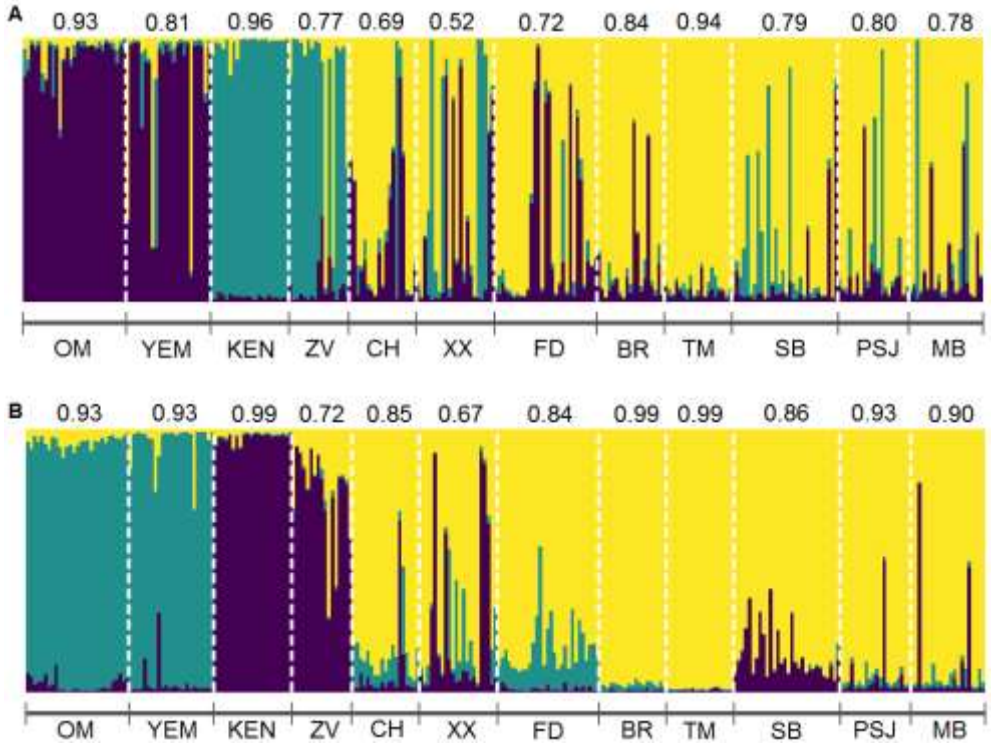


Figure 3.4 STRUCTURE plots for the *P. homarus* dataset, (A) using admixture model with no locprior and (B) admixture model with locprior. The probabilities for belonging to the dominant cluster at each location are also indicated. Abbreviated site names are as per Figure 2.1.

The pairwise Φ_{ST} values for COI for the *P. homarus* mitochondrial DNA dataset ranged from -0.004 between Oman and Yemen to 0.77 between Chidenguele and Oman (Table S3.3). For CR, it ranged from -0.009 between Fort Dauphin and Chidenguele to > 0.800 between Fort Dauphin and Arabian localities (Table S3.4). Pairwise F_{ST} values based on microsatellite data for *P. homarus* ranged from -0.007 between Mdumbi and Xai Xai, to 0.094 between Blood Reef and Kenya (Table S3.5).

AMOVA (Figure 3.5) found significant genetic differentiation among the three subspecies using COI (51.5%, F_{CT} = 0.52, P < 0.01, Table S3.9). Using CR, significant genetic differentiation was observed within populations (52%, F_{ST} = 0.47, P < 0.01, Table S3.9). For the microsatellites, genetic differentiation was significant at all levels, but it was highest within populations (94%, F_{ST} = 0.02, P < 0.01, Table S3.9) of the three subspecies. These statistics support the results of the clustering analyses.

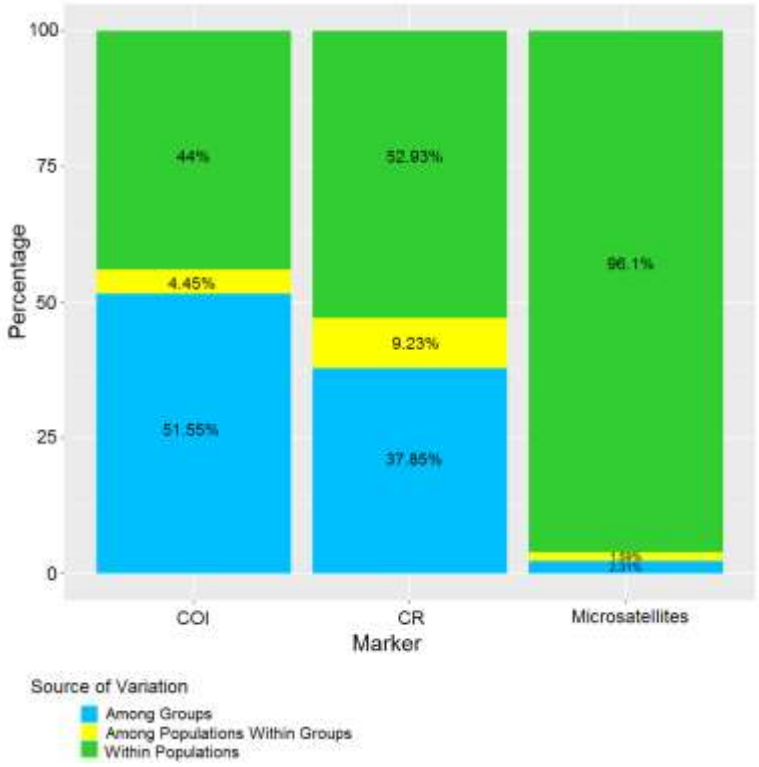


Figure 3.5 AMOVA for *P. homarus* using COI, CR and the microsatellites. Groupings were tested by region; Arabia (Oman and Yemen), Kenya, Mozambique (Zavora, Chidenguele, Xai Xai), Madagascar and South Africa (Blood Reef, Tinley Manor, Scottburgh, Port St Johns and Mdumbi).

Significant correlation between genetic and geographic distance among localities was observed for the entire *P. homarus* COI dataset (Figure 3.6 A, R^2 = 0.45, p = 0.003). A weak, but significant correlation among localities was noted for the entire *P. homarus* microsatellite dataset (Figure 3.6 C, R^2 = 0.23, p = 0.04). For CR, the comparison received weak and not statistically significant correlations (Figure 3.6 B, R^2 = 0.004, p = 0.46) which was possibly due to CR being highly variable. These results suggest that geographic distance only plays a minor role in genetic differentiation between the subspecies.

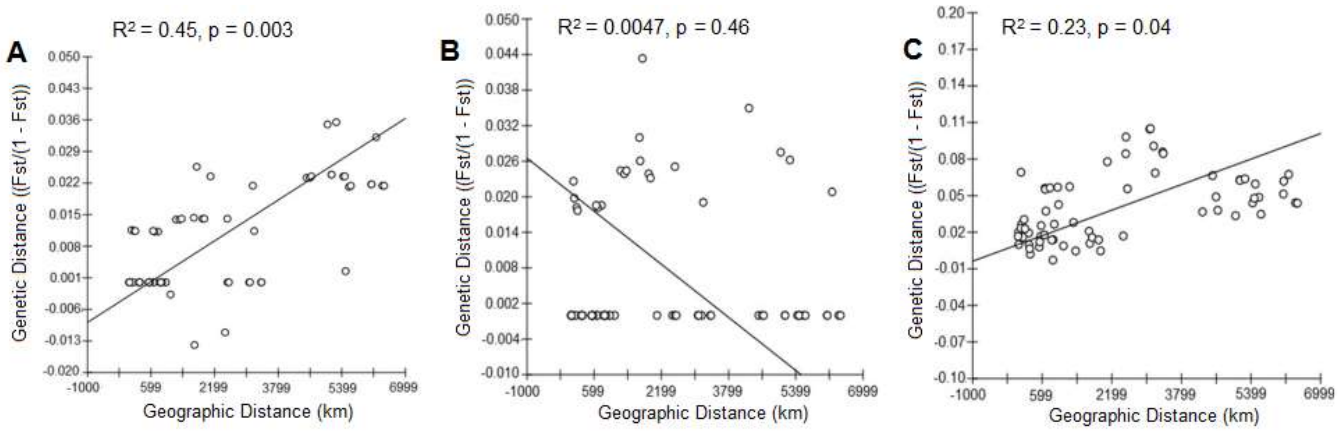


Figure 3.6 IBD analysis for *Panulirus homarus* using (A) COI, (B) CR and (C) microsatellites. The correlation coefficient (R^2) and the p-value are indicated. Negative genetic distances were changed to 0.001 by the program.

3.3.3 Gene flow: *P. h. homarus* + *P. h. megasculptus*

The Migrate-n analysis of historical gene flow between populations of *P. h. homarus* and *P. h. megasculptus* grouped by country, using COI and CR datasets and a simple island model of migration, indicated that there was considerable gene flow between Mozambique, Kenya, Oman and Yemen in the past. At COI, Kenya and Oman appeared to receive the most migrants per generation (> 600, Figure 3.7 A), while Yemen received the least (< 40). At CR, Mozambique received the most migrants per generation (Figure 3.7 B, > 600) and Yemen received the least (< 300).

Recent gene flow based on the microsatellite data and estimated using BayesAss for the *P. h. homarus* + *P. h. megasculptus* group of populations indicated that the majority of gene flow originated within each country (Figure 3.7 C). For the *P. h. homarus* + *P. h. megasculptus* group, Oman received the most migrants (13%) from each of the other countries while Yemen, Kenya and Mozambique each received approximately 7% of migrants (Figure 3.7 C).

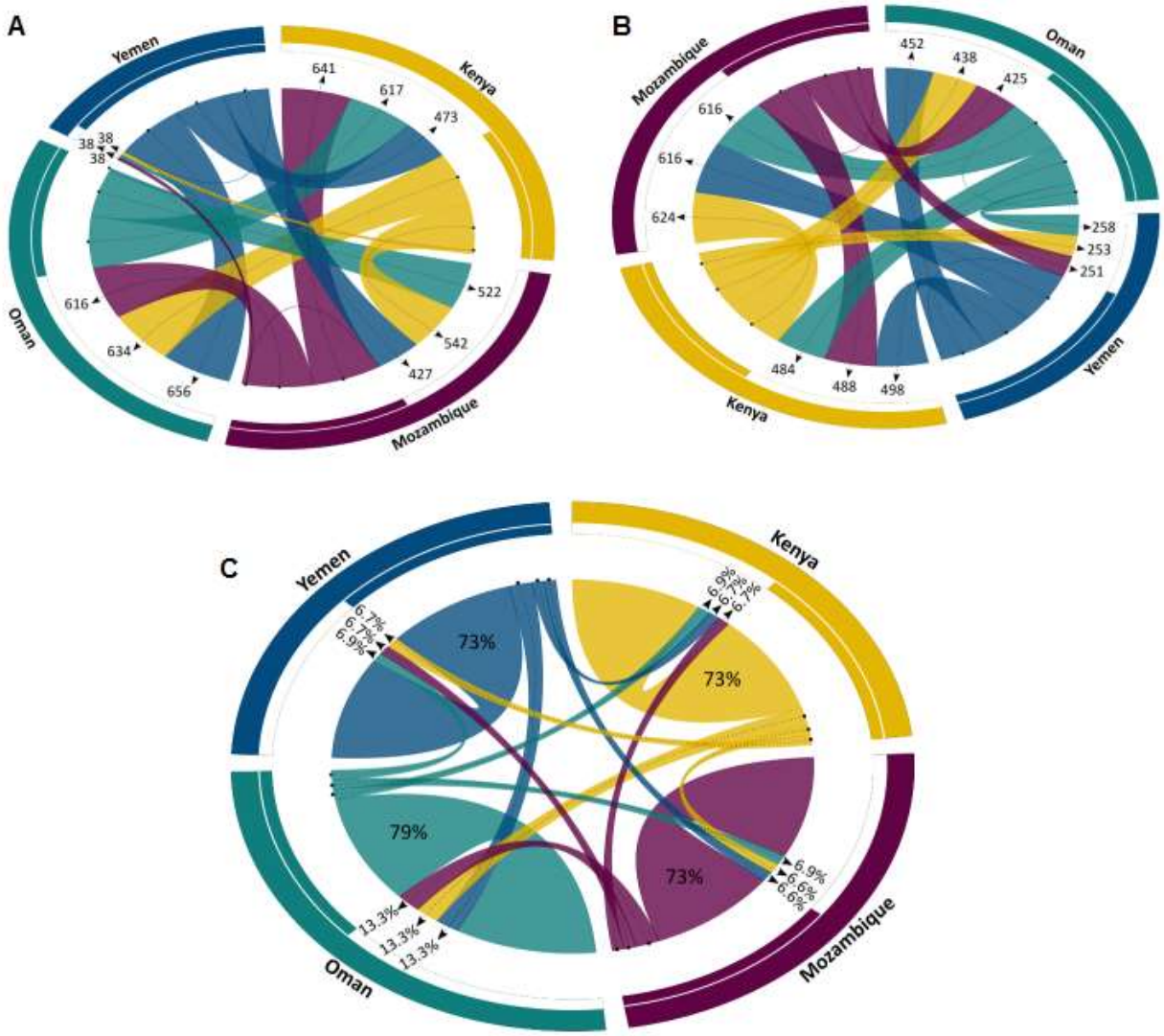


Figure 3.7 Historical gene flow analysis for *P. h. homarus* + *P. h. megasculptus* using Migrate-n (A) based on COI and (B) based on CR. (C) Contemporary gene flow analysis using the program BayesAss and microsatellite data.

3.3.4 Historical demography

Significantly negative values ($P < 0.05$) of Fu's Fs and Tajima's D were observed using COI (Table 3.1) for the *P. homarus* data at the subspecies level. This could indicate that populations of these subspecies have experienced a recent population expansion and possibly diversifying selection. For CR (Table 3.2), the overall Fu's Fs statistic was significantly

negative for the *P. homarus* subspecies. This was also significantly negative for the *P. h. rubellus* CR dataset.

The overall mismatch distribution for *P. homarus* was bimodal for both mitochondrial markers (Figure 3.8 A and C). One mode corresponded to the number of pairwise differences among the subspecies, and the other to the differences among individuals within subspecies. A multimodal distribution generally indicates a stable population over a long period. The Bayesian skyline analyses revealed that a demographic expansion occurred for all three subspecies within the last 100 000 years using COI (Figure 3.8 B), and a demographic decline and then expansion occured within the last 50 000 years when using CR (Figure 3.8 D). These dates correspond to the Pleistocene.

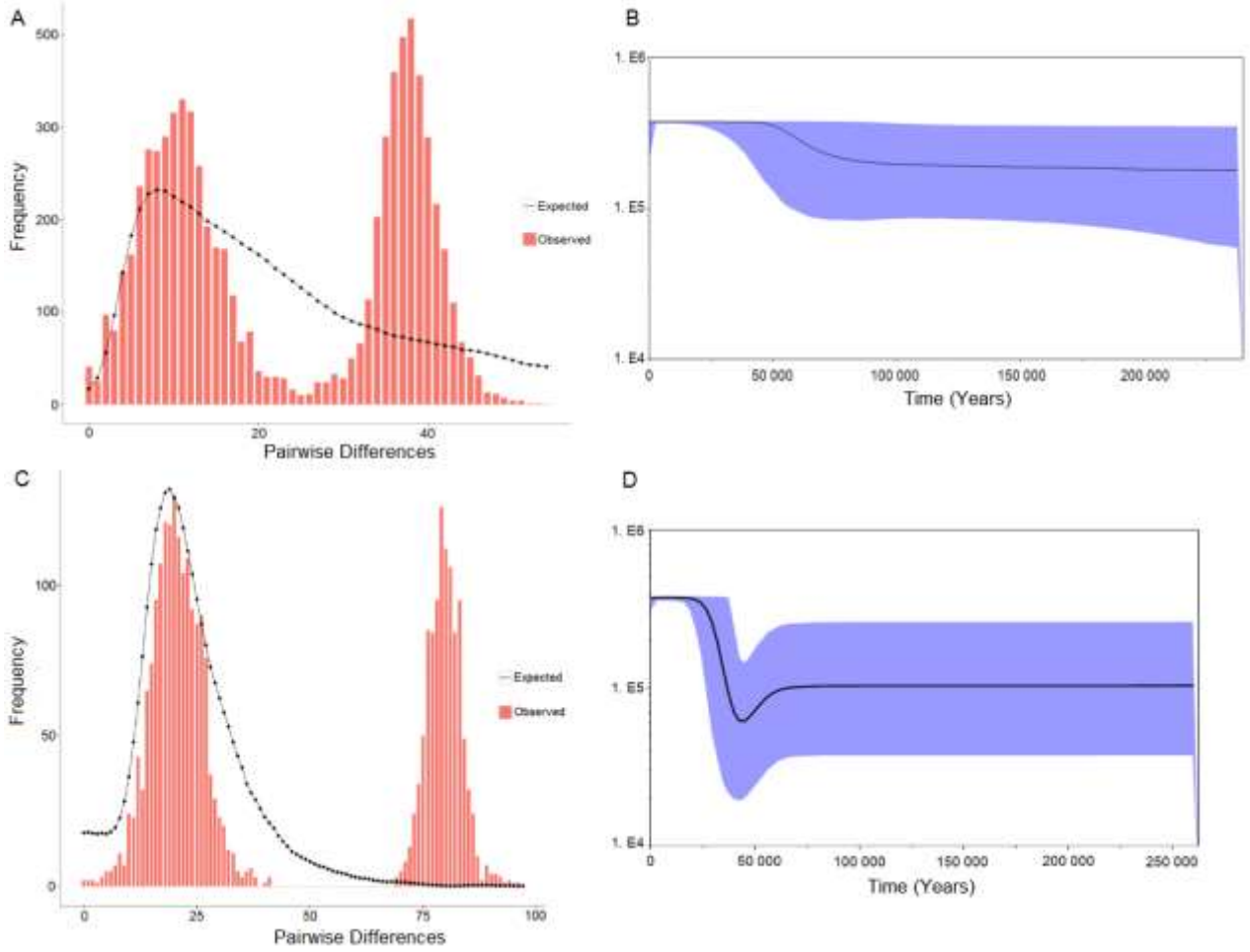


Figure 3.8 Historical demography analyses for *P. homarus*. (A) Mismatch distribution using COI and (C) CR. The histograms indicate the frequency of each pairwise difference, and dotted lines indicate the expected frequencies under the sudden expansion model. (B) Bayesian skyline plot using COI and (D) using CR, showing past population decline and expansion. The black line shows the median posterior effective population size through time. The 95% HPDI is shown by the purple area.

3.3.5 Population genetic structure within *P. h. rubellus*

BAPS Analysis of *P. h. rubellus* individuals using COI showed that there were three genetic clusters, one which was unique to some individuals from Zavora and Chidenguele, and the other two clusters contain individuals from many different populations (Figure 3.9 A). The analysis of the *P. h. rubellus* CR data supported the results of the COI data, which clustered some individuals from Zavora and Chidenguele into a single group, while all other individuals were included in a second cluster. (Figure 3.9 B).

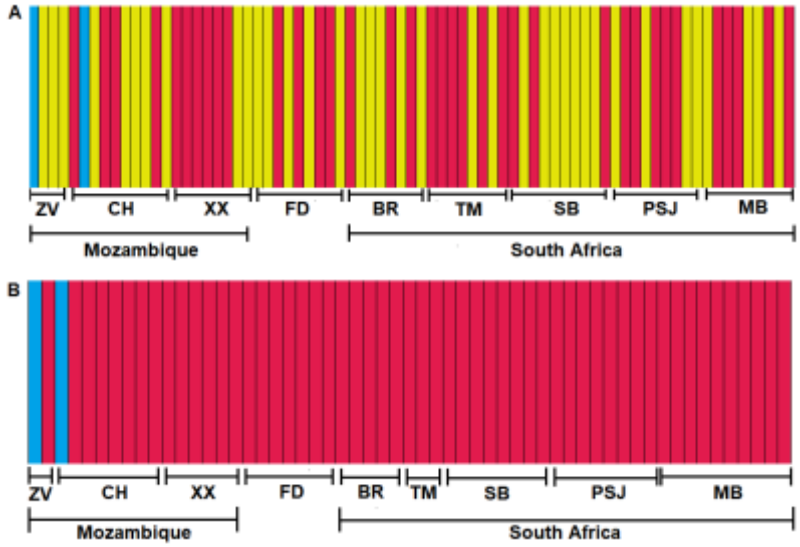


Figure 3.9 BAPS plot for *P. h. rubellus* using (A) COI and (B) CR. Abbreviated site names are as per Figure 2.1.

The DAPC analysis indicated that the optimal number of principle components to retain for the *P. h. rubellus* dataset was 9, the optimal number of clusters was K = 3 (Figure 3.10 A and B), and the admixture analysis indicated that 27 out of 175 (15%) of individuals were admixed.

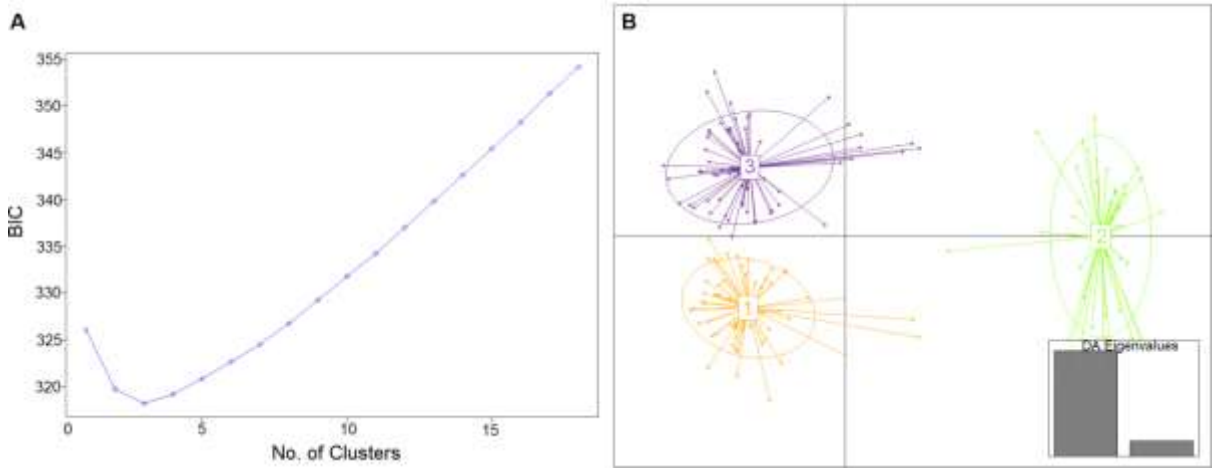


Figure 3.10 DAPC analysis for *P. h. rubellus* using microsatellite data. The lowest BIC score indicates that the optimal K = 3. (B) Scatterplot of individual genotypes from the DAPC analysis and the clusters they belong to.

For the *P. h. rubellus* STRUCTURE analysis, the optimal K was 2 (Figure 3.11 A and B). Without the locprior model, all populations showed approximately equal levels of admixture. The Scottburgh population showed differentiation from the rest of the populations when using locprior as a prior.

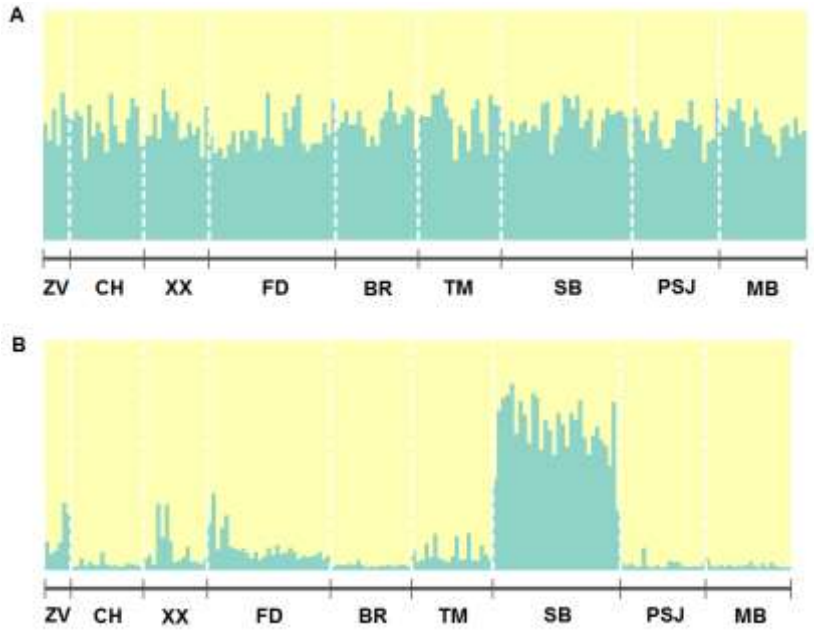


Figure 3.11 STRUCTURE plots for *P. h. rubellus* using microsatellite data. The optimal number of clusters was two. (A) Admixture without locprior. (B) Admixture with locprior. Abbreviated site names are as per Figure 2.1.

The pairwise Φ_{ST} and F_{ST} values were low between populations of *P. h. rubellus*. Pairwise Φ_{ST} ranged from from -0.00007 between Port St Johns and Tinley Manor, to 0.119 between Scottburgh and Tinley Manor based on COI (Table S3.6), and from -0.009 between Fort Dauphin and Chidenguele to 0.304 between Mdumbi and Zavora based on CR (Table S3.7). For the microsatellite data, pairwise F_{ST} values ranged from -0.006 between Xai Xai and Zavora, to 0.077 between Chidenguele and Zavora (Table S3.8).

AMOVA conducted among sampling localities of *P. h. rubellus* (Figure 3.12) found that using COI, there was no significant genetic differentiation at any level (Table S3.10), while using CR, there was low but significant differentiation among populations within groups (9%, $F_{SC} = 0.09$, $P < 0.01$, Table S3.10) and high differentiation within populations (93%, $F_{ST} = 0.07$, $P < 0.01$, Table S3.10). The microsatellite data also indicated low but significant

differentiation among populations within groups (1.3%, F_{SC} = 0.01, P < 0.01, Table S3.10) and high significant differentiation (99%, F_{ST} = 0.01, P < 0.01, Table S3.10) within populations.

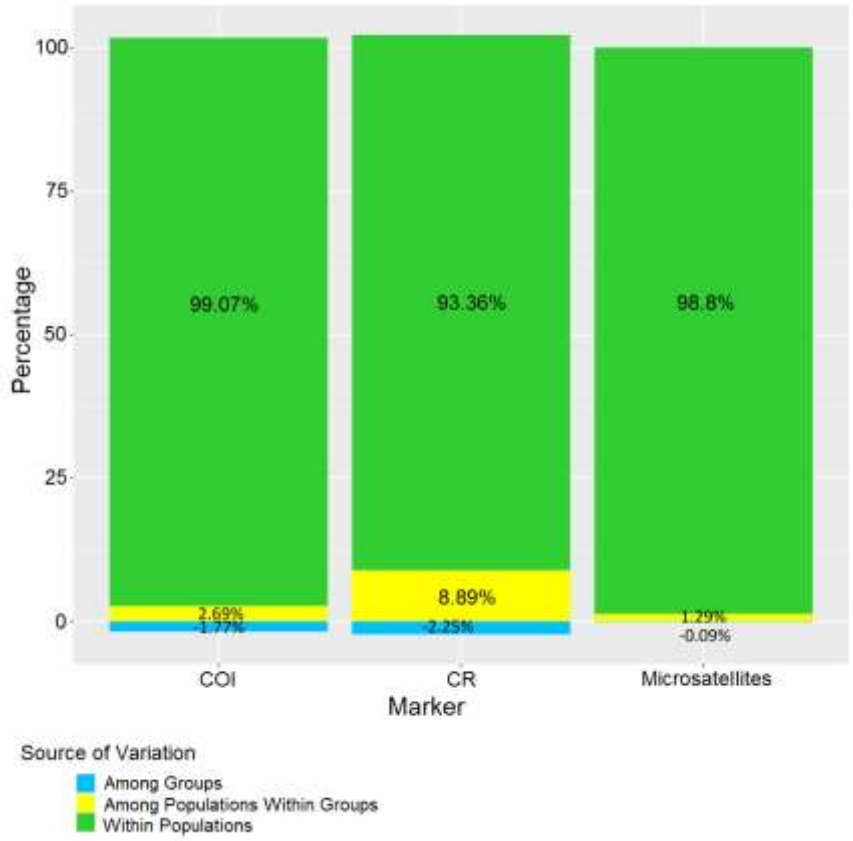


Figure 3.12 AMOVA for *P.h. rubellus* using COI, CR and the microsatellites. Groupings were tested by region; Mozambique (Zavora, Chidenguele, Xai Xai), Madagascar and South Africa (Blood Reef, Tinley Manor, Scottburgh, Port St Johns and Mdumbi).

There was no significant IBD between populations of *P. h. rubellus* at any of the markers used in this study (Figure 3.13 A, B and C).

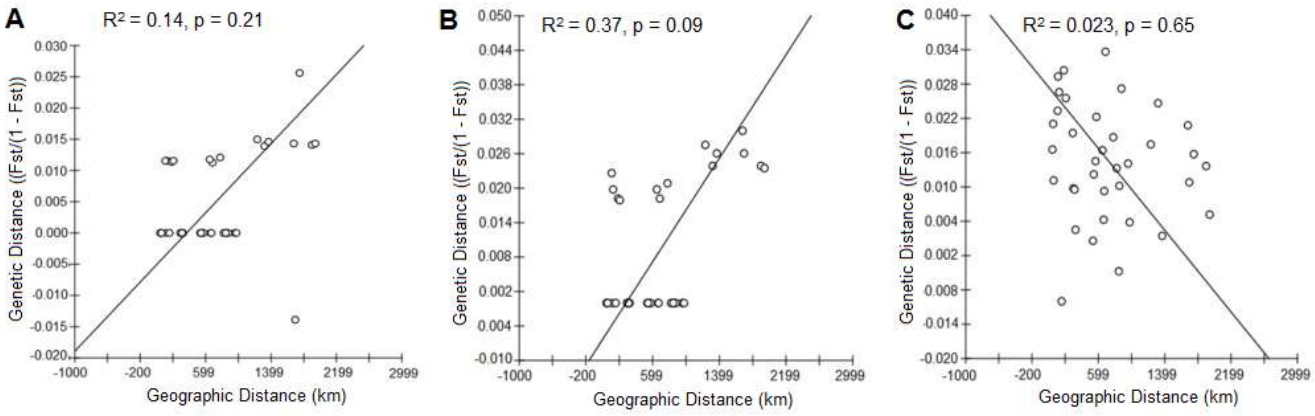


Figure 3.13 IBD analysis for *P. h. rubellus* using (A) COI, (B) CR and (C) microsatellites. The correlation coefficient (R^2) and the p-value are indicated. Negative genetic distances were changed to 0.001 by the program.

3.3.6 Gene flow: *P. h. rubellus*

For the *P. h. rubellus* populations grouped by country, historical gene flow was highest from Mozambique to South Africa (658 migrants per generation, Figure 3.14 A) and lowest from South Africa to Madagascar (18 migrants per generation) using COI. Using CR, historical gene flow was highest from South Africa to Mozambique (732 migrants per generation, Figure 3.14 B) and lowest from South Africa and Mozambique to Madagascar (2 migrants per generation). Contemporary gene flow for the *P. h. rubellus* populations, grouped by country, indicated that the majority of the gene flow is entrained within the originating populations. Mozambique received more migrants (17%) compared to South Africa or Madagascar (Figure 3.14 C). Overall, there is gene flow between the *P. h. rubellus* populations from Madagascar, Mozambique and South Africa, implying connectivity across the Mozambique Channel. However, historical gene flow occurred predominantly between Mozambique and South Africa.

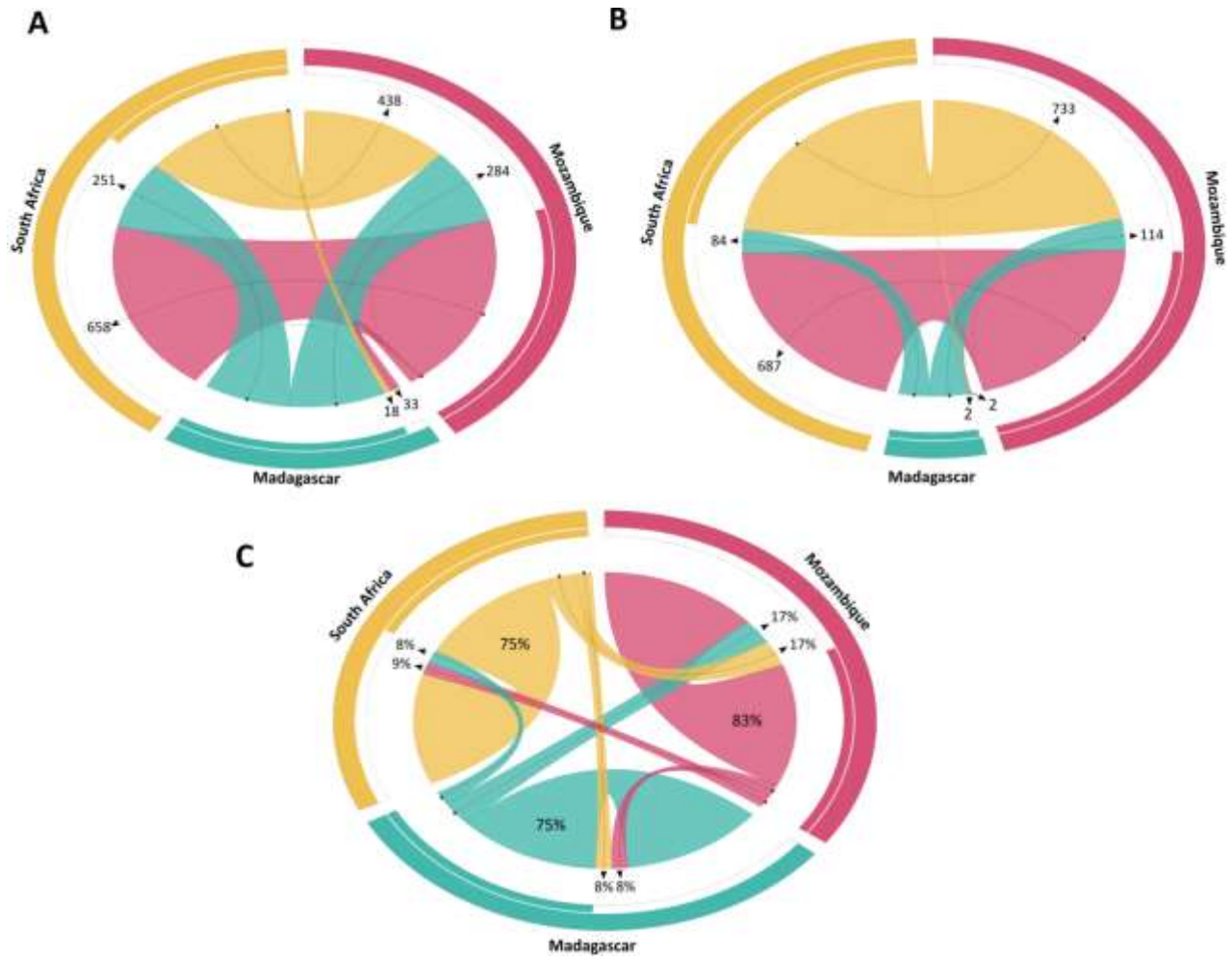


Figure 3.14 Historical gene flow analysis for *P. h. rubellus* using Migrate-n (A) using COI and (B) using CR. (C) Contemporary gene flow analysis using the program BayesAss and microsatellite data.

3.3.7 Historical demography: *P. h. rubellus*

The *P. h. rubellus* dataset had a unimodal mismatch distribution for both COI (Figure 3.15 A) and CR (Figure 3.15 C), which is a signature of recent population size expansion. Non-significant p-values for the SSD and HRI indices indicate that the population expansion is recent (Table 3.1 and 3.2). The Bayesian skyline analyses indicated that a demographic expansion also occurred for *P. h. rubellus* within the last 100 000 years using COI (Figure 3.15 B), and a demographic decline and then expansion occured within the last 50 000 years when using CR (Figure 3.15 D).

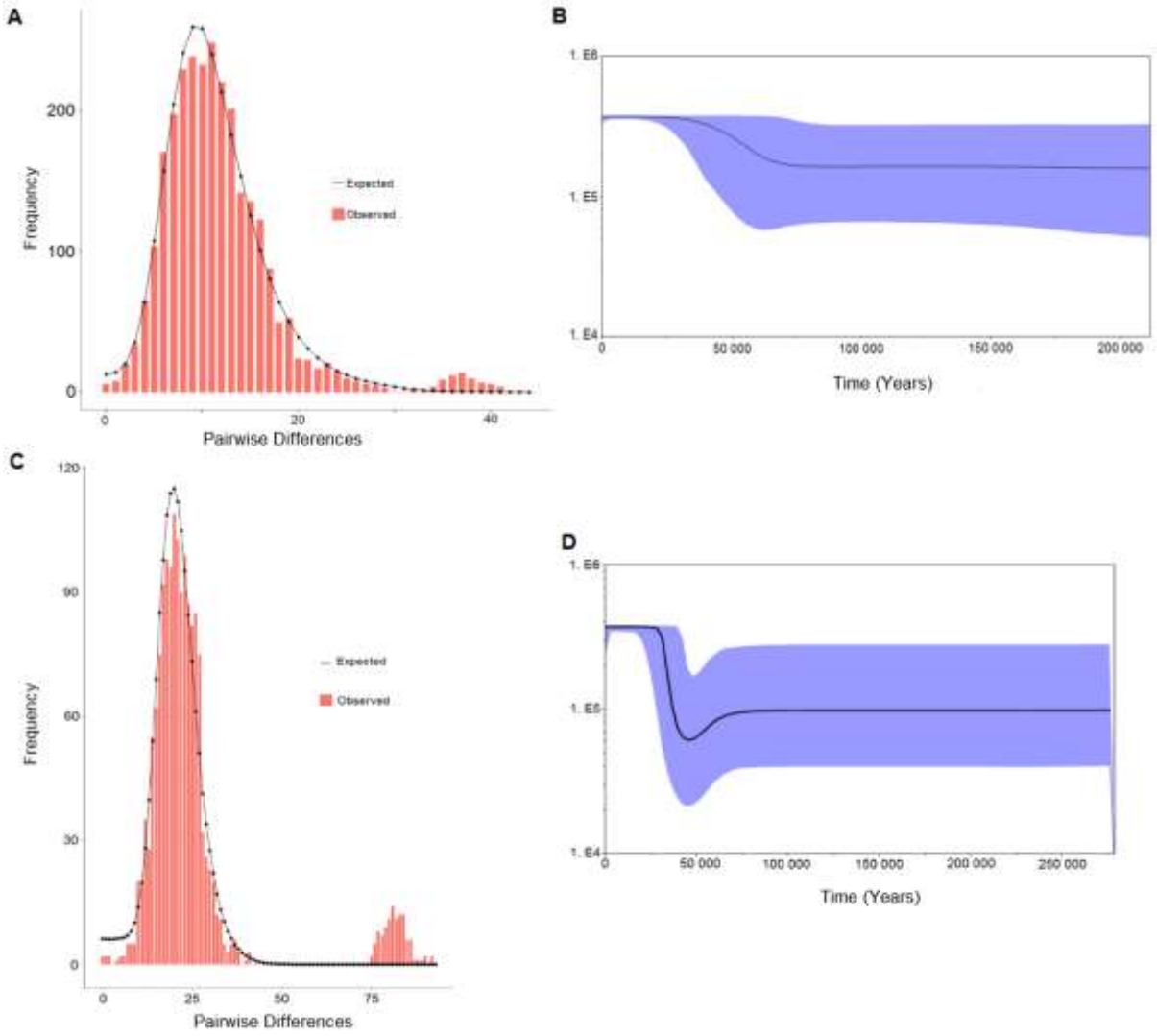


Figure 3.15 Historical demography analyses for *P. h. rubellus*. (A) Mismatch distribution using COI and (C) CR. The histograms indicate the frequency of each pairwise difference, and dotted lines indicate the expected frequencies under the sudden expansion model. (B) Bayesian skyline plot using COI and (D) using CR, showing past population decline and expansion. The black line shows the median posterior effective population size through time. The 95% HPDI is shown by the purple area.

3.4 Discussion

3.4.1 Genetic differentiation between subspecies

This chapter examines the finer-scale population genetic structure and phylogeography of *P. homarus* in the Western Indian Ocean using fast evolving mitochondrial markers and a suite of nuclear microsatellite markers. Genetic structure analysis using BAPS for the mitochondrial DNA, and DAPC and STRUCTURE for the microsatellite DNA, indicates differentiation between *P. h. rubellus* and the other two subspecies. The spatial analyses indicate that *P. h. rubellus* and *P. h. homarus* are sympatric in Mozambique, but there was little conclusive evidence of molecular hybridization in the data. The high degree of genetic differentiation between *P. h. rubellus* from *P. h. homarus* and *P. h. megasculptus* in both the mitochondrial DNA markers and microsatellite loci, coupled with little geneflow between *P. h. rubellus* and *P. h. homarus* where they are sympatric, provides a strong case for *P. h. rubellus* to be elevated to species rank. This supports the conclusion of Chapter 2 (Singh et al. 2017), in which the species name *Panulirus rubellus* is suggested.

In addition, microsatellite loci suggest that *P. h. homarus* and *P. h. megasculptus* could also be separately evolving lineages, having diverged much more recently. Farhadi et al. (2013) first demonstrated that *P. h. homarus* lobsters from Tanzania were genetically different from *P. h. megasculptus* in Oman and Iran, based on a portion of the hypervariable CR. However, this variation was not picked up by COI or CR in the study by Lavery et al. (2014) or by the same mitochondrial markers in the present study. Also using microsatellite data, Farhadi et al. (2017) indicated that there was some evidence of genetic divergence between Arabian populations and the rest of the *P. h. homarus* populations. Given the considerable morphological differences between *P. h. homarus* and *P. h. megasculptus*, and their different geographic distributions, the separation of these subspecies at the microsatellite markers could be due to a contemporary speciation event, as indicated by faster evolving microsatellites. The large effective population size of marine species increases the time needed for genetic differences to become fixed in markers such as COI and CR, while allele frequency difference at microsatellite loci would become fixed much more rapidly.

Panulirus homarus megasculptus in Oman has a prolonged breeding season with multiple broods, extending for 9 months, from the onset of the SW monsoon in May/June to the end of

the NE monsoon in January (Al-Marzouqi et al. 2007, 2008). Drifting larvae are therefore present in the Arabian Sea throughout the year. Oceanic circulation in the Arabian Sea is influenced by the seasonally reversing Somali Current and eddies which develop along the coasts of Oman and Somalia (Schott and McCreary 2001). This system may lead to larval retention in that area, thereby giving rise to the incipient genetic structure between *P. h. homarus* and *P. h. megasculptus* (Pollock 1993, Farhadi et al. 2013). Vogler et al. (2012) also suggested that the Somali Current would prevent the northward dispersal of the starfish *Acanthaster planci* larvae from east Africa into the northern Arabian Sea.

The Φ_{ST} for CR and microsatellite F_{ST} in this study were comparable to those obtained by Farhadi et al. (2017). Microsatellite F_{ST} estimates were also lower than the mitochondrial Φ_{ST} estimates, but higher than that obtained by Farhadi et al. (2017). For example, genetic differentiation between southern Africa and east Africa (Kenya) in this study was 0.07 while Farhadi et al. (2017) obtained 0.027. This is likely due to using more microsatellite loci and including more individuals from South Africa, Madagascar, Mozambique, Kenya and Yemen in the present study. The discrepancy between mitochondrial and nuclear differentiation could be in part due to the mitochondrial DNA reflecting only matrilineal divergence, while nuclear DNA reveals male and female divergence patterns. Several hypotheses were put forward to explain why mitochondrial DNA divergence was greater than the nuclear microsatellite divergence between *P. h. rubellus* and *P. h. homarus* (Farhadi et al. 2017). These hypotheses were differences in migratory behaviour due to sex, putative asymmetric developmental fitness of hybrids, and the effect of strong environmental selection on differentially adapted phenotypes (Farhadi et al. 2017). Given that there is no definitive evidence for hybrids between the two forms, environmental selection on differentially adapted phenotypes (red megasculpta form and green microsculpta form) appeared to be the most likely hypothesis. This selection gradient could be contributing to reproductive isolation between the two lineages and is discussed in detail below.

3.4.2 Contact zone and isolation by distance

From a biogeographic perspective (Spalding et al. 2007), *P. h. homarus* and *P. h. megasculptus* inhabit the Western Indo-Pacific region, whereas *P. h. rubellus* inhabits both the Western Indo-Pacific and Temperate Southern African region. At a different (ecoregion)

level, *P. h. rubellus* extends southwards from the Delagoa Bight ecoregion in Mozambique, to the Natal and Agulhas Bank ecoregions in South Africa, and to the Southeast Madagascar ecoregion (Spalding et al. 2007). The Mozambique sampling sites for *P. h. homarus* and *P. h. rubellus* lie within the Delagoa Bight ecoregion, which is a likely contact zone between these two subspecies. The Delagoa Bight is a coastal indentation just south of the Mozambique Channel, and both its location and unique oceanographic features may play a role in larval dispersal. It is affected by waters moving gradually southwards through the Mozambique Channel, and also by cross-channel eddies originating from the East Madagascar Current, on its path around the southern tip of Madagascar (Lutjeharms 2006, Cossa et al. 2016). Additionally, the Delagoa Bight is also located along the boundary of the upper reaches of the Agulhas Current (Lutjeharms 2006). The confluence of oceanic features in this region are likely to play a major role in maintaining the boundary zone between the *P. h. homarus* (north) and *P. h. rubellus* (south) subspecies.

A quasi-stationary and topographically-induced lee-eddy, the Delagoa Bight Eddy at approximately 26°S (Saetre and da Silva 1984, Lutjeharms and da Silva 1988, Lamont et al. 2010, Cossa et al. 2016, Halo et al. 2017), creates strong upwelling and a cool Delagoa Bight cell, with near-surface temperatures of < 16 °C at 150 m depth. The cell can potentially form a boundary restricting gene flow between the two subspecies, but it is intermittent and therefore unlikely to be impermeable. The mixture of equatorial and sub-tropical waters in this area may also contribute to this contact zone – in which larvae of *P. h. homarus* from further north and of *P. h. rubellus* originating locally (in the bight) or from southeastern Madagascar, after crossing the channel in eddies, would settle. Successful reproduction between settlers that survive to adulthood may then give rise to hybrids in this contact zone, although we saw no evidence for this in our data.

The decreasing temperature as climate transitions from tropical to subtropical conditions could also account for the observed genetic break and speciation, with *P. h. homarus* preferring tropical waters while *P. h. rubellus* prefers subtropical waters (Berry 1974a). Another important phylogeographic break in southeast Africa, close to Cape St Lucia, was identified by Teske et al. (2009). A selection gradient is thought to exist at this break due to the diminishing effects of the Agulhas Current on nearshore waters to the south of St Lucia, where the Natal Bight deflects the current away from the coast, resulting in lower SSTs and potential changes in circulation patterns, such as the formation of eddies and nearshore return

currents (Teske et al. 2011). A similar break is suggested to occur for *P. h. homarus* and *P. h. rubellus*, but further to the north, at the Delagoa Bight. However, hybrids may be rare because of reproductive isolation between the two *P. homarus* lineages, low survival rates of settled post-larvae originating from elsewhere, or preference of conspecifics as seen in lobsters such as *Homarus americanus* and *H. gammarus* (van der Meeren et al. 2008).

Isolation by distance did not play a crucial role in influencing population genetic structure among the subspecies. For example, the lobsters in Zavora (northern edge of Delagoa Bight) were more closely related to those in Oman, Yemen and Kenya which are thousands of kilometres away (Φ_{ST} and F_{ST}, Tables S3.3 – S3.8) than to those in Chidenguele, which is only 114 km southwards from Zavora, within the Delagoa Bight. The Zavora population was also more closely related to the population from Xai Xai (also within the Bight) which is 258 km from Zavora. This further elucidates the presence of the contact zone between subspecies possibly being close to Zavora in the Delagoa Bight ecoregion and also indicates cohesion within subspecies over large spatial scales, contrasted with breaks between subspecies over shorter distance scales.

3.4.3 Gene flow among subspecies and among populations of *P. h. rubellus*

Analysis using COI and CR indicate gene flow between *P. h. homarus* and *P. h. megasculptus* suggesting that these two subspecies were connected in the past, probably during the Miocene (see Singh et al. 2017) (Figure 3.7 A and B). Recent gene flow patterns obtained using BayesAss and the microsatellite data show that most individuals remain within populations, with fewer migrants moving between populations (Figure 3.7 C). This has important implications for the management of commercial fisheries of these species, because overfishing in one area could deplete local populations.

The patterns of genetic differentiation between the *P. homarus* subspecies could be attributed to the prevailing currents in the Western Indian Ocean, particularly the Mozambique Channel. A large, seasonal anticyclonic cell prevails at the northern entrance to the channel (Donguy and Piton 1991), and centrally, a succession of mesoscale cyclonic and anticyclonic eddies are found along the Mozambican coast (Schouten et al. 2003, Swart et al. 2010). They create strong dynamic gradients that could profoundly affect the early passive stages of larval

dispersal. Silva et al. (2010) speculated that a major component of the flow through the Mozambique Channel is caused by the anti-cyclonic circulation pattern which has the effect of randomizing larval dispersal in the mangrove crab *Perisesarma guttatum*, resulting in panmictic populations to the North of the channel. Madeira et al. (2012) identified that the Agulhas Current between southern Mozambique and South Africa acts as a transporter of the gastropod *Cerithidea decolata* larvae southwards, and could be responsible for homogenizing populations in southeast Africa.

Further genetic evidence that populations to the north of the Mozambique Channel are differentiated from those at the south has been documented for shallow-water prawns *Fenneropenaeus indicus* and *Metapenaeus monoceros* (Mkare et al. 2017), green turtle *Chelonia mydas* (Bourjea et al. 2007), the swordfish *Xiphias gladius* (Muths et al. 2009), reef fish *Myripristis berndti* (Muths et al. 2011), reef grouper *Epinephelus merra* (Muths et al. 2015), and corals (Montoya-Maya et al. 2016). Studies of population genetics and genetic differentiation in the Western Indian Ocean region have uncovered cases where southeast African marine populations of the prawn *Penaeus monodon* (Forbes et al. 1999, Duda and Palumbi 1999, Benzie et al. 2002) and crab *Scylla serrata* (Fratini and Vannini 2002), lack significant population genetic structure, in contrast to populations in the Red Sea and more northern Indian Ocean locations, which exhibit clearer genetic structure (Ridgway and Sampayo 2007). A phylogeographic study of *Acanthaster planci*, using COI and CR, highlighted two distinct genetic lineages - one in the northern Indian Ocean with highly structured populations, and a southern Indian Ocean lineage, exhibiting high connectivity and little regional structure (Vogler et al. 2012). This is in agreement with the patterns of diversity obtained among *P. homarus* subspecies, particularly between *P. h. homarus* in the north and *P. h. rubellus* in the south.

For *P. h. rubellus* populations, migration analysis using COI and CR suggest that gene flow was predominantly between South Africa and Mozambique, with fewer migrants originating from Madagascar. Gene flow from South Africa and Mozambique to Madagascar was virtually absent (Figure 3.14 A and B). Pollock (1993) postulated that intensified westerly winds during the last glacial period might have impeded the flow of the south equatorial current and the East Madagascar current towards the southeast coast of Africa. These flows usually feed into the Agulhas Current, but during the glacial period, the influence of the Agulhas Current may have decreased along the southeast African coast, because of the

reduced leakage from the other two current systems (Hutson 1980, Pollock 1993). Contrary to what was originally thought, the phylogenetic relationships suggested by COI pseudogene amplified by Reddy et al. (2014), which suggested that the populations of *P. h. rubellus* in Madagascar were genetically distinct from the southeast African shelf populations, might be more ancestral than the relationships suggested by the mitochondrial COI amplified in the present study. The phylogenetic relationships recovered using the pseudogene may represent relationships in the past where alleles were not shared across the Mozambique Channel. Pollock (1993) suggested that retroflection of the east Madagascar current during the last glacial period may have resulted in isolation of larvae of a southeastern Madagascan population of *P. homarus* in another gyre system in the South Indian Ocean, promoting the formation of the *P. h. rubellus* lineage.

Migration analysis for *P. h. rubellus* populations detected that Mozambique received a higher percentage of migrants from South Africa and Madagascar, possibly because it is centrally located (Figure 3.14 C). There is little genetic structure seen between the populations of *P. h. rubellus* at any of the molecular markers used, and the populations from South Africa, Madagascar and Mozambique appear genetically connected. Even though gene flow was asymmetric, very few migrants per generation are required to sustain the genetic connectivity between populations. Pollock (1989, 1993) suggested that Madagascan and southeast African stocks of *P. h. rubellus* are panmictic, connected through an East Madagascar-Agulhas circulating current system, in which long-lived larvae would become entrained and dispersed widely by the South Indian Ocean gyre system. However, recent physical oceanography shows westward propagating eddies, which may pick up chlorophyll-a rich waters and other biological material from the southeastern Madagascar shelf and transport them across the Mozambique Channel, towards the southeast African coast (de Ruijter et al. 2004, Quartly and Srokosz 2004, Ridderinkhof et al. 2013). Marsac et al. (2014) proposed the 'suitcase hypothesis', for the transport of biological particles from Madagascar to the African shelf. In this hypothesis, water from the shelf on the southeast coast of Madagascar laden with biological particles is taken up by passing mesoscale eddies, and transported across the Mozambique Channel, colliding with the African Coast off southern Mozambique and along the coast of KwaZulu-Natal in South Africa. Several other physical oceanography studies have also demonstrated that mesoscale eddies interact with the southern Madagascar coast and transport biological particles to Mozambique and South Africa (Morris et al. 2013, Braby 2014, Halo et al. 2014).

Mesoscale eddies could plausibly be vectors for the transport of *P. h. rubellus* larvae from southeast Madagascar across the southern Mozambique Channel to southern Mozambique and down the east coast of South Africa, which results in the genetic connectivity between populations of *P. h. rubellus*. Oceanographic studies observe that the cyclonic eddy elongates and moves down the Agulhas Current when it interacts with the coast (Morris et al. 2013). In addition, data from gliders deployed in the Agulhas Current region showed that submesoscale cyclonic eddies form at the inshore boundary due to shear instability and drive plumes of warm water towards the continental shelf (Krug et al. 2017). The resulting filament of water is thought to prevent organisms such as larvae from getting swept away in the Agulhas Current (Morris et al. 2013, Braby 2014). The regular influx of larvae transported by eddies from southeastern Madagascar potentially homogenizes genetic variability between the *P. h. rubellus* populations over time as they arrive in the vicinity of the Delagoa Bight and are then transported southwards along the coast. This east to west pattern of connectivity and gene flow from Madagascar across the Mozambique Channel is reflected in the genetic data presented for *P. h. rubellus* in this study – not only in the Migrate analyses, but also as low diversity estimates using CR for Madagascar. The latter suggests that Madagascar is a source of *P. h. rubellus* larvae, but that this population does not receive migrants from elsewhere.

3.4.4 Demographic history

The high haplotype and low nucleotide diversities observed (Table 3.1 and 3.2) for both mitochondrial markers is consistent with a recent population expansion after a bottleneck (Grant and Bowen 1998). Significantly negative Fu's Fs and Tajima's D for each subspecies using COI (Table 3.1), significantly negative Fu's Fs using CR (Table 3.2) for the *P. homarus* dataset and for *P. h. rubellus*, and the non-significant HRI analysis, are all indicative of a recent population expansion. The timing of the population decline and subsequent expansion was estimated by the Bayesian skyline analyses to have occurred within the last 100 000 years, during the Pleistocene, using both COI and CR data. The Pleistocene glacial period was characterised by a decline in sea level, subsequent reductions in marine habitats, changes in water column thermal dynamics and changes in the strength of ocean currents (Pollock 1992). These factors must have affected the distribution of marine life along the coast (Lenoir and Svenning 2015). The contribution of alternating glacial and interglacial

events during the Pleistocene, and the corresponding effects on sea-levels, have been implicated in speciation of *Palinurus* and *Jasus* species (George and Main 1967, Pollock 1990, 1992, 1993, Groeneveld et al. 2007). Other studies on southern African spiny lobster species have also uncovered population expansion events within the last 100 000 years, based on mitochondrial DNA analyses (Tolley et al. 2005, Gopal et al. 2006, Matthee et al. 2007, von der Heyden et al. 2007b). Tolley et al. (2005) hypothesized that the range expansion observed for the deeper water lobster, *P. gilchristi* using hypervariable CR, could have been due to a habitat increase following the submergence of the Agulhas Bank after the last glacial period. In the case of *P. homarus*, which inhabits the intertidal and shallow subtidal habitats up to about 30 m depth, an increase in water temperature after the last glacial period could have contributed to the southwards expansion of this lobster in the Southwestern Indian Ocean, extending along the eastern coast of South Africa.

To conclude, Chapter 3 demonstrated that there is clear speciation of the *P. h. rubellus* lineage based on mitochondrial and nuclear microsatellite DNA. *P. h. homarus* and *P. h. megasculptus* also showed divergence at the microsatellite level. The circulation within the Mozambique Channel is potentially a barrier to gene flow between *P. h. homarus* populations in the north and *P. h. rubellus* populations in the south, with the Delagoa Bight Eddy intermittently contributing to contact between the two subspecies. Different species should be sampled across this region to determine if the Delagoa Bight serves as a more general biogeographical barrier – from the perspectives of oceanographic features (upwelling systems, quasi-permanent cool-water cell, SST gradient from tropical to subtropical environments) and also as the northernmost point where the cross-channel eddies originating from southern Madagascar, and carrying biological particles such as larvae, are likely to arrive at the southeast African coast.

The key finding of this chapter, that the westward pattern of gene flow from Madagascar to the southeast African coast across the Mozambique Channel promotes connectivity within the *P. h. rubellus* lineage, is further investigated in Chapter 4, where biophysical ocean circulation models are developed to simulate larval dispersal patterns – and hence to test the hypotheses of gene flow and diversity developed in Chapter 3.

3.5 References

Al-Marzouqi, A., A. Al-Nahdi, N. Jayabalan, and J. Groeneveld. 2007. An assessment of the spiny lobster *Panulirus homarus* fishery in Oman — Another decline in the Western Indian Ocean? *Western Indian Ocean Journal of Marine Science* **6**: 159-174.

Al-Marzouqi, A., J. C. Groeneveld, A. Al-Nahdi, and A. Al-Hosni. 2008. Reproductive season of the scalloped spiny lobster *Panulirus homarus* along the coast of Oman: management implications. *Agricultural and Marine Sciences, Sultan Qaboos University* **13:** 33-42.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press. 464 pages.

Beerli, P., and M. Palczewski. 2010. Unified framework to evaluate panmixia and migration direction among multiple sampling locations. *Genetics* **185**: 313-326.

Bentzen, P., C. T. Taggart, D. E. Ruzzante, and D. Cook. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. *Canadian Journal of Fisheries and Aquatic Sciences* **53**: 2706-2721.

Benzie, J. A. H., E. Ballment, A. T. Forbes, N. T. Demetriades, K. Sugama, S. Haryanti, and S. Moria. 2002. Mitochondrial DNA variation in Indo-Pacific populations of the giant tiger prawn, *Penaeus monodon*. *Molecular Ecology* **11**: 2553-2569.

Berry, P. F. 1974a. A revision of the *Panulirus homarus*-group of spiny lobsters (Decapoda, Palinuridae). *Crustaceana* **27**: 31-42.

Berry, P. F. 1974b. Palinurid and scyllarid lobster larvae of the Natal coast, South Africa. *South African Association for Marine Biological Research, Investigational Report* **34**: 3-44.

Bouckaert, R., J. Heled, D. Kuhnert, T. Vaughan, C.-H. Wu, D. Xie, M. A. Suchard, A. Rambaut, and A. J. Drummond. 2014. BEAST 2: A software platform for Bayesian evolutionary analysis. *PLoS Computational Biology* **10**: e1003537.

Bourjea, J., S. Lapegue, L. Gagnevin, D. Broderick, J. A. Mortimer, S. Ciccione, D. Roos, C. Taquet, and H. Grizel. 2007. Phylogeography of the green turtle, *Chelonia mydas*, in the

Southwest Indian Ocean. *Molecular Ecology* **16**: 175-186.

Braby, L. 2014. Dynamics, interactions and ecosystem implications of mesoscale eddies formed in the southern region of Madagascar. MSc Thesis, University of Cape Town.

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

Chapuis, M.-P., and A. Estoup. 2007. Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* **24**: 621-631.

Corander, J., P. Waldmann, and M. J. Sillanpää. 2003. Bayesian analysis of genetic differentiation between populations. *Genetics* **163**: 367-374.

Cossa, O., S. Pous, P. Penven, X. Capet, and C. J. C. Reason. 2016. Modelling cyclonic eddies in the Delagoa Bight region. *Continental Shelf Research* **119**: 14-29.

Cowen, R. K., K. M. M. Lwiza, S. Sponaugle, C. B. Paris, and D. B. Olson. 2000. Connectivity of marine populations: open or closed? *Science* **287**: 857-859.

Dao, H. T., E. V. Todd, and D. R. Jerry. 2013. Characterization of polymorphic microsatellite loci for the spiny lobster *Panulirus* spp. and their utility to be applied to other *Panulirus* lobsters. *Conservation Genetics Resources* **5**: 43-46.

Dao, H. T., C. Smith-Keune, E. Wolanski, C. M. Jones, D. R. Jerry, and N. Suzuki. 2015. Oceanographic currents and local ecological knowledge indicate, and genetics does not refute, a contemporary pattern of larval dispersal for the ornate spiny lobster, *Panulirus ornatus* in the South-East Asian Archipelago. *PLoS ONE* **10**. DOI: 10.1371/journal.pone.0124568.

Darriba, D., G. L. Taboada, R. Doallo, and D. Posada. 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods* **9**: 772.

Delghandi, M., S. Goddard, D. R. Jerry, H. T. Dao, H. S. Afzal, and S. Al-Jardani. 2015. Isolation, characterization, and multiplexing of novel microsatellite markers for the tropical scalloped spiny lobster (*Panulirus homarus*). *Genetics and Molecular Research* **14**: 19066-19070.

De Ruijter, W. P. M., H. M. van Aken, E. J. Beier, J. R. E. Lutjeharms, R. P. Matano, and M.

W. Schouten. 2004. Eddies and dipoles around South Madagascar: Formation, pathways and large-scale impact. *Deep Sea Research Part I: Oceanographic Research Papers* **51**: 383-400.

Donguy, J.-R., and B. Piton. 1991. The Mozambique Channel revisited. *Oceanologica Acta* **14**: 549-558.

Duda, T. F., and S. R. Palumbi. 1999. Population structure of the black tiger prawn, *Penaeus monodon*, among western Indian Ocean and western Pacific populations. *Marine Biology* **134**: 705-710.

Earl, D. A., and B. M. von Holdt. 2012. STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**: 359-361.

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

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin suite v. 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**: 564-567.

Farhadi, A., H. Farahmand, M. A. Nematollahi, A. G. Jeffs, and S. D. Lavery. 2013. Mitochondrial DNA population structure of the scalloped lobster *Panulirus homarus* (Linnaeus 1758) from the West Indian Ocean. ICES Journal of Marine Science **70**: 1491-1498.

Farhadi, A., A. G. Jeffs, H. Farahmand, T. S. Rejiniemon, G. Smith, and S. D. Lavery. 2017. Mechanisms of peripheral phylogeographic divergence in the indo-Pacific: lessons from the spiny lobster *Panulirus homarus*. *BMC Evolutionary Biology* **17**: 195.

Farrington, L. W., C. M. Austin, and P. C. Coutin. 2000. Allozyme variation and stock structure in the black bream, *Acanthopagrus butcheri* (Munro) (Sparidae) in southern Australia: implications for fisheries management, aquaculture and taxonomic relationship with *Acanthopagrus australis* (Gunther). *Fisheries Management and Ecology* **7**: 265-279.

Forbes, A. T., N. T. Demetriades, J. A. H. Benzie, and E. Ballment. 1999. Allozyme frequencies indicate little geographic variation among stocks of giant tiger prawn *Penaeus monodon* in the south-west Indian Ocean. *South African Journal of Marine Science* **21**: 271-277.

Francis, R. M. 2017. pophelper : an R package and web app to analyse and visualize population structure. *Molecular Ecology Resources* **17**: 27-32.

Fratini, S., and M. Vannini. 2002. Genetic differentiation in the mud crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean. *Journal of Experimental Marine Biology and Ecology* **272**: 103-116.

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**: 915-925.

George, R. W., and A. R. Main. 1967. The evolution of spiny lobsters (Palinuridae): A study of evolution in the marine environment. *Evolution* **21**: 803-820.

Gopal, K., K. A. Tolley, J. C. Groeneveld, and C. A. Matthee. 2006. Mitochondrial DNA variation in spiny lobster *Palinurus delagoae* suggests genetically structured populations in the southwestern Indian Ocean. *Marine Ecology Progress Series* **319**: 191-198.

Goudet, J. 1995. FSTAT (Version 1.2): A Computer Program to Calculate F-Statistics. *Journal of Heredity* **86**: 485-486.

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

Groeneveld, J. C., C. L. Griffiths, and A. P. van Dalsen. 2006. A new species of spiny lobster, *Palinurus barbarae* (Decapoda, Palinuridae) from Walters Shoals on the Madagascar Ridge. *Crustaceana* **79**: 821-833.

Groeneveld J. C., K. Gopal, R. W. George, and C. A. Matthee. 2007. Molecular phylogeny of the spiny lobster genus *Palinurus* (Decapoda: Palinuridae) with hypotheses on speciation in the NE Atlantic/Mediterranean and SW Indian Ocean. *Molecular Phylogenetics and Evolution* **45**: 102-110.

Halo, I., P. Penven, B. Backeberg, I. Ansorge, F. Shillington, and R. Roman. 2014.

Mesoscale eddy variability in the southern extension of the East Madagascar Current: Seasonal cycle, energy conversion terms, and eddy mean properties. *Journal of Geophysical Research: Oceans* **119**: 7324-7356.

Halo, I., B. Malauene, and M. Ostrowski. 2017. Physical oceanography. In: *The RV Dr Fridtjof Nansen in the Western Indian Ocean: Voyages of marine research and capacity development.* J. C. Groeneveld and K. A. Koranteng (Eds). FAO, Rome, Italy. Pages 37-50.

Haye, P. A., N. I. Segovia, N. C. Muñoz-Herrera, F. E. Gálvez, A. Martínez, A. Meynard, M. C. Pardo-Gandarillas, E. Poulin, and S. Faugeron. 2014. Phylogeographic Structure in Benthic Marine Invertebrates of the Southeast Pacific Coast of Chile with Differing Dispersal Potential. *PLoS ONE* **9**. DOI: 10.1371/journal.pone.0088613.

Hutson, W. H. 1980. The Agulhas Current during the late Pleistocene: analysis of modern faunal analogs. *Science* **207**: 64-66.

Iacchei, M., J. M. O'Malley, and R. J. Toonen. 2014. After the gold rush: population structure of spiny lobsters in Hawaii following a fishery closure and the implications for contemporary spatial management. *Bulletin of Marine Science* **90**: 331-357.

Jensen, J. L., A. J. Bohonak, and S. T. Kelley. 2005. Isolation by distance, web service. *BMC Genetics* **6**: 1-6.

Jombart, T. 2008. adegenet: an R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**: 1403-1405.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology* **16**: 1099-1106.

Kennington, W. J., S. A. Cadee, O. Berry, D. M. Groth, M. S. Johnson, and R. Melville-Smith. 2013a. Maintenance of genetic variation and panmixia in the commercially exploited western rock lobster (*Panulirus cygnus*). *Conservation Genetics* **14**: 115-124.

Kennington, W. J., R. Melville-Smith, and O. Berry. 2013b. Genetics of Wild and Captive Lobster Populations. *In: Lobsters Biology, Management, Aquaculture and Fisheries*. Wiley-Blackwell. Pages 36-54.

King, T. L., S. T. Kalinowski, W. B. Schill, A. P. Spidle, and B. A. Lubinski. 2001. Population structure of Atlantic salmon (*Salmo salar L.*): a range-wide perspective from microsatellite DNA variation. *Molecular Ecology* **10**: 807-821.

Krug, M., S. Swart, and J. Gula (2017). Submesoscale cyclones in the Agulhas Current. *Geophysical Research Letters* **44**. DOI:10.1002/2016GL071006.

Laikre, L., S. Palm, and N. Ryman. 2005. Genetic population structure of fishes: Implications for Coastal Zone Management. *AMBIO: A Journal of the Human Environment* **34**: 111-119

Lamont, T., M. J. Roberts, R. G. Barlow, T. Morris, and M. A. van den Berg. 2010. Circulation patterns in the Delagoa Bight, Mozambique, and the influence of deep ocean eddies. *African Journal of Marine Science* **32**: 553-562.

Lane, H. S., J. E. Symonds, and P. A. Ritchie. 2016. The phylogeography and population genetics of *Polyprion oxygeneios* based on mitochondrial DNA sequences and microsatellite DNA markers. *Fisheries Research* **174**: 19-29.

Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**: 1695-1701.

Lavery, S. D., A. Farhadi, H. Farahmand, T. Y. Chan, A. Azhdehakoshpour, V. Thakur, and A. G. Jeffs. 2014. Evolutionary divergence of geographic subspecies within the scalloped spiny lobster *Panulirus homarus* (Linnaeus 1758). *PLoS ONE* **9**. DOI: 10.1371/journal.pone.0097247.

Lenoir, J., and J.-C. Svenning. 2015. Climate-related range shifts - a global multidimensional synthesis and new research directions. *Ecography* **38**: 15-28.

Lutjeharms, J. R. E. 2006. The Agulhas Current. Springer-Verlag Berlin Heidelberg. 329 pages.

Lutjeharms, J. R. E., and J. da Silva. 1988. The Delagoa Bight eddy. *Deep Sea Research II* **35**: 619-634.

Madeira, C., M. Judite Alves, N. Mesquita, S. E. Silva, and J. Paula. 2012. Tracing geographical patterns of population differentiation in a widespread mangrove gastropod:

genetic and geometric morphometrics surveys along the eastern African coast. *Biological Journal of the Linnean Society* **107**: 647-663.

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

Marsac, F., R. Barlow, J. F. Ternon, F. Ménard, and M. Roberts. 2014. Ecosystem functioning in the Mozambique Channel: Synthesis and future research. *Deep-Sea Research Part II: Topical Studies in Oceanography* **100**: 212-220.

Marshall, T. C., J. Slate, L. E. B. Kruuk, and J. M. Pemberton. 1998. Statistical confidence for likelihood-based paternity inference in natural populations. *Molecular Ecology* **7**: 639-655.

Matthee, C. A., A. C. Cockcroft, K. Gopal, and S. von der Heyden. 2007. Mitochondrial DNA variation of the west-coast rock lobster, *Jasus lalandii*: marked genetic diversity differences among sampling sites. *Marine and Freshwater Research* **58**: 1130-1135.

Mkare, T. K., S. von der Heyden, J. C. Groeneveld, and C.A. Matthee. 2014. Genetic population structure and recruitment patterns of three sympatric shallow-water penaeid prawns in Ungwana Bay, Kenya, with implication for fisheries management. *Marine and Freshwater Research* **65**: 255-266.

Mkare, T. K., J. C. Groeneveld, P. R. Teske, and C. A. Matthee. 2017. Comparative genetic structure in two high-dispersal prawn species from the south-west Indian Ocean. *African Journal of Marine Science* 39: 467-474.

Montoya-Maya, P. H., M. H. Schleyer, and A. H. H. Macdonald. 2016. Limited ecologically relevant genetic connectivity in the south-east African coral populations calls for reef-level management. *Marine Biology* **163**: 171.

Morgan, E. M. J., B. S. Green, N. P. Murphy, and J. M. Strugnell. 2013. Investigation of genetic structure between deep and shallow populations of the southern rock lobster, *Jasus edwardsii* in Tasmania, Australia. *PLoS ONE* **8**. DOI: 10.1371/journal.pone.0077978.

Moritz, C. 1994. Applications of mitochondrial DNA analysis in conservation: a critical review. *Molecular Ecology* **3**: 401-411.

Morris, T., T. Lamont, and M. J. Roberts. 2013. Effects of deep-sea eddies on the northern KwaZulu-Natal shelf, South Africa. *African Journal of Marine Science* **35**: 343-350.

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

Muths, D., E. Tessier, G. Gouws, M. Craig, M. Mwale, J. Mwaluma, A. Mwandya, and J. Bourjea. 2011. Restricted dispersal of the reef fish *Myripristis berndti* at the scale of the SW Indian Ocean. *Marine Ecology Progress Series* **443**: 167-180.

Muths, D., E. Tessier, and J. Bourjea. 2015. Genetic structure of the reef grouper *Epinephelus merra* in the West Indian Ocean appears congruent with biogeographic and oceanographic boundaries. *Marine Ecology* **36**: 447-461.

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

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**: 288-295.

Pollock, D. E. 1989. Spiny lobsters. *In: Oceans of Life off Southern Africa*. Payne, A. I. L. and R. J. M. Crawford (Eds). Cape Town; Vlaeberg: 70-80.

Pollock, D. E. 1990. Palaeoceanography and speciation in the spiny lobster genus *Jasus*. *Bulletin of Marine Science* **46**: 387-405.

Pollock, D. E. 1992. Palaeoceanography and speciation in the spiny lobster genus *Panulirus* in the Indo-Pacific. *Bulletin of Marine Science* **51**: 135-146.

Pollock, D. E. 1993. Speciation in spiny lobsters - clues to climatically-induced changes in ocean circulation patterns. *Bulletin of Marine Science* **53**: 937-944.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945-59.

Quartly, G. D. and M. A. Srokosz. 2004. Eddies in the southern Mozambique Channel. *Deep Sea Research Part II: Topical Studies in Oceanography* **51**: 69-83.

Rambaut, A., and A. J. Drummond. 2007. Tracer v. 1.6. http://tree.bio.ed.ac.uk/software/tracer/.

R Development Core Team, R. 2011. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. 3-900051-07-0.

Reddy M. M, A. H. H. MacDonald, J. C. Groeneveld, and M. H. Schleyer. 2014. Phylogeography of the scalloped spiny-lobster *Panulirus homarus rubellus* in the Southwest Indian Ocean. *Journal of Crustacean Biology* **34**: 773-781.

Reiss, H., G. Hoarau, M. Dickey-Collas, and W. J. Wolff. 2009. Genetic population structure of marine fish: mismatch between biological and fisheries management units. *Fish and Fisheries* **10**: 361-395.

Ridderinkhof, W., D. Le Bars, A. S. von der Heydt, and W. P. M. de Ruijter. 2013. *Dipoles of the South East Madagascar Current. Geophysical Research Letters* **40**: 558-562.

Ridgway, T., and E. M. Sampayo. 2007. Population genetic status of the Western Indian Ocean: What do we know? *Western Indian Ocean Journal of Marine Science* **4**: 1-10.

Saetre, R., and da Silva, A. J. 1984. The circulation of the Mozambique Channel. *Deep-Sea Research* **31**: 485-508.

Schott, F. A., and J. P. McCreary. 2001. The monsoon circulation of the Indian Ocean. *Progress in Oceanography* **51**: 1-123.

Schouten, M. W., W. P. M. de Ruijter, P. J. van Leeuwen, and H. Ridderinkhof. 2003. Eddies and variability in the Mozambique Channel. *Deep-Sea Research Part II: Topical Studies in Oceanography* **50**: 1987-2003.

Senevirathna, J. D. M., D. H. N. Munasinghe, and P. B. Mather. 2016. Assessment of genetic structure in wild populations of *Panulirus homarus* (Linnaeus, 1758) across the South Coast of Sri Lanka inferred from mitochondrial DNA sequences. *International Journal of Marine Science* **6**: 1-9.

Silva, I. C., N. Mesquita, J. Paula. 2010. Genetic and morphological differentiation of the mangrove crab *Perisesarma guttatum* (Brachyura: Sesarmidae) along an East African latitudinal gradient. *Biological Journal of the Linnean Society* **99**: 28-46.

Singh, S. P. Groeneveld, J. C. Al-Marzouqi, A., and Willows-Munro, S. 2017. A molecular phylogeny of the spiny lobster *Panulirus homarus* highlights a separately evolving lineage from the Southwest Indian Ocean. *Peer J* **5:e3356**. DOI 10.7717/peerj.3356.

Slate, J., T. Marshall, and J. Pemberton. 2000. A retrospective assessment of the accuracy of the paternity inference program Cervus. *Molecular Ecology* **9**: 801-808.

Spalding, M. D., H. E. Fox, G. R. Allen, N. Davidson, Z. A. Ferdaña, M. Finlayson, B. S. Halpern, M. A. Jorge, A. Lombana, S. A. Lourie, K. D. Martin, E. McManus, J. Molnar, C. A. Recchia, and J. Robertson. 2007. Marine ecoregions of the world: A bioregionalization of coastal and shelf areas. *BioScience* **57**: 573-583.

Steyn, E., and M. Schleyer. 2011. Movement patterns of the East Coast rock lobster *Panulirus homarus rubellus* on the coast of KwaZulu-Natal, South Africa. *New Zealand Journal of Marine and Freshwater Research* **45**: 85-101.

Swart, N. C., J. R. E. Lutjeharms, H. Ridderinkhof, and W. P. M. de Ruijter. 2010. Observed characteristics of Mozambique Channel eddies. *Journal of Geophysical Research: Oceans* **115**.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585-595.

Teske, P. R., H Winker, C. D. McQuaid, and N. P. Barker. 2009. A tropical/subtropical biogeographic disjunction in southeastern Africa separates two evolutionarily significant units of an estuarine prawn. *Marine Biology* **156**: 1265-1275.

Teske, P. R., I. Papadopoulos, K. L. Mmonwa, T. G. Matumba, C. D. McQuaid, N. P. Barker, and L. B. Beheregaray. 2011. Climate-driven genetic divergence of limpets with different life histories across a southeast African marine biogeographic disjunction: Different processes, same outcome. *Molecular Ecology* **20**: 5025-5041.

Teske, P. R., I. Papadopoulos, N. P. Barker, C. D. McQuaid, and L. B. Beheregaray. 2014. Mitonuclear discordance in genetic structure across the Atlantic/Indian Ocean biogeographical transition zone. *Journal of Biogeography* **41**: 392-401.

Tolley, K. A., J. C. Groeneveld, K. Gopal, and C. A. Matthee. 2005. Mitochondrial DNA panmixia in spiny lobster *Palinurus gilchristi* suggests a population expansion. *Marine*

Ecology Progress Series **297**: 225-231.

Truelove, N. K., K. Ley-Cooper, I. Segura-García, P. Briones-Fourzán, E. Lozano-Álvarez, B. F. Phillips, S. J. Box, and R. F. Preziosi. 2015. Genetic analysis reveals temporal population structure in Caribbean spiny lobster (*Panulirus argus*) within marine protected areas in Mexico. *Fisheries Research* **172**: 44-49.

van der Meeren, G. I., A. Chandrapavan, and T. Breithaupt. 2008. Sexual and aggressive interactions in a mixed species group of lobsters *Homarus gammarus* and *H. americanus*. *Aquatic Biology* **2**: 191-200.

van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**: 535-538.

Vogler, C., J. Benzie, P. H. Barber, M. V. Erdmann, M. Ambariyanto, C. Sheppard, K. Tenggardjaja, K. Gérard, and G. Wörheide. 2012. Phylogeography of the crown-of-thorns starfish in the Indian Ocean. *PLoS ONE* **7**. DOI: 10.1371/journal.pone.0043499.

von der Heyden, S., M. R. Lipinski, and C. A. Matthee. 2007a. Mitochondrial DNA analyses of the Cape hakes reveal an expanding, panmictic population for *Merluccius capensis* and population structuring for mature fish in *Merluccius paradoxus*. *Molecular Phylogenetics and Evolution* **42**: 517-527.

von der Heyden, S., J. Groeneveld, and C. Matthee. 2007b. Long current to nowhere? — Genetic connectivity of *Jasus tristani* populations in the southern Atlantic Ocean. *African Journal of Marine Science* **29**: 491-497.

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

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

Wilson, G. A., and B. Rannala. 2003. Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* **163**: 1177-91.

Wright, J. M., and P. Bentzen. 1994. Microsatellites : genetic markers for the future. *Reviews in Fish Biology and Fisheries* **4**: 384-388.

3.6 Supplementary information

Table S3.1 Multiplex PCR primer combinations for the microsatellites.

Multiplex	**Primers**
A	Orn 4, Orn 16, Orn 21
B	Orn 5, Orn 11
C	Orn 12 and Orn 32
D	Orn 17
E	G01, G32, G35, G36, G53
F	G03, G21, G42, G58
G	G22, G25, G27, G30

Table S3.2 A Microsatellite summary statistics by population and locus for the Dao et al (2013) primer set. N = No. of individuals, Na = no. of alleles, Ar = allelic richness, Ho = observed heterozygosity, He = expected heterozygosity, p(HWE) = probability of deviation from HWE, PHH = *P. h. homarus*, PHM = *P. h. megsasculptus*, PHR = *P. h. rubellus*, PIC = polymorphic information content.

Location/ Subspecies	**N**		**Orn 4**	**Orn 5**	**Orn 11**	**Orn 12**	**Orn 16**	**Orn 17**	**Orn 21**	**Orn 32**
All	271	**Na**	4.167	7.750	11.167	14.00	7.583	4.000	1.917	2.250
		Ar	4.087	7.685	10.548	13.62	7.338	4.600	1.964	3.015
		Ho	0.651	0.848	0.812	0.768	0.554	0.934	0.079	0.408
		He	0.660	0.833	0.884	0.925	0.761	0.619	0.077	0.348
		p(HWE)	0.962	**0**	0.058	**0**	**0**	**0**	1	**0**
OM	29	**Na**	5	7	13	18	7	6	2	3
		Ar	4.403	6.324	10.98	14.06	6.13	4.86	1.517	2.513
		Ho	0.538	0.964	0.857	0.483	0.517	0.964	0.034	0.276
		He	0.683	0.758	0.908	0.926	0.739	0.677	0.034	0.246
		p(HWE)	0.132	**0**	**0**	**0**	0.015	**0**	1	1
YEM	24	**Na**	4	8	9	14	7	5	2	4
		Ar	3.983	7.309	8.433	12.04	5.859	4.533	1.75	3.304
		Ho	0.917	0.909	0.773	0.565	0.542	0.957	0.05	0.652
		He	0.677	0.838	0.852	0.913	0.743	0.650	0.05	0.492
		p(HWE)	0.032	**0**	0.083	**0**	0.057	**0**	1	0.125
KEN	22	**Na**	4	4	13	13	7	6	2	3
		Ar	3.904	3.429	10.64	11.81	5.949	5.99	1.998	3

Location/ Subspecies	N		Orn 4	Orn 5	Orn 11	Orn 12	Orn 16	Orn 17	Orn 21	Orn 32
		Ho	0.714	0.524	0.864	1	0.409	1	0.227	0.909
		He	0.617	0.502	0.864	0.909	0.751	0.818	0.206	0.609
		p(HWE)	0.858	1	0.218	0.739	**0**	0.003	1	0.004
ZV	17	**Na**	4	5	9	14	6	6	1	2
		Ar	4	4.979	8.763	13.26	5.754	5.882	1	1.989
		Ho	0.625	0.765	0.941	0.941	0.529	0.941	NA	0.118
		He	0.601	0.706	0.886	0.914	0.745	0.789	NA	0.114
		p(HWE)	0.926	0.075	0.953	0.206	0.024	0.034	NA	1
CH	19	**Na**	5	9	12	16	7	2	1	1
		Ar	4.81	8.329	10.49	14.89	6.736	2.000	1.000	1.000
		Ho	0.833	0.895	0.789	0.895	0.632	0.947	NA	NA
		He	0.711	0.862	0.851	0.940	0.718	0.512	NA	NA
		p(HWE)	0.829	**0**	0.487	0.454	0.594	0.0003	NA	NA
XX	22	**Na**	4	9	9	14	7	4	2	2
		Ar	3.904	8.017	7.938	12.15	6.543	3.904	1.904	2
		Ho	0.636	0.955	0.727	0.864	0.682	0.952	0.091	0.364
		He	0.630	0.849	0.817	0.909	0.744	0.617	0.089	0.304
		p(HWE)	0.719	0.002	0.494	0.941	0.193	**0**	1	1
FD	29	**Na**	5	9	13	14	9	3	3	3
		Ar	4.071	7.512	9.953	11.71	7.601	2.577	2.442	2.959

Location/ Subspecies	N		Orn 4	Orn 5	Orn 11	Orn 12	Orn 16	Orn 17	Orn 21	Orn 32
		Ho	0.607	0.893	0.759	0.857	0.607	0.885	0.143	0.679
		He	0.662	0.848	0.857	0.894	0.796	0.519	0.137	0.495
		p(HWE)	0.103	**0**	0.003	**0**	**0**	**0**	1	0.058
BR	19	**Na**	5	8	11	14	6	2	1	2
		Ar	4.954	7.783	10.59	13.12	5.539	2	1	1.999
		Ho	0.684	0.944	0.944	0.947	0.474	0.944	NA	0.211
		He	0.707	0.857	0.884	0.920	0.646	0.513	NA	0.193
		p(HWE)	0.829	**0**	0.653	0.037	0.070	0.001	NA	1
TM	19	**Na**	4	9	9	10	9	3	1	1
		Ar	4	8.629	8.866	9.956	8.105	2.96	1.000	1.000
		Ho	0.467	0.842	0.895	0.588	0.632	0.737	NA	NA
		He	0.494	0.838	0.872	0.907	0.677	0.512	NA	NA
		p(HWE)	0.098	0.009	0.006	**0**	0.307	0.076	NA	NA
SB	30	**Na**	3	5	11	15	9	4	2	2
		Ar	2.999	4.797	8.619	12.10	7.087	3	1.944	2
		Ho	0.433	0.586	0.586	0.793	0.367	1	0.133	0.92
		He	0.538	0.669	0.836	0.918	0.632	0.541	0.127	0.507
		p(HWE)	0.279	0.487	**0**	**0**	**0**	**0**	1	**0**
PSJ	20	**Na**	3	9	13	13	9	4	3	2
		Ar	3	8.223	11.72	12.74	8.525	3.831	2.5	2

Location/ Subspecies	N		Orn 4	Orn 5	Orn 11	Orn 12	Orn 16	Orn 17	Orn 21	Orn 32
		Ho	0.722	0.950	0.850	0.563	0.632	0.889	0.1	0.222
		He	0.667	0.829	0.919	0.909	0.844	0.589	0.099	0.203
		p(HWE)	0.933	0.078	0.006	**0**	0.097	0.021	1	1
MB	21	**Na**	4	11	12	13	8	3	3	2
		Ar	3.923	9.759	10.68	12.19	7.537	2.833	2.429	1.993
		Ho	0.714	1	0.905	0.789	0.714	0.944	0.095	0.158
		He	0.624	0.876	0.875	0.915	0.819	0.538	0.094	0.149
		p(HWE)	0.956	0.007	0.841	0.135	0.055	0.001	1	1
PHH	43	**Na**	4	9	14	19	9	7	2	3
		Ar	4	9	13.93	19	8.93	7	2	3
		Ho	0.714	0.690	0.837	0.952	0.512	1	0.116	0.558
		He	0.662	0.677	0.888	0.907	0.740	0.796	0.111	0.447
		p(HWE)	0.758	**0**	0.093	0.144	0.004	**0**	1	0.150
PHM	53	**Na**	5	8	13	19	9	6	3	5
		Ar	4.98	8	12.96	18.77	8.769	5.96	3	4.827
		Ho	0.72	0.940	0.82	0.519	0.528	0.961	0.041	0.442
		He	0.681	0.806	0.883	0.922	0.738	0.663	0.041	0.371
		p(HWE)	0.480	**0**	0.008	0.011	**0**	**0**	1	0.141
PHR	175	**Na**	4.000	7.778	10.56	12.67	7.333	3.111	1.889	1.778
		Ar	5	11.95	15.94	20.96	9.942	5.988	5.81	3

Location/ Subspecies	N		Orn 4	Orn 5	Orn 11	Orn 12	Orn 16	Orn 17	Orn 21	Orn 32
		Ho	0.614	0.860	0.803	0.800	0.572	0.909	0.080	0.358
		He	0.624	0.829	0.868	0.912	0.753	0.535	0.078	0.310
		p(HWE)	0.707	**0**	0.052	**0**	**0**	**0**	1	0.059
PIC			0.605	0.809	0.872	0.917	0.730	0.550	0.072	0.314

Table S3.2 B Microsatellite summary statistics by population and locus for the Delghandi et al (2015) primer set. N = No. of individuals, Na = no. of alleles, Ar = allelic richness, Ho = observed heterozygosity, He = expected heterozygosity, p(HWE) = probability of deviation from HWE, PHH = *P. h. homarus*, PHM = *P. h. megsasculptus*, PHR = *P. h. rubellus*, PIC = polymorphic information content.

Location/ Subspecies	**N**		**G01**	**G03**	**G21**	**G22**	**G25**	**G27**	**G30**	**G32**	**G35**	**G36**	**G42**	**G53**	**G58**
All	271	**Na**	5.333	4.000	4.167	5.833	8.667	2.250	4.500	3.583	10.667	9.083	2.083	7.917	1.917
		Ar	5.187	4.124	4.525	5.723	8.227	2.514	4.462	3.881	10.37	9.377	2.161	7.744	2
		Ho	0.668	0.556	0.309	0.742	0.733	0.157	0.490	0.194	0.874	0.950	0.989	0.735	0.299
		He	0.712	0.516	0.657	0.669	0.849	0.196	0.538	0.551	0.871	0.857	0.506	0.806	0.369
		p(HWE)	0.025	**0**	**0**	**0**	0.118	**0**	0.283	**0**	0.379	**0**	**0**	**0**	0.004
OM	29	**Na**	5	6	5	6	9	3	5	4	14	16	3	12	2
		Ar	4.665	5.021	4.542	5.687	8.593	2.906	4.772	3.495	10.59	12.99	2.894	9.12	1.517
		Ho	0.689	0.689	0.655	0.821	1	0.357	0.607	0.379	0.828	1	1	0.828	0.034
		He	0.719	0.617	0.619	0.712	0.874	0.382	0.570	0.426	0.883	0.919	0.556	0.857	0.034
		p(HWE)	0.511	0.385	0.208	0.241	0.929	0.017	0.568	0.016	0.062	0.021	**0**	0.443	1
YEM	24	**Na**	5	4	4	7	9	2	6	4	12	13	2	11	1
		Ar	4.304	3.979	3.625	6.072	8.378	1.989	4.979	3.884	10.65	11.73	2	9.798	1
		Ho	0.652	0.75	0.375	0.565	0.609	0	0.458	0.391	1	0.636	1	0.909	NA
		He	0.647	0.602	0.576	0.673	0.816	0.162	0.569	0.658	0.884	0.891	0.511	0.879	NA
		p(HWE)	0.699	0.339	0.006	0.249	0.183	0.001	0.646	0.009	0.985	**0**	**0**	0.546	NA
KEN	22	**Na**	4	2	4	4	10	2	4	5	7	5	2	7	2
		Ar	3.364	2	3.902	3.586	8.859	1.682	3.694	4.896	6.548	4.965	2	6.351	1.999

Location/ Subspecies	N		G01	G03	G21	G22	G25	G27	G30	G32	G35	G36	G42	G53	G58
		Ho	0.5	0.364	0.182	0.864	0.818	0.045	0.190	0.364	0.727	0.955	1	0.762	0.238
		He	0.470	0.304	0.625	0.555	0.818	0.045	0.372	0.719	0.696	0.737	0.512	0.777	0.215
		p(HWE)	0.664	1	**0**	0.001	0.406	1	0.011	**0**	0.776	0.026	**0**	0.242	1
ZV	17	**Na**	5	4	4	4	7	2	3	4	8	7	2	5	2
		Ar	4.872	3.882	3.882	3.765	6.743	2	2.765	3.999	7.529	6.871	2.000	4.989	2.000
		Ho	0.529	0.588	0.118	1	0.529	0.529	0.118	0.235	0.647	1	1	0.647	0.235
		He	0.611	0.506	0.620	0.572	0.750	0.401	0.116	0.672	0.693	0.834	0.515	0.739	0.214
		p(HWE)	0.653	1	**0**	**0**	0.031	0.288	1	0.0001	0.161	0.114	**0**	0.392	1
CH	19	**Na**	6	3	4	7	8	2	3	3	10	8	2	5	2
		Ar	5.92	3.000	3.833	6.619	7.497	1.976	3	2.999	9.742	7.86	2.000	4.833	2.000
		Ho	0.737	0.278	0.111	0.611	0.611	0.111	0.556	0.053	0.824	1	1	0.833	0.556
		He	0.787	0.417	0.576	0.705	0.779	0.108	0.603	0.605	0.893	0.852	0.514	0.779	0.508
		p(HWE)	0.812	0.01	**0**	0.028	0.296	1	0.340	**0**	0.373	0.088	**0**	0.945	1
XX	22	**Na**	5	4	5	7	11	3	7	4	11	9	2	6	2
		Ar	4.938	3.902	4.364	6.171	9.305	2.904	6.238	3.68	9.478	8.169	2	5.336	2.000
		Ho	0.591	0.500	0.136	0.864	0.591	0.364	0.591	0.227	0.864	1	0.955	0.773	0.318
		He	0.648	0.481	0.651	0.719	0.859	0.376	0.626	0.560	0.808	0.848	0.511	0.729	0.426
		p(HWE)	0.439	0.513	**0**	0.007	0.011	0.039	0.520	0.0005	0.072	**0**	**0**	0.919	0.318
FD	29	**Na**	6	4	5	7	9	3	5	4	14	10	2	9	2
		Ar	5.577	3.948	4.772	5.472	8.392	2.885	4.325	3.439	11.30	8.535	2	7.532	2

Location/ Subspecies	N		G01	G03	G21	G22	G25	G27	G30	G32	G35	G36	G42	G53	G58
		Ho	0.679	0.586	0.357	0.778	0.852	0.111	0.607	0.214	0.926	1	1	0.741	0.241
		He	0.765	0.554	0.658	0.674	0.855	0.238	0.684	0.342	0.904	0.796	0.509	0.819	0.390
		p(HWE)	0.113	0.170	0.001	**0**	0.423	0.002	0.191	0.034	0.440	0.124	**0**	0.633	0.055
BR	19	**Na**	5	4	3	5	9	2	4	3	10	9	2	8	2
		Ar	4.749	3.973	3	4.667	8.637	1.997	3.81	2.789	9.59	8.738	2	7.151	2
		Ho	0.421	0.667	0.188	0.667	0.889	0.167	0.333	0.105	1	1	1	0.684	0.667
		He	0.669	0.522	0.522	0.662	0.865	0.157	0.383	0.459	0.876	0.827	0.514	0.767	0.508
		p(HWE)	0.025	0.647	0.0005	0.009	0.854	1	0.655	**0**	0.941	0.049	0.0005	0.070	0.334
TM	19	**Na**	5	4	4	5	7	2	4	2	10	6	2	8	2
		Ar	4.954	3.947	3.789	4.789	6.742	1.96	3.959	2	9.458	5.539	2	7.702	2
		Ho	0.895	0.421	0.368	0.684	0.526	0	0.684	0	0.947	1	1	0.474	0.421
		He	0.724	0.371	0.644	0.650	0.812	0.102	0.558	0.273	0.883	0.728	0.513	0.811	0.478
		p(HWE)	0.587	1	0.009	0.233	0.001	0.025	0.934	**0**	0.198	0.025	**0**	**0**	0.643
SB	30	**Na**	7	4	5	8	9	2	4	3	12	9	2	9	2
		Ar	5.88	3.535	4.892	6.085	7.436	1.517	3.872	2.517	10.15	7.615	2	7.451	2
		Ho	0.7	0.571	0.357	0.759	0.655	0.034	0.517	0.034	0.867	1	0.964	0.759	0.321
		He	0.771	0.537	0.718	0.642	0.842	0.034	0.556	0.416	0.873	0.793	0.508	0.797	0.431
		p(HWE)	0.683	0.066	**0**	0.101	0.176	1	0.628	**0**	0.929	0.077	**0**	0.067	0.229
PSJ	20	**Na**	5	5	3	4	7	2	4	3	10	9	2	7	2
		Ar	4.783	4.635	3	3.994	6.996	1.789	3.748	2.75	9.733	8.615	2	6.873	2

Location/ Subspecies	N		G01	G03	G21	G22	G25	G27	G30	G32	G35	G36	G42	G53	G58
		Ho	0.789	0.55	0.389	0.389	0.875	0.053	0.600	0.05	1	0.882	1	0.500	0.400
		He	0.739	0.486	0.608	0.579	0.855	0.053	0.506	0.537	0.891	0.752	0.514	0.782	0.385
		p(HWE)	0.114	0.372	0.012	0.032	0.439	1	0.882	**0**	1	0.963	0.0001	0.026	1
MB	21	**Na**	6	4	4	6	9	2	5	4	10	8	2	8	2
		Ar	5.766	3.998	3.831	5.539	8.536	1.993	4.539	3.638	9.251	6.989	2	7.124	2
		Ho	0.809	0.600	0.222	0.842	0.737	0.158	0.474	0.190	0.857	0.905	0.947	0.762	0.421
		He	0.700	0.622	0.537	0.706	0.859	0.149	0.496	0.475	0.877	0.746	0.512	0.819	0.478
		p(HWE)	0.799	0.231	**0**	0.091	0.162	1	0.098	0.001	0.608	0.266	0.0005	0.205	0.654
PHH	43	**Na**	5	4	4	5	11	2	5	5	9	9	2	8	2
		Ar	5	3.977	4	4.999	10.95	2	5	5	8.953	8.93	2.000	8.000	2.000
		Ho	0.488	0.442	0.139	0.884	0.628	0.209	0.214	0.372	0.628	0.977	0.977	0.762	0.190
		He	0.553	0.386	0.596	0.582	0.758	0.189	0.336	0.721	0.654	0.776	0.506	0.740	0.248
		p(HWE)	0.507	0.806	**0**	**0**	0.115	1	0.017	**0**	0.192	**0**	**0**	0.376	0.178
PHM	53	**Na**	5	6	5	7	9	3	7	4	16	16	3	12	2
		Ar	5	5.849	4.995	6.961	9	3	6.939	4	15.84	15.96	3	11.96	1.925
		Ho	0.673	0.717	0.528	0.706	0.824	0.196	0.538	0.385	0.902	0.843	1	0.863	0.019
		He	0.688	0.608	0.595	0.702	0.856	0.294	0.568	0.545	0.881	0.908	0.532	0.869	0.019
		p(HWE)	0.296	0.017	0.031	0.325	0.632	0.001	0.867	**0**	0.685	**0**	**0**	0.219	1
PHR	175	**Na**	5.444	4.000	4.000	5.778	8.333	2.222	4.222	2.667	10.22	8.000	2.000	7.222	2.000
		Ar	6.942	4.998	5	10.95	12.98	3	7.927	4.942	17.94	11.96	2	11.98	2

Location/ Subspecies	N		G01	G03	G21	G22	G25	G27	G30	G32	G35	G36	G42	G53	G58
		Ho	0.711	0.535	0.282	0.717	0.732	0.132	0.544	0.092	0.929	0.976	0.988	0.689	0.414
		He	0.735	0.514	0.641	0.668	0.855	0.167	0.564	0.446	0.877	0.788	0.501	0.793	0.452
		p(HWE)	0.086	0.001	**0**	**0**	0.097	**0**	0.406	**0**	0.675	**0**	**0**	**0**	0.336
PIC			0.664	0.484	0.589	0.629	0.829	0.185	0.500	0.502	0.857	0.840	0.386	0.782	0.302

Table S3.3 Pairwise Φ_{ST} values for the COI *P. homarus* dataset. Values in bold indicate significance.

	1	2	3	4	5	6	7	8	9	10	11	12
1. OM	*											
2. YEM	**-0.004**	*										
3. KEN	**0.161**	**0.199**	*									
4. ZV	**0.313**	**0.252**	**0.230**	*								
5. CH	**0.769**	**0.754**	**0.749**	**0.352**	*							
6. XX	**0.677**	**0.621**	**0.626**	**0.193**	-0.037	*						
7. FD	**0.761**	**0.739**	**0.734**	**0.335**	-0.023	-0.027	*					
8. BR	**0.761**	**0.736**	**0.732**	**0.314**	-0.054	-0.037	-0.044	*				
9. TM	**0.762**	**0.736**	**0.732**	**0.335**	0.005	-0.0007	0.014	0.006	*			
10. SB	**0.762**	**0.751**	**0.748**	**0.341**	0.027	-0.001	0.051	0.003	**0.113**	*		
11. PSJ	**0.769**	**0.753**	**0.748**	**0.345**	-0.049	-0.044	-0.035	-0.054	-0.005	0.005	*	
12. MB	**0.684**	**0.638**	**0.637**	**0.194**	0.032	-0.031	-0.003	0.018	0.036	**0.067**	0.038	*

Table S3.4 Pairwise Φ_{ST} values for the CR *P. homarus* dataset. Values in bold indicate significance.

CR Fst	1	2	3	4	5	6	7	8	9	10	11	12
1. OM	*											
2. YEM	-0.023	*										
3. KEN	0.147	0.190	*									
4. ZV	0.078	-0.038	0.038	*								
5. CH	**0.773**	**0.762**	**0.763**	**0.463**	*							
6. XX	**0.604**	**0.555**	**0.612**	**0.280**	-0.034	*						
7.FD	**0.807**	**0.805**	**0.789**	**0.469**	-0.009	-0.005	*					
8.BR	**0.778**	**0.764**	**0.765**	**0.419**	-0.062	-0.057	-0.016	*				
9. TM	**0.766**	0.736	**0.756**	0.375	0.002	-0.084	0.014	-0.061	*			
10. SB	**0.769**	**0.759**	**0.762**	**0.464**	**0.099**	0.020	**0.151**	0.048	0.031	*		
11. PSJ	**0.748**	**0.731**	**0.739**	**0.437**	-0.037	-0.054	0.002	-0.035	-0.032	0.021	*	
12. MB	**0.658**	**0.629**	**0.654**	**0.350**	-0.025	-0.067	-0.026	-0.042	-0.057	**0.059**	-0.025	*

Table S3.5 Pairwise F_{ST} values for the microsatellite *P. homarus* dataset. Values in bold indicate significance.

	1	2	3	4	5	6	7	8	9	10	11	12
1. OM	*											
2. YEM	**0.008**	*										
3. KEN	**0.082**	**0.072**	*									
4. ZV	**0.056**	**0.064**	**0.016**	*								
5. CH	**0.045**	**0.050**	**0.090**	**0.066**	*							
6. XX	**0.033**	**0.037**	**0.051**	**0.015**	**0.019**	*						
7. FD	**0.031**	**0.036**	**0.077**	**0.055**	**0.029**	0.004	*					
8. BR	**0.046**	**0.060**	**0.093**	**0.052**	**0.015**	0.005	**0.010**	*				
9. TM	**0.055**	**0.059**	**0.094**	**0.052**	**0.023**	**0.010**	**0.020**	**0.011**	*			
10. SB	**0.064**	**0.060**	**0.065**	**0.055**	**0.037**	**0.016**	**0.015**	**0.025**	**0.023**	*		
11. PSJ	**0.035**	**0.038**	**0.075**	**0.051**	**0.014**	0.007	**0.012**	0.006	**0.015**	**0.025**	*	
12. MB	**0.038**	**0.044**	**0.075**	**0.039**	**0.024**	-0.007	0.002	0.002	0.004	**0.020**	**0.008**	*

Table S3.6 *P. h. rubellus* pairwise Φ_{ST} for COI. Values in bold are significant.

	1	2	3	4	5	6	7	8	9
1.ZV	*								
2. CH	0.103	*							
3. XX	0.063	-0.060	*						
4. FD	0.099	-0.023	-0.038	*					
5. BR	0.053	-0.054	-0.052	-0.044	*				
6. TM	**0.115**	0.008	0.010	0.016	0.010	*			
7. SB	0.090	0.027	-0.016	0.051	0.003	**0.119**	*		
8. PSJ	0.101	-0.049	-0.061	-0.035	-0.054	-0.00007	0.005	*	
9. MB	0.039	-0.004	-0.017	-0.037	-0.008	0.019	0.044	0.006	*

Table S3.7 *P. h. rubellus* pairwise Φ_{ST} for CR.

	1	2	3	4	5	6	7	8	9
1. ZV	*								
2. CH	0.270	*							
3. XX	0.216	-0.045	*						
4. FD	0.281	-0.009	0.032	*					
5. BR	0.204	-0.062	-0.062	-0.016	*				
6. TM	0.134	0.002	-0.074	0.014	-0.061	*			
7. SB	**0.288**	**0.099**	0.0007	**0.151**	0.048	0.031	*		
8. PSJ	0.234	-0.037	-0.071	0.002	-0.035	-0.032	0.021	*	
9. MB	0.304	-0.023	-0.022	-0.013	-0.021	-0.011	**0.099**	-0.017	*

Table S3.8 *P. h. rubellus* pairwise F_{ST} for microsatellites.

	1	2	3	4	5	6	7	8	9
1. ZV	*								
2. CH	0.028	*							
3. XX	-0.006	0.013	*						
4. FD	0.021	**0.018**	0.003	*					
5. BR	0.011	0.009	0.005	**0.016**	*				
6. TM	0.017	**0.019**	0.012	0.008	0.009	*			
7. SB	0.013	**0.023**	0.008	**0.012**	**0.019**	0.005	*		
8. PSJ	0.017	0.009	**0.014**	**0.017**	**0.014**	**0.016**	**0.024**	*	
9. MB	0.017	**0.017**	-0.004	0.001	0.011	0.008	**0.012**	**0.011**	*

Table S3.9 AMOVA results for *P. homarus* subspecies, grouped by country, showing fixation indices and significance values.

Level	Fixation Indices	COI	CR	Microsatellites
Among groups	F_{CT}	0.52, 0.0004	0.38, 0.004	0.02, 0.0009
APWG	F_{SC}	0.09, 0.004	0.15, 0.003	0.02, 0.000
Within populations	F_{ST}	0.56. 0.000	0.47, 0.000	0.04, 0.000

Table S3.10 AMOVA results for *P. h. rubellus*, grouped by country, showing fixation indices and significance values.

Level	Fixation Indices	COI	CR	Microsatellites
Among groups	F_{CT}	-0.02, 0.76	-0.02, 0.35	-0.0008, 0.04
APWG	F_{SC}	0.03, 0.11	0.09, 0.0008	0.01, 0.000
Within populations	F_{ST}	0.009, 0.16	0.07, 0.0003	0.01, 0.000

Chapter Four: Linking population genetic, environmental and geospatial variation using a seascape genetics approach

Abstract

Linking population genetic, environmental and spatial variation to answer the question of what drives genetic variation in species has been widely applied in terrestrial landscapes, but is understudied in the marine environment. A seascape genetics approach was used to test the effects of physical oceanography, spatial and environmental variables on the genetic variability of *P. homarus* in the Western Indian Ocean. A simple, coupled-biophysical ocean circulation model was used to simulate larval dispersal with a Lagrangian particle tracking tool for the years 2009 and 2010, when adult lobsters genotyped in this study would have been drifting larvae. The results of the dispersal model were used to test the hypotheses postulated in Chapter 3: that the prevailing ocean currents in the Western Indian Ocean are responsible for the incipient speciation between *P. h. homarus* and *P. h. megasculptus*; for genetic differentiation between *P. h. homarus* and *P. h. rubellus*; and for genetic connectivity among *P. h. rubellus* populations. The dispersal model was able to partially reproduce the observed patterns of genetic diversity. Spatial genetic clustering based on microsatellite data confirmed the existence of three distinct genetic clusters corresponding to the subspecies, and pinpointed the locations of spatial genetic discontinuities. The distance-based redundancy analyses (dbRDA) indicated that latitude and minimum sea surface temperature were significantly associated with the genetic differentiation. The GEnetic STructure inference based on genetic and Environmental data (GESTE) analysis also showed how environmental variables, such as sea surface temperature, chlorophyll-a and turbidity could play subtle, but important roles in the genetic differentiation of organisms in the marine environment.

4.1 Introduction

Chapters 2 and 3 established that *P. h. rubellus* is a separately evolving lineage in the southwestern part of the Western Indian Ocean, and that *P. h. homarus* and *P. h. megasculptus* may also be separately evolving lineages which have diverged much more recently, based on microsatellite DNA data. In this chapter, environmental, ecological and oceanographic information is used, in conjunction with microsatellite data, to infer how the patterns of genetic variability observed among the three subspecies, and the connectivity of *P. h. rubellus* populations, may have originated.

Despite the predominance and importance of aquatic environments to Earth, studies focusing on 'waterscape genetics' – which encompass rivers, lakes and oceans, are underrepresented in the scientific literature, with only 6% of these studies conducted in marine systems (Storfer et al. 2010, Selkoe et al. 2016). Even fewer seascape genetics studies have been conducted in the Western Indian Ocean, which is acknowledged as a biogeographic hotspot (Hoareau et al. 2013, Postaire et al. 2014). In contrast to terrestrial environments, oceans have few visible barriers to dispersal that may act to limit genetic exchange between populations (Carr et al. 2003, Groeneveld et al. 2012).

Gene flow can be hindered by geographic distance, or isolation by distance, when genes are exchanged mainly among neighbouring populations. As a result, genetic differentiation frequently increases with geographic distance (Wright 1943, Kimura and Weiss 1964, Rousset 1997). Vicariance events can also impede gene flow, leading to genetic discontinuities (Wiley 1988). In the marine realm, this is often caused by sea-level fluctuations occurring over evolutionary time scales, which give rise to range fragmentations (Benzie 1999, McCartney et al. 2000, Ludt and Rocha 2015).

Ocean features that can potentially shape genetic connectivity or diversity in marine organisms include the positions of landmasses and topology of coastlines (Riginos and Liggins 2013). Upon interaction with the continental shelf or coastal landscape features, the flow of ocean currents may morph into other hydrodynamic features, such as eddies, fronts or upwelling systems (Mann and Lazier 2006). These water movements may then act to concentrate larvae in a specific area, resulting in spatially explicit patterns of recruitment (Mace and Morgan 2006, Morgan et al. 2011). Ocean currents can play an important, but its effects on population genetic structure are not easily observed or quantified. As the main

force driving planktonic larval dispersal, ocean circulation can actively promote genetic exchange, or constitute a barrier to gene flow, even when populations are geographically close to each other (Palumbi 1994, Ayre and Dufty 1994, Teske et al. 2008).

Habitat diversity can pose a barrier to gene flow, irrespective of the distance between populations (McGaughran et al. 2014). Environmental isolation, associated with tolerance of factors such as sea surface temperature (SST), chlorophyll-a concentration, turbidity or salinity, can then give rise to genetic differentiation and structured populations (Mendez et al. 2010, Amaral et al. 2012, Riccioni et al. 2013).

Panulirus homarus display both benthic and pelagic life history phases, which determine movement patterns and habitats. For example, in the planktonic larval stage, which lasts for 4–6 months (Berry 1971a) they are capable of vertical movement in the water column, but rely on ocean currents for horizontal movement (Booth 2002, George 2005). Ocean currents, gyres and eddies therefore have profound effects on larval dispersal, acting to either retain or return them to a particular site, or to facilitate wide dispersal. On the other hand, benthic juveniles and adult *P. homarus* inhabit the shallow subtidal, and undertake only short movements on the seafloor, for feeding, shelter or reproduction (Steyn and Schleyer 2011). Gene flow among distant populations is therefore reliant on dispersal during the drifting larval stage.

Planktonic larval dispersal is influenced by the complex ocean current system in the Western Indian Ocean (see map in Chapter 2 for a depiction of the main current systems), as well as larval behaviour (Butler et al. 2011). In Chapter 3, the observed genetic structuring between *P. homarus* populations was attributed to the prevailing ocean currents in the region. Circulation along the coast of Somalia and Oman is influenced by the seasonally reversing Somali Current, which is influenced by the southwest monsoon during June to September (flows northwards), and the northeast monsoon during December to February (flows southwards) (Schott and McCreary 2001). The reversing current system potentially acts to retain *P. h. megasculptus* larvae in Arabian Sea waters, thus promoting incipient speciation of *P. h. homarus* and *P. h. megasculptus*.

The South Equatorial Current (SEC) flows westward in equatorial waters of the Western Indian Ocean, and splits into northern and southern filaments where it collides with the African coast in Tanzania and northern Mozambique. The resultant East African Coastal Current (EACC) flows northwards, along Tanzanian and Kenya, before feeding into the

Somali Current, or retroflecting towards the east to form an Equatorial return current. The southern branch enters the Mozambique Channel, forming a series of eddies which propagate southwards through the Channel (Otwoma and Kochzius 2016, Halo et al. 2017). Near the southern end of the Channel, these waters are augmented by eddies originating from the East Madagascar Current, after crossing the southern part of the Channel (de Ruijter et al. 2004, Ridderinkhof et al. 2013). This confluence, near the origin of the upper Agulhas Current, appears to form a barrier to larval dispersal, with *P. h. homarus* distributed to its north, and *P. h. rubellus* to its south. The distribution of *P. h. rubellus* benthic stages is restricted to the coasts of southern Mozambique, southeastern Madagascar and eastern South Africa. Connectivity and gene flow between *P. h. rubellus* populations, along the African coast and across the Mozambique Channel, is therefore facilitated by water movements in this area.

Coupled biophysical ocean circulation models (CBOCM's) bring together physical oceanographic models with biological species and life history information, in order to realistically simulate larval dispersal (Werner et al. 2007, Liggins et al. 2013). These models utilise particle tracking, which is defined as the movement of individual particles within a fluid, and uses a particle-specific Lagrangian modelling framework (McDonald et al. 2006). Lagrangian particle tracking models therefore simulate particle movements spatially and temporally using the flow of the ocean (Lange and van Sebille 2017). Linking information from these models with ecological information and population genetics can assist in understanding the processes that drive population differentiation or connectivity in the marine environment (Werner et al. 2007). This interdisciplinary approach can aid in ensuring more robust and informative spatially explicit gene flow predictions (Liggins et al. 2013).

A major caveat of simulating larval dispersal with CBOCM's is that they are very rarely cross-validated with empirical data, such as data from population genetics studies. Therefore, in this study, biological, habitat and hydrodynamic ocean modelling data were used to explain the genetic structure observed among the subspecies of *P. homarus*. Specifically, the aims of this chapter were to (i) generate larval dispersal simulations using coupled physical-biological ocean circulation models, and compare their outputs to genetic structure observed from the microsatellite analysis (see Chapter 3); and (ii) investigate whether genetic structure correlates with selected environmental variables.

4.2 Materials and methods

4.2.1 *P. homarus* larval dispersal simulations at subspecies level

To investigate larval dispersal dynamics of *P. homarus*, a particle tracking tool called Parcels v.0.9 (Probably A Really Computationally Efficient Lagrangian Simulator) (Lange and van Sebille 2017) was used to perform the simulations. Parcels uses velocity data to move the synthetic particles and calculates their Lagrangian trajectories with the equation (Lange and van Sebille 2017):

$$X(t + \Delta t) = X(t) + \int_{t}^{t+\Delta t} v(x, \tau) d\tau$$

where X is the three-dimensional position of a particle and v(x,t) is the three-dimensional velocity field at that location from an ocean general circulation model.

Ocean velocity data for the Parcels tracking tool was obtained from satellite derived altimetry data (GlobCurrent Project; http://www.globcurrent.org/) which provides surface ocean geostrophic current estimates for the world's ocean (Danielson et al. 2017). These estimates were obtained by merging data from multiple altimeter missions with gravity models and *in situ* data, to derive a gridded product of absolute dynamic topography (the sum of the sea-level anomalies and mean dynamic topography).

The u- and v-component geostrophic velocities are defined by the following equations (Saraceno et al. 2008):

$$\upsilon_{geo} = -\left(\frac{g}{f}\right) . \frac{\delta\eta_{y[0]} - \delta\eta_{y[1]}}{\delta y}$$

$$\nu_{geo} = \left(\frac{g}{f}\right) . \frac{\delta\eta_{x[0]} - \delta\eta_{x[1]}}{\delta x}$$

where υ represents the zonal component, ν the meridional component, $\delta\eta$ is the variation in the sea surface height, $\delta x/\delta y$ the distance between grid points, g the gravitational acceleration and f the Coriolis force.

These products are combined with the Ekman currents which are estimated using Argo floats and drifter data in conjunction with wind stress estimates from the European Centre for Medium-Range Weather Forecasts (ECMWF). At depth z (0 m and 15 m), the Ekman response ($\vec{v}_{ek}$) to the wind stress ($\vec{\tau}$) is expressed by Rio et al. (2014):

$$\vec{v}_{ek}(z) = \beta(z).\vec{\tau}.e^{i\theta(z)}$$

The combined geostrophic current components constitute the GlobCurrent ocean current data. This data provides 3-hourly, global ocean currents at the surface and at 15 m depth with a 0.25° spatial resolution, covering the period 1993 – 2015 (Danielson et al. 2017).

The reproductive season of *P. homarus* extends for 8 months, with egg-bearing females first appearing in August, proportionally increasing up to January, and declining in February and March (Berry 1971a). Hatching of eggs and the release of larvae into the water column therefore peaks in January to March. The pelagic larval duration (PLD) has been estimated as 4 – 6 months (Berry 1971a). Hence larvae released in January to March would settle on the seafloor as the post-larval puerulus stage in April to July (Berry 1974a). Based on the reproductive biology of *P. homarus*, simple exploratory simulations were performed by deploying 100 000 synthetic particles, starting at 12 am on the 1st of January in the years 2009 and 2010 (years in which adult lobsters sampled for the genetic component of this study in 2013 and 2014 would presumably have been drifting larvae). The simulations were carried out for 4 months (120 days) and the final locations of larvae were plotted. To quantify the number of simulated larvae that reached the coast, a 0.25 by 0.25 degree grid was placed around each site and the particles within the grid counted. This CBOCM is based on on-going work by Hart-Davis et al. (In review).

4.2.2 Genetic data

The microsatellite dataset amplified and genotyped in Chapter 3 was used for data analysis in this chapter. The dataset consisted of 271 *P. homarus* lobsters genotyped at 21 microsatellite loci. Of these, 43 were *P. h. homarus* (Kenya = 22, Mozambique = 20, South Africa = 1), 53 were *P. h. megasculptus* (Oman = 29, Yemen = 24), and 175 were *P. h. rubellus* (Mozambique = 38, Madagascar = 29, South Africa = 108).

The R-package diveRsity (Keenan et al. 2013) implemented using R (R Development Core Team 2011), was used to produce population genetic connectivity plots based on pairwise F_{ST} estimates. The difPlot function was used to plot the diversity partitioning and F_{ST} in order to compare population genetic connectivity to larval dispersal connectivity.

4.2.3 Seascape genetics: spatial clustering

The combined use of genetic data, geospatial data and statistics is characteristic in seascape genetics studies. Two spatially explicit clustering programs, the Geneland v.4.0.3 R-package (Guillot et al. 2005) and TESS v.2.3.1 (Chen et al. 2007), were used to refine cluster predictions from Chapter 3, and to infer the role of seascape features in population genetic structure by incorporating multilocus microsatellite genotype data and geospatial information. Both methods implement geographical co-ordinates as priors, enabling the detection of subtle population structure, and also to infer the locations of genetic discontinuities or genetic transition zones (Guillot et al. 2005). The Geneland analysis consisted of 10 independent runs of 1 000 000 MCMC iterations, sampling every 100 generations. The number of predefined genetic clusters (K) ranged from 1 – 12. The spatial model was employed, with and without accounting for null alleles. The correlated allele frequencies model was chosen to detect subtle genetic structure, because the uncorrelated model does not detect subtle structure in populations with low genetic differentiation (Guillot 2008).

TESS was run using the admixture BYM model with 50 000 sweeps and a burnin period of 10 000, with K ranging from 2 – 12. The optimal value of K was then chosen by plotting the Deviance Information Criterion (DIC) against values of K, and then choosing the K with the smallest DIC value. This value of K was then run for 100 iterations and summarized using the program CLUMPP (Jakobsson and Rosenberg 2007). The results were visualized using R-code adapted from (François 2016)

The program GenAlEx v.6.5.0.3 (Peakall and Smouse 2006) was used to perform spatial autocorrelation analysis in order to identify the spatial scale of genetic structure. Pairwise geographic and squared genetic distance matrices were used to calculate the spatial autocorrelation coefficient *r* among the 271 *P. homarus* individuals within 20 even distance class bins encompassing the entire sampling range. Even sample-classes were selected because the program selects integer classes that incorporate an equal number of samples in each distance class. This option can be useful to reduce noisy confidence limits resulting from uneven sample sizes in the data (Blyton and Flanagan n.d.). The statistical significance of the analysis and 95% confidence interval for the null hypothesis of no spatial genetic structure was assessed based on 1000 permutations and bootstrap replicates.

4.2.4 Effect of environment and ecology

Monthly composite oceanic variable data of ocean productivity (chlorophyll-a; CHL-a, $mg.m^{-3}$), sea surface temperature (°C, SST) and turbidity (diffuse attenuation coefficient KD490; m^{-1}), were obtained from the GMIS Marine Geodatabase (mcc.jrc.ec.europa.eu), based on the NASA MODIS terra satellite at a 4-km spatial resolution. Data were collected for 2012 – 2015, during which the adult lobsters genotyped in this study were sampled. SST was chosen because the distribution of the subspecies appears to be correlated with temperature, *P. h. homarus* and *P. h. megasculptus* are distributed in tropical waters whereas *P. h. rubellus* occurs in sub-tropical waters (Berry 1971b, 1974b). CHL-a was chosen because it is a measure of ocean productivity, and turbidity was selected because *P. homarus* is noted to inhabit turbid water (Holthuis 1991). Using latitude and longitude as factors enable the detection of an increase in genetic variation between local and ancestral populations, resulting from successive founder events during gradual range expansions (Foll and Gaggiotti 2006).

The SST values for each month were averaged for the year, and then the mean SST value was calculated over the four years. The minimum and maximum temperatures were chosen for each of the four years and then averaged (Canales-Aguirre et al. 2016). The mean CHL-a concentration ($mg.m^{-3}$) and turbidity (KD490, m^{-1}) were also calculated over the four years. Each of these values were then used in the subsequent analyses.

The effect of environmental variables on population genetic structure was tested using the hierarchical Bayesian method implemented in the program GESTE v.2.0 (Foll and Gaggiotti 2006). F_{ST} values are calculated and related to factors using generalised linear models. Posterior probabilities are then estimated for each model, the model that best fits the data was selected based on the highest posterior probability. Factors used in the analyses were latitude and longitude and the interaction between them, mean, minimum and maximum sea surface temperatures, turbidity and CHL-a. Larval recruitment (based on estimates from ocean modelling simulations as the number of larvae that reach settlement on the coast) was also used as a parameter in the analysis. The analysis was done using 10 pilot runs with a run length of 5000 to obtain the proposal distributions. Thereafter, the posterior probabilities were estimated by running a rjMCMC with a sample size of 100 000, a thinning interval of 20 and 50 000 burnin iterations.

Distance-based redundancy analysis (dbRDA) is a multivariate, constrained ordination method based on principal co-ordinates analysis (PCoA) (Legendre and Anderson 1999), and it was also used to infer the effects of environmental and spatial variables on genetic differentiation between *P. homarus* subspecies. This method uses a dissimilarity matrix of a response variable, which is the pairwise genetic differentiation in this case (F_{ST}). Classical multidimensional scaling is performed on the matrix, and thereafter a redundancy analysis is performed on the ordination results in order to estimate the amount of genetic variation explained by the explanatory environmental and spatial variables. In this analysis, a matrix of genetic differentiation between each sampling site (F_{ST}) was used as the response variable, and mean measurements of environmental variables (CHL-a, mean, max and min SST's, KD490), latitude and longitude, and larval recruits were used as the explanatory variables. The dbRDA analysis was carried out using the vegan package (Oksanen et al. 2015) in R, and was run using three models, (1) a full model taking into account all environmental factors and spatial information, (2) a partial model whereby geography explains genetic differentiation under the condition of the environmental variables, and (3) another partial model in which environmental variables explain genetic differentiation conditioned on spatial information. This approach can enable detection of how much genetic differentiation is due to environmental factors alone, geography alone, or both geography and environmental factors.

For this analysis, the correlation among variables was measured using Pearson's correlation coefficient performed in vegan. Environmental features which are strongly correlated results in models that are interdependent. This can have an effect on the estimation of the importance of different factors, as it tends to inflate the variance (Rellstab et al. 2015). Where variable pairs were highly correlated, the biologically more relevant one was chosen for the rest of the analyses.

4.3 Results

4.3.1 CBOCM larval dispersal simulations

4.3.1.1 Arabian Sea

The results of the larval dispersal simulations for January 2009 and 2010 are presented in the supplementary figures where A is the simulation showing how the larvae disperse and B shows the final locations after 120 days. In supplementary figures S4.1 – S4.5, most of the

particles that were released in Oman and Yemen remained in the Arabian Sea after 120 days, but a few did remain close to the coast and appeared to have settled. Particles that were released at sites in Oman, and remained close to the coast after 120 days, returned to the sites from which they were released, with a few also settled upstream or downstream from their release site (Figures S4.1 – S4.4) and also along the shore of southern Iran. An important observation is that no particles settled in the Sea of Oman, as *P. homarus* is not known to occur there. The surviving particles from Mirbat (Figure S4.3) and Dhalkhut (Figure S4.4) also show settlement on the coast of Yemen, concordant with the genetic results, which show that the populations of *P. h. megasculptus* from Oman and Yemen are connected. Some of the particles released in Dhalkhut (Figure S4.4) and Yemen (Figure S4.5), which are the southernmost sites in the Arabian Sea, appeared to have crossed the Gulf of Aden and settled along the Horn of Africa, along the coast of Somalia. The dispersal patterns also show a clear influence of eddies and gyres in the Arabian Sea, acting to entrain the particles, which end up staying within the eddies after the simulation. There was also a large inter-annual difference between 2009 (Figures S4.1 – S4.5 A) and 2010 (Figures S4.1 – S4.5 B), at all the Arabian Sea sites.

The larval dispersal connectivity plots for the simulation in 2009 and 2010 indicated connectivity between the sites Mirbat and Dhalkhut in Oman, and between Dhalkhut and Yemen in 2010 (Figure 4.1 A and B).

4.3.1.2 Kenya

In Kenya, very few simulated larval particles settled on the Kenyan coast (Figure S4.6). A big proportion of the particles appeared to get swept out to sea. The simulations show that these particles did not disperse into the Mozambique Channel. The south equatorial return current may have had a prominent effect on the particle dispersal from the Kenyan site, especially in January when it is at its strongest due to the Northeast monsoon season. The site in Kenya did not exhibit connectivity with any of the other sites in the connectivity plots (Figure 4.1 A and B)

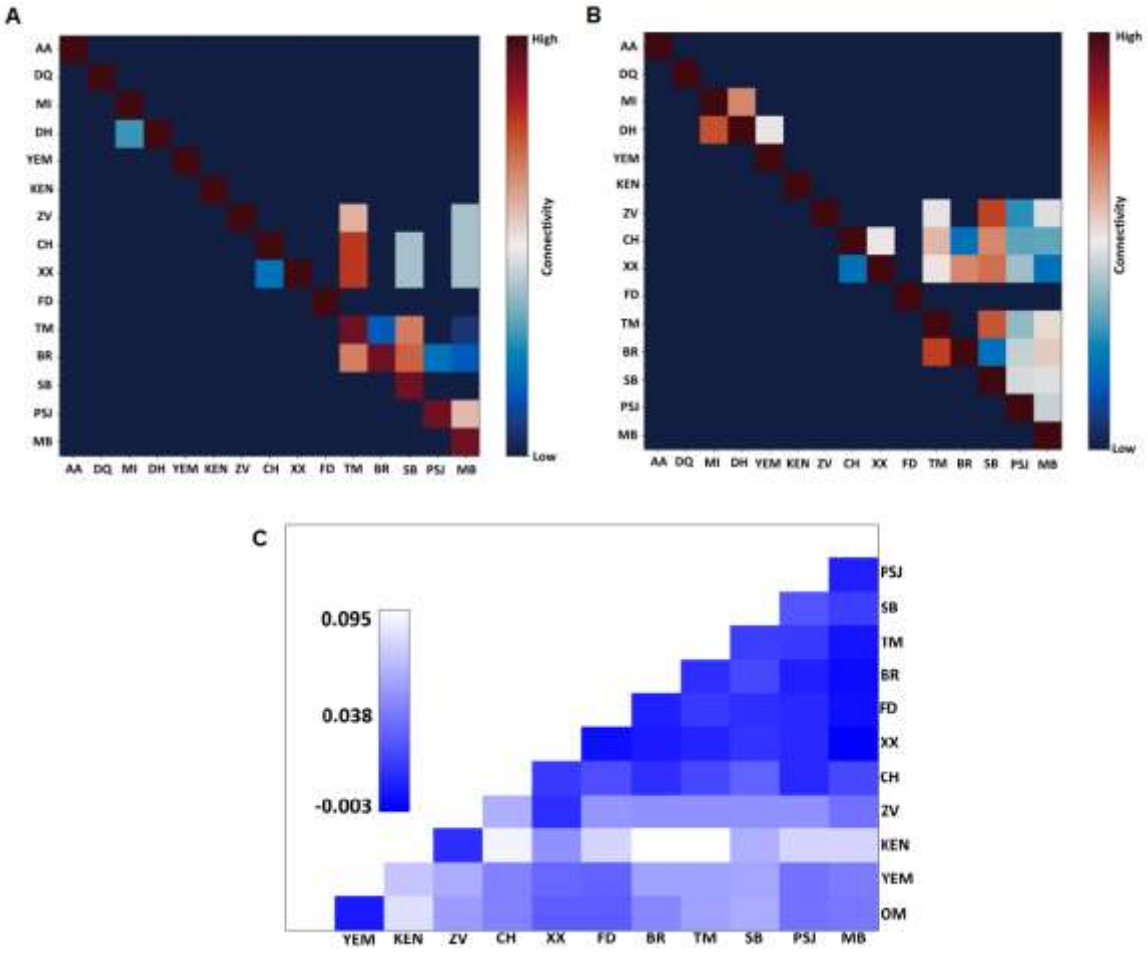


Figure 4.1 *P. homarus* larval dispersal simulation connectivity matrix for (A) 2009 and (B) 2010 for each site sampled in the Western Indian Ocean. (C) represents the pairwise genetic differentiation (F_{ST}) connectivity matrix for each site (locations in Oman were grouped together because of low sample sizes in some locations). A high F_{ST} value indicates poor connectivity while a low F_{ST} value means high connectivity.

4.3.1.3 Mozambique

Irrespective of the site in Mozambique, a large proportion of the simulated particles were caught up in the Agulhas Current system and remained lost at sea after 120 days. In Zavora (Figure S4.7), some particles settled close to their release origin, but more reached sites further to the south, in southern Mozambique and South Africa. The same pattern was observed for particles released at Chidenguele and Xai Xai (Figures S4.8 and S4.9). This can also be seen in the connectivity plots (Figure 4.1 A and B). The sites in Mozambique provided more particles to South African sites such as Blood Reef, Tinley Manor, Scottburgh and Mdumbi than to the other Mozambique locations. Nevertheless, most particles were

swept up in the Agulhas Current, and transported rapidly southwestwards along the shelfbreak, either retroflecting eastwards into the Southwest Indian Ocean (presumably lost), or leaking into the Southeast Atlantic basin, sometimes within large anticyclonic eddies, called Agulhas rings (Olson and Evans 1986). Villar et al. (2015) describe taxonomic and functional plankton assemblages inside Agulhas rings, but did not investigate decapod zooplankton. It is, however, highly unlikely that *P. homarus* phyllosomas will survive for long in these rings, where plankton communities gradually converge with those of surrounding South Atlantic waters.

4.3.1.4 Madagascar

Particles that were released at Fort Dauphin on the southeast coast of Madagascar travelled across the Mozambique Channel but did not quite reach the mainland coast along southern Mozambique or eastern South Africa (Figure S4.10). It is important to note that the CBOCM resolution is coarse, and that larvae which appear close to the coast may still reach it, because later stage larvae exhibit directional swimming (see discussion). There also appears to be some self-recruitment near southeast Madagascar. This is essential, as we found no evidence of the Madagascar population receiving larval recruits from other regions. Most particles were lost at sea after 4 months. The connectivity plots for Fort Dauphin in Madagascar showed no connectivity to the other southern hemisphere sites (Figure 4.1 A and B).

4.3.1.5 South Africa

Similar to the Mozambique scenario, the majority of particles released at sites along the east coast of South Africa were swept southwestwards by the Agulhas Current, and were either retroflected into the Southwest Indian Ocean, or leaked into the Southeast Atlantic. As larvae, these are considered lost after 120 days, and would not be expected to return to the coast. Some particles released in eastern South Africa do reach the coast after 120 days, and these presumably simulate settlement locations and proportions of viable post-larvae. Nearly all of these settled to the south of their release locations, and it also appears as if particles released from sites furthest to the south, such as Scottburgh (Figure S4.13), Port St Johns (Figure S4.14) and Mdumbi (Figure S4.15), contributed very little to posterity, i.e., most of them

were swept away and lost. This pattern indicates that these southerly sites represent sink populations, and that they receive larvae from sites further to the north.

In the connectivity plots, Tinley Manor showed high connectivity to Scottburgh, Blood Reef displayed high connectivity with Tinley Manor, and Scottburgh and Port St Johns demonstrated high connectivity with Mdumbi (Figure 4.1 A and B) – the latter thus illustrating that larval settlement at the most-southerly Mdumbi site might depend on upstream larval sources.

The percentage of simulated larvae from the southern hemisphere sites that reach the east coast of South Africa for the years 2009 and 2010, is presented in Figure 4.2. Remarkably, a higher percentage of larvae from Mozambican sites reach the east coast of South Africa when compared to the number of simulated larvae from South African sites that make it back to the east coast of South Africa. Little to no simulated larvae released in Madagascar made it to sites along the South African coast.

The larval dispersal simulation connectivity matrix shows that there is connectivity between South Africa and Mozambique. The only major difference between genetic connectivity and simulated larval dispersal connectivity was that genetic data showed Madagascan populations to be connected to Mozambique and South Africa, but the simulated data indicated weak connectivity. This could be due to the coarse resolution of the CBOCM and because the larval dispersal simulation considers the particles to be passive drifters and does not account for larval behaviour, such as directed swimming. The larval dispersal model also fails to account for connectivity between Kenya and Mozambique populations of *P. h. homarus*.

The pairwise F_{ST} genetic connectivity plot among *P. homarus* sampling locations indicate as expected that *P. h. megasculptus* populations in Oman and Yemen were closely connected, *P. h. homarus* populations in Kenya were closely connected to those in Zavora. Zavora exhibited moderate genetic connectivity with *P. h. rubellus* sampling sites, indicating that this is the point of the genetic transition and contact zone between *P. h. homarus* and *P. h. rubellus* and potentially the area where Madagascan larvae arrive at the southeast African coast and are dispersed towards the South African east coast. There was a high degree of genetic connectivity between *P. h. rubellus* populations in South Africa and the site in Madagascar (Figure 4.1 C). These genetic connectivity results closely parallel the larval connectivity plots derived from the larval dispersal simulations (Figure 4.1 A and B).

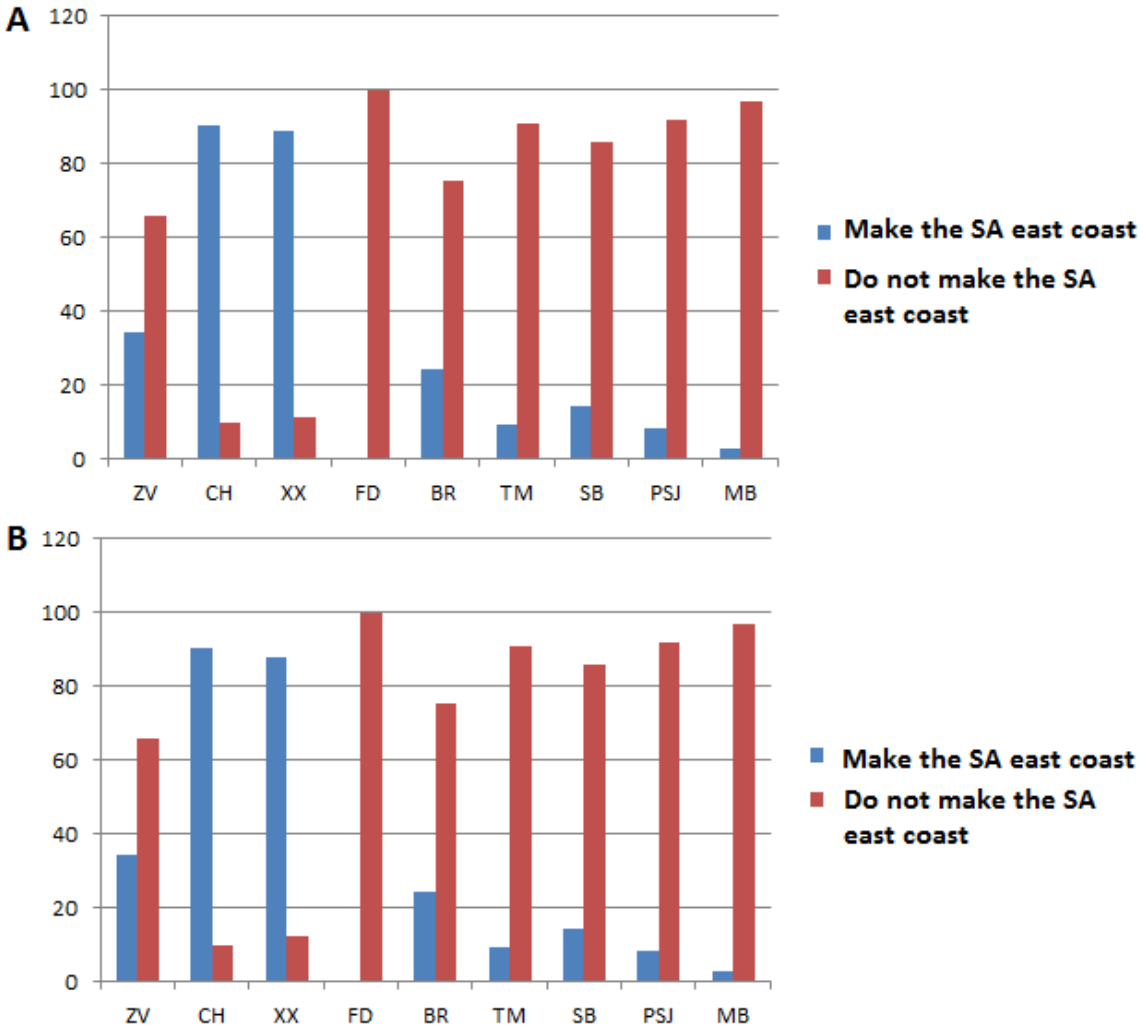


Figure 4.2 The percentage of larvae that reach the east coast of South Africa from southern hemisphere sampling sites in (A) 2009 and (B) 2010.

4.3.2 Spatial genetic clustering and autocorrelation

For the Geneland analysis, the MCMC chains indicated good mixing and the posterior distribution of the number of genetic clusters showed a clear mode at three, which is the optimal number of genetic clusters found (Figure 4.3 A). The first genetic cluster corresponded to individuals from Oman and Yemen which are the *P. h. megasculptus* lobsters (Figure 4.3 B). The second genetic cluster was the *P. h. homarus* individuals from Kenya and a few from Mozambique (Figure 4.3 C), and the third cluster contained *P. h. rubellus* individuals from Mozambique, South Africa and southern Madagascar (Figure 4.3 D). Steep contour lines on the maps in Figure 4.3 C and D indicate genetic discontinuity. This

zone of discontinuity corresponds to the periphery of *P. h. homarus* and *P. h. rubellus* distributions, and as noted in Chapter 3, form a likely contact zone between these two subspecies.

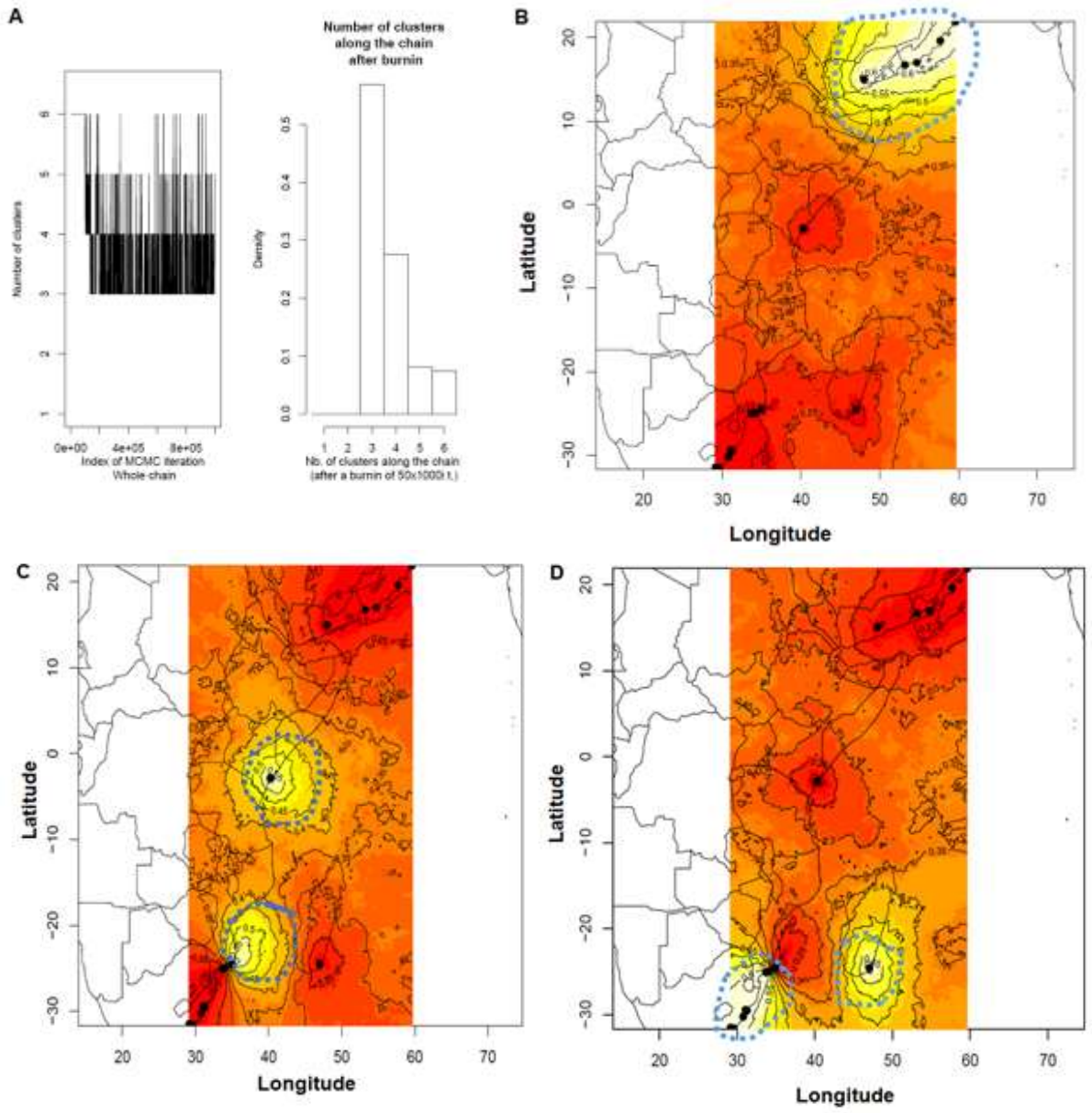


Figure 4.3 Results of the Geneland analysis. (A) Posterior distribution of the number of genetic clusters, showing a clear mode at K = 3. (B) Contour plot showing the posterior probabilities of the inferred clusters corresponding to *P. h. megasculptus* populations, (C) *P. h. homarus*, (D) *P. h. rubellus*. The highest membership values are in white to yellow, and the contour lines depict the spatial position of genetic discontinuities.

The spatial clustering analysis using TESS also indicated that there were three genetic clusters (Figure 4.4). The map plot of the ancestry coefficients show the probability of individuals belonging to one of the three clusters. Cluster one corresponded to the *P. h. megasculptus* individuals, cluster two to the *P. homarus* individuals and *P. h. rubellus*

individuals were assigned to cluster 3. The TESS clustering analysis also uncovered a genetic transition zone between *P. h. homarus* and *P. h. rubellus* in southern Mozambique.

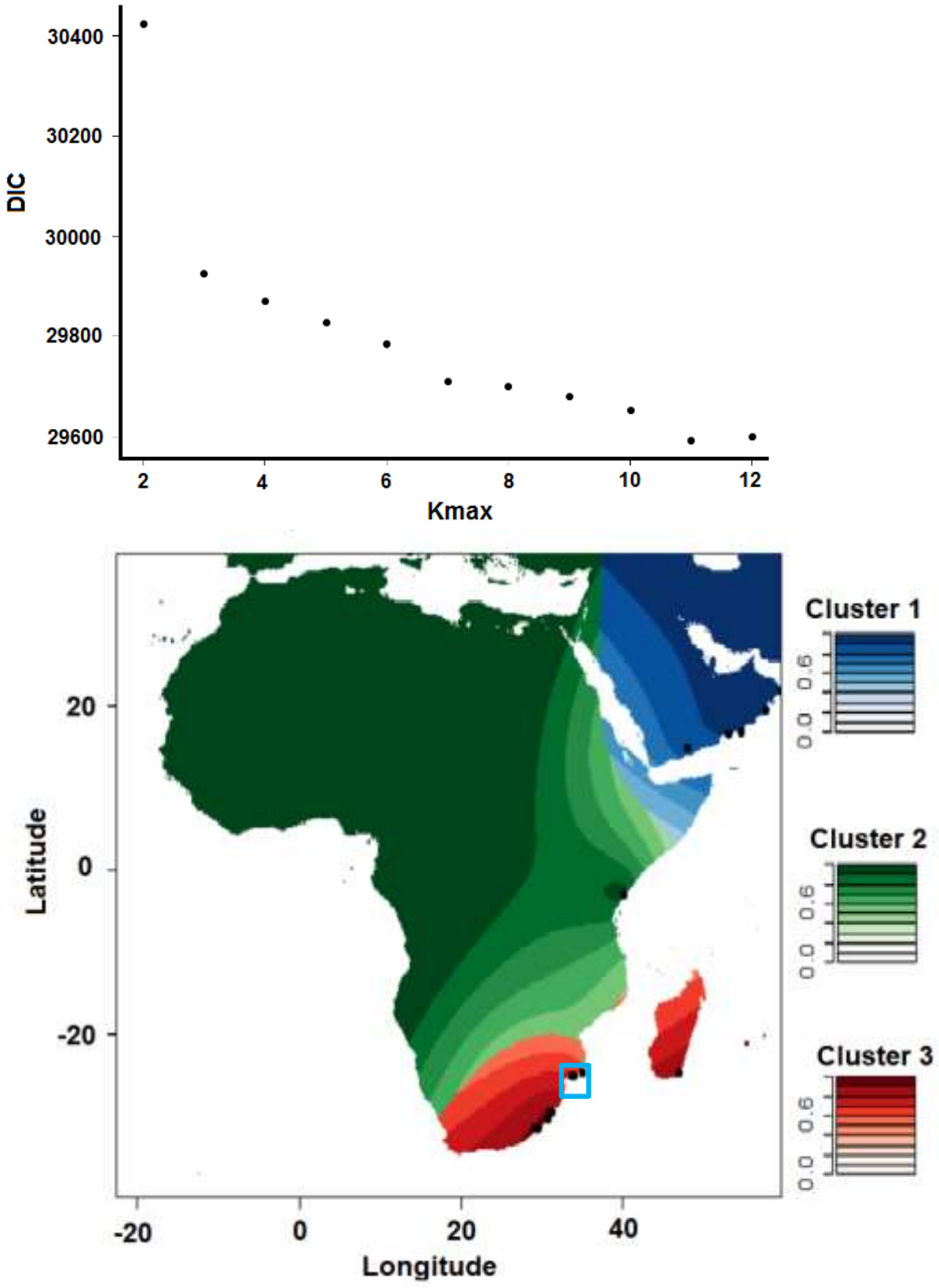


Figure 4.4 Results of the TESS analysis. A plot of the DIC vs. K to determine the optimal K. The plateau started at 3, so a geographic map of ancestry coefficients was plotted using K = 3 ancestral populations. The clusters are colour coded on the map. Cluster 1 corresponds to the *P. h. megasculptus* populations, cluster 2 to the *P. h. homarus* populations, and cluster 3 to the *P. h. rubellus* populations. A genetic transition zone from green to red is observable near Zavora in Mozambique. The blue square represents the Delagoa Bight region, adapted from Cossa et al (2016).

The results of the spatial structure analysis for *P. homarus* using the spatial autocorrelation method showed significantly positive spatial autocorrelation at some smaller distance classes (50.5 km and 249 – 433.5 km), which rejects the null hypothesis of panmixia, suggesting that there is genetic structure (Figure 4.5 A). In this case, the genetic structure observed corresponds to the locations in Mozambique where *P. h. homarus* and *P. h. rubellus* co-occur. There is a significantly negative spatial autocorrelation from distance classes 2898 – 6207 km. Negative correlation at the most distant distance classes suggests a signature of a gradual change in genetic variation due to selection. There was no consistent evidence of significant spatial autocorrelation for the *P. h. homarus* subspecies, indicating that there is no spatial genetic structure among populations of *P. h. homarus* in the Western Indian Ocean (Figure 4.5 B). This pattern was also seen for *P. h. megasculptus* and *P. h. rubellus* populations, which indicates that there is spatial structure between subspecies but not within them (Figure 4.5 C and D).

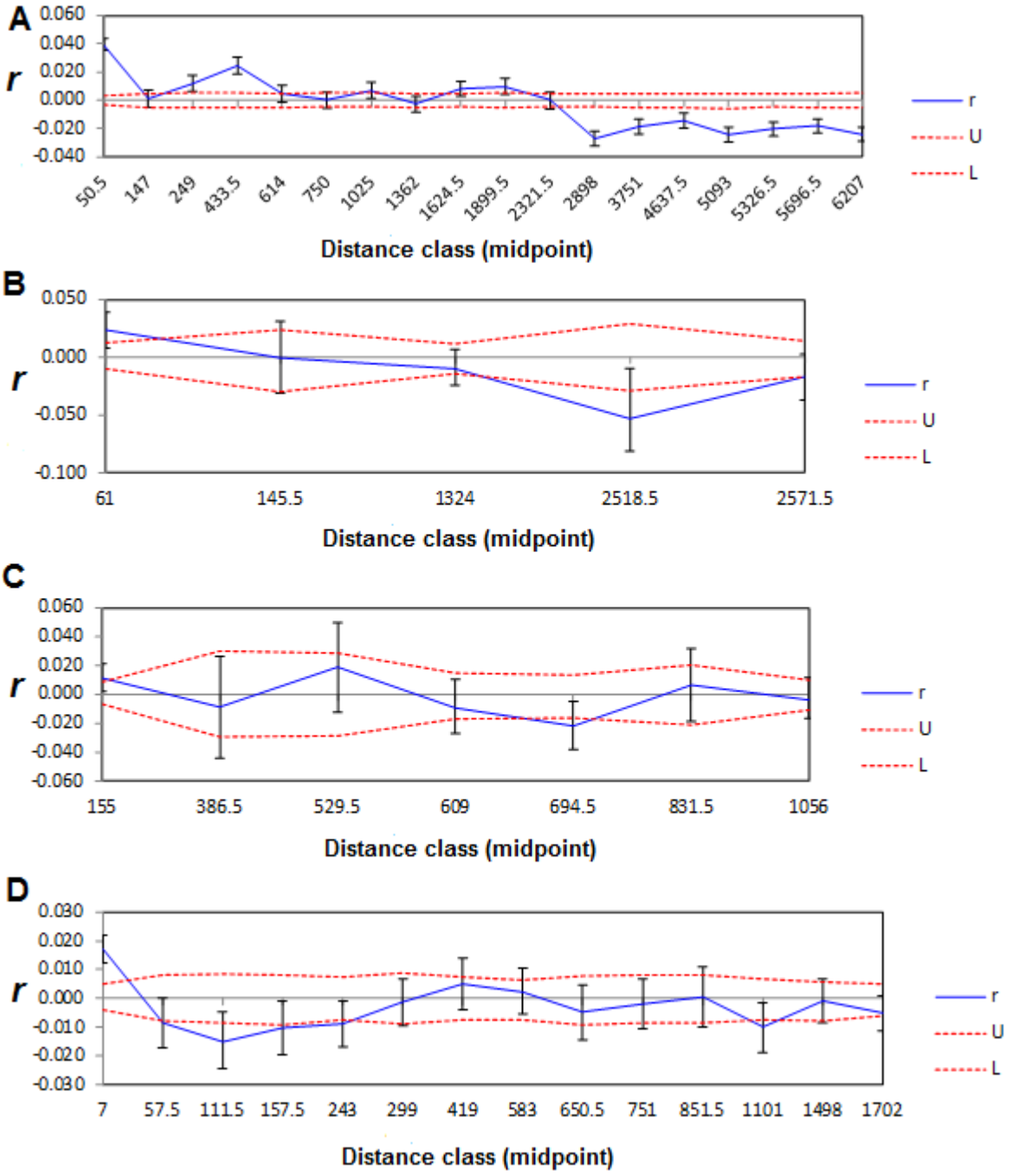


Figure 4.5 Correlogram of spatial autocorrelation analysis results where r is the spatial correlation coefficient as a function of geographic distance across (A) the entire sampled range of *P. homarus*, (B) *P. h. homarus* sampling locations, (C) *P. h. megasculptus* sampling locations and (D) *P. h. rubellus* sampling locations. The whiskers depict the standard errors of r and the dashed red lines depict the upper and lower 95% confidence intervals for the hypothesis of no spatial autocorrelation.

4.3.3 Effects of environmental factors on genetic differentiation

The GESTE analysis selected the constant model as the best-fit to the genetic data with a probability of > 0.89 (Table 4.1). The constant model excludes all other factors as being the best-fit for the data. This indicates that none of the environmental factors tested can alone explain the observed genetic structure. There was no significant genetic differentiation attributed to latitude and longitude, which suggests that a gradual spatial range expansion was not responsible for the genetic differentiation between the subspecies. Although no factor alone could explain the genetic variation, using the mean SST as a factor explained 11% of the genetic differentiation, which could mean that SST does have a partial role in the genetic variation between the *P. homarus* subspecies. Turbidity (KD420) also seemed to have a small effect on genetic differentiation (8.9%, Table 4.1), followed by productivity (CHL-a: 8%, Table 4.1).

Table 4.1 Results of the GESTE analysis based on environmental and spatial data.

Environmental Variables	**Factors**	**Posterior Probability**
Spatial range expansion	Constant	0.896
	Latitude	0.0539
	Constant, Latitude	0.0436
	Longitude	0.0592
	Constant, Longitude	0.0489
	Constant, Latitude, Longitude	0.0103
	Constant, Latitude, Longitude, Latitude*Longitude	0.0008
Oceanographic Variables	Constant	0.939
	Min SST (°C)	0.0612
	Constant	0.883
	Mean SST (°C)	0.117
	Constant	0.94
	Max SST (°C)	0.0596
	Constant	0.911
	Turbidity (KD490 m^{-1})	0.0886
	Constant	0.918
	Chlorophyll-a ($mg.m^{-3}$)	0.0817
Larval Recruitment	Constant	0.945
	Larvae 2009	0.055
	Constant	0.947
	Larvae 2010	0.0525

For the dbRDA analysis, the CHL-a variable was excluded because it was highly correlated with turbidity (KD490). Mean, minimum and maximum SST were all highly correlated, so the minimum SST was chosen for the dbRDA analysis. Finally, latitude and longitude were also strongly correlated (0.93, Table 2) so latitude was chosen because the sample sites were spaced along a latitudinal gradient spanning from the tropics to subtropical and warm temperate waters.

Table 4.2 Pearson correlation coefficients computed for each pair of variables. Values in bold indicate significance at a 95% significance level.

Variables	Chl-a	KD490	Mean SST	Min SST	Max SST	Latitude	Longitude	Larval Recruits
Chl-a	*							
KD490	**0.99**	*						
Mean SST	-0.418	-0.45	*					
Min SST	0.202	0.168	**0.775**	*				
Max SST	0.206	-0.006	**0.852**	**0.964**	*			
Latitude	0.499	0.475	0.41	**0.787**	**0.739**	*		
Longitude	0.507	0.488	0.333	**0.718**	**0.672**	**0.93**	*	
Larval Recruits	-0.238	-0.229	-0.226	-0.35	-0.294	-0.315	-0.344	*

ANOVA tests selected model 1 which was the full model including minimum SST, latitude KD490 and larval recruits, as being the most statistically significant (F = 9.18, p = 0.001, Table 4.3). This model indicated that 78.6% of the genetic variation can be accounted for by the environmental factors (Figure 4.6). In model 1, latitude (F = 20.53, p = 0.001, Table 4.3) and minimum SST (F = 15.67, p = 0.001) were significantly correlated with genetic differentiation. In model 2 (Figure S4.16), when conditioned on the environmental variables, latitude was still significantly correlated with F_{ST} (F = 17.58, p = 0.002). In model 3 (Figure S4.17), the environmental variables were conditioned on latitude and minimum SST was still significantly associated with genetic differentiation (F = 15.67, p = 0.007).

Table 4.3. Statistical significance using ANOVA, of models and factors evaluated in the dbRDA analyses.

Model	Variable	F-statistic	P-value
Model 1	Overall	9.18	**0.001**
	Min SST	15.67	**0.001**
	Latitude	20.53	**0.001**
	Mean KD490	-0.36	0.995
	Larval Recruits	0.909	0.407
Model 2	Overall	17.58	**0.004**
	Latitude	17.58	**0.002**
Model 3	Overall	5.40	0.058
	Min SST	15.67	**0.007**
	Mean KD490	-0.36	0.946
	Larval Recruits	0.91	0.305

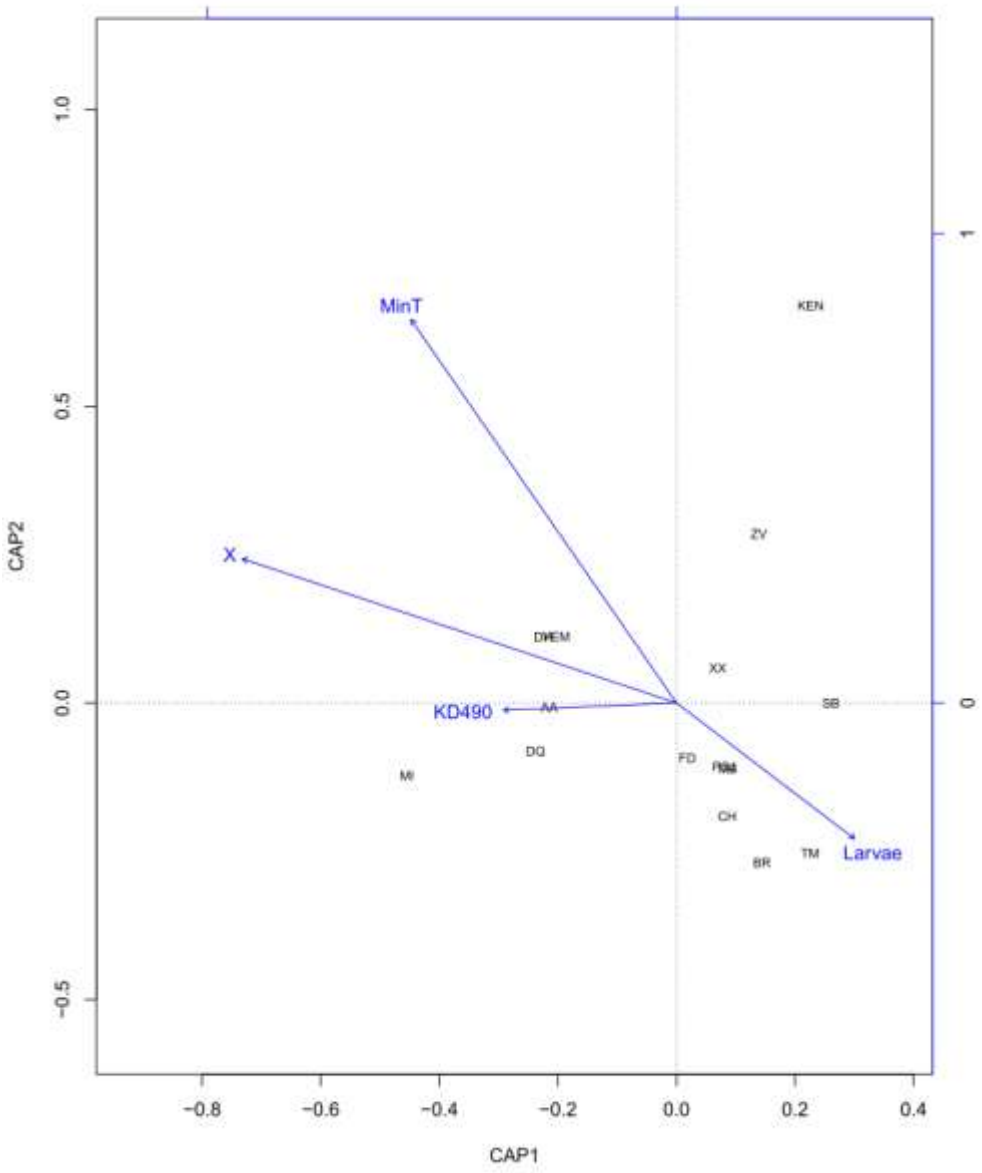


Figure 4.6 dbRDA plot of the results for the most statistically significant model considering all the variables. The arrows indicate the direction of the maximum correlation and the length of the arrow represents the strength of the correlation. X = latitude.

4.4 Discussion

The combination of genetic data with spatial information, multivariate statistical techniques using environmental and spatial data, along with oceanographic larval dispersal simulations have shown how a multitude of variables and conditions within the oceanic environments can influence genetic differentiation among the three subspecies of *P. homarus* in the Western Indian Ocean.

4.4.1 CBOCM's of larval dispersal vs. empirical genetic data

The results obtained in this study highlight that patterns of larval dispersal and connectivity can be predicted by physical oceanography. Patterns observed with the larval dispersal simulations broadly corresponded with many of the patterns reconstructed using the genetic data. The larval dispersal model corroborated the conclusion derived from the genetic data, that larvae from sites in Oman and Yemen may be retained in eddies within the Arabian Sea circulation (see Bruce et al. 1994), which is driven by the monsoon climate and the seasonally reversing Somali Current. Even though the genetic connectivity between *P. h. homarus* populations in Kenya and Mozambique were not reflected in the larval dispersal simulation, southern Mozambique may receive *P. h. homarus* larvae from sites that were not sampled in the present study such as northern Mozambique and Tanzania.

Larval connectivity modelled using CBOCM's and genetic data showed correlation on a broad scale in the rock scallop *Spondylus calcifer* (Soria et al. 2012), the mussel *Mytilus edulis* (Gilg and Hilbish 2008) and corals in the Caribbean (Foster et al. 2012). Examples of other spiny lobsters include the congeneric *P. ornatus* in the South-East Asian Archipelago, where larval dispersal models correlated with the observed genetic connectivity between sites, indicating a panmictic population (Dao et al. 2015). In *P. argus*, CBOCM's demonstrated that genetic structure was correlated with biophysical connectivity rather than isolation by distance (Truelove et al. 2017). A larval dispersal model was developed for the rock lobster *Jasus edwardsii* to test whether Australia contributed larvae to New Zealand populations across the Tasman Sea (Chiswell et al. 2003). The model indicated that there was trans-Tasman larval flow to connect Australian and New Zealand populations of *J. edwardsii*. In 2013, a genetic study of *J. edwardsii* using microsatellite data showed that the dispersal

model developed by Chiswell et al. (2003) was congruent with the genetic data (Thomas and Bell 2013).

The present study confirmed that *P. h. rubellus* lobsters from Mozambique, Madagascar and South Africa represent a shared stock from both genetic and oceanographic perspectives. The model shows that *P. h. rubellus* larvae from Madagascar can cross the Mozambique Channel, with some larvae reaching the Mozambique and South African coasts. This presents further evidence that the "suitcase hypothesis" of larval transport from southeast Madagascar across to Mozambique and South Africa may be acting to promote connectivity between populations of *P. h. rubellus* (Marsac et al. 2014). In contrast, larvae released at sites in Mozambique and eastern South Africa do not reach the Madagascan coast. This is in agreement with the patterns of gene flow found in Chapter 3. These results suggest that the southeastern Madagascan populations largely rely on self-recruitment to sustain the populations because they do not receive larvae from Mozambique and South Africa. The dispersal model confirms that some particles released in southeastern Madagascar do return to their area of release, albeit in low numbers. This has important consequences for the management of the species in southeast Madagascar.

Although the larval dispersal model used in this study was able to predict the observed genetic structure between the subspecies it is too simplistic to account for the finer scale population-specific patterns of connectivity between *P. h. rubellus* sampling sites. The larval dispersal model confirms that larvae from Mozambique can travel down to the east coast of South Africa, but it does not show how larvae released at South African sites are transported to Mozambique. This is in contrast to the gene flow pattern shown in Chapter 3, which indicates that Mozambique receives more immigrants from South Africa, than the other way around.

While at a coarse scale, there was significant correlation between modelled and empirical estimates of genetic data and oceanography in a Caribbean corals seascape genetics study, but it also highligted differences between modelled and empirical data (Foster et al. 2012). This implies that other intricate processes, besides passive larval dispersal, occurs at those sites. Likewise, in the present study, differences between modelled larval dispersal and empirical genetic data suggest that some processes were not accounted for in the model. For example, inshore return currents that flow in the opposite direction to the Agulhas Current may play a role in dispersing larvae in a northerly direction, from eastern South Africa towards

Mozambique. Although these reverse currents over the shelf are well-documented (Gründlingh 1974), the resolution of the CBOCM model is too coarse to accurately incorporate these nearshore water movements.

4.4.2 CBOCM limitations and improvements that could be made

A major limitation of the CBOCM is that particles are passive drifters. In reality, lobster larvae are able to swim, and position themselves in the water column to benefit from water movements at different depths (Chiswell and Booth 1999, 2005, 2008, Paris and Cowen 2004, Bradford et al. 2005). Lobster larvae exhibit ontogenetic vertical migratory behaviour, in which they can vertically orient themselves in the water column at later stages of development, to achieve control over their transport and remain in the vicinity of their settlement sites (Paris and Cowen 2004, Bradford et al. 2005). They can also undertake diel vertical migrations, in which they inhabit deeper strata during the day, to escape predation, and rise to the surface at night to feed (Bradford et al. 2005, Butler et al. 2011). Late stage larvae receive sensory cues directing them towards the shore (Jeffs et al. 2005) - these cues may include acoustic reef sounds (Hinojosa et al. 2016), chemical (Hadfield and Paul 2001), celestial, magnetic field or electrosense signals (Jeffs et al. 2005), and cannot yet be incorporated into a CBOCM. The lobster puerulus stage is also a strong swimmer (Phillips and Olsen 1975, Phillips 2013), and is able to cross the shelf using stored energy reserves (Wilkin and Jeffs 2011). Adding these directional movements to a passive drifter could substantially alter dispersal patterns. For example, in the present case, directional movements of larvae across the shelf may have assisted larvae originating from Madagascar to reach the coasts of Mozambique and South Africa, instead of being swept away by the Agulhas Current.

It is also important to note that the simulations reproduced a single scenario, reflecting only the peak January reproductive season – when most larvae are presumably released. In reality, *P. homarus* females can carry up to four batches of eggs per year, releasing them during different months. Seasonal variations in current strength and position (Williams et al. 1984, Lobe1 1989, Caputi et al. 1996), and in physio-chemical characteristics of nearshore waters (Hadfield and Paul 2001, Dubois et al. 2007) will affect dispersal patterns, and hence settlement patterns. Inter-annual variation in ocean currents and environmental variables have

often been implicated in recruitment patterns of species with drifting larvae (Chelton et al. 1982, Botsford 2001, Griffin et al. 2001), and clear differences were also seen in the outputs from the 2009 and 2010 particle dispersal models in the present study.

The CBOCM models used in Chapter 4 can be improved for future studies by incorporating aspects of larval biology, such as ontogenetic vertical migration, diel vertical migration, variation in reproductive timing, seasonality, and directional swimming behaviour (Soria et al. 2012). Modelling experiments on coral reef fish larvae showed that larval behaviour is just as important as eddies in determining connectivity patterns (Paris et al. 2007). Furthermore, the vertical migrations can potentially reduce larval loss, because it can promote retention of the larvae near natal sites (Paris et al. 2007). Similarly, Lagrangian simulations of Irish Sea shellfish larvae showed that vertical migration synchronised with tides or the earth's rotation acted to retain them near the coast (Robins et al. 2013). Thus, including small-scale nearshore factors that may influence dispersal, recruitment and survival would improve the predictive accuracy of the spiny lobster CBOCM (Foster et al. 2012, Teske et al. 2015, 2016). Nevertheless, even this simple implementation of the dispersal model provided new insights into the scale of larval dispersal and settlement of *P. homarus* in the Western Indian Ocean.

4.4.3 Spatial clustering and autocorrelation

The results of the spatial clustering methods Geneland and TESS confirmed that there was a genetic transition zone near the Delagoa Bight in Mozambique, where *P. h. homarus* and *P. h. rubellus* occur sympatrically (Figure 4.3 and 4.4). Using spatially explicit clustering methods is very important to empirically identify the hydrological features or vicariant barriers that may affect gene flow.

Studies on broadcast spawning gastropods demonstrated that positive spatial autocorrelation at short distance classes could be a signature of self-recruitment of larvae, back to the sites from which they were released (Teske et al. 2015, 2016). The occurrence of autocorrelation at short distance classes in *P. homarus* (Figure 4.5 A) also suggests that self-recruitment occurs at most sites. Larval simulations supported the return of larvae to near their natal sites after 4 months of passive drift (Supplementary figures).

4.4.4 Environmental factors vs. genetic structure

General linear models and multivariate statistics such dbRDA to investigate the correlation between environmental, spatial and genetic data were useful in identifying environmental drivers of genetic variability across the seascape. SST was an important facilitator of genetic differentiation between *P. homarus* subspecies (Table 4.1 and Figure 4.6). A study on the small pelagic fish *Sprattus fuegensis* from Patagonia detected two genetic clusters which likely arose because of oceanographic factors, with environmental variables such as SST and nitrate correlating with allele frequency differences (Canales-Aguirre et al. 2016). SST and ocean productivity were also correlated with genetic structure in the short-beaked common dolphin in the Atlantic, Indian and Pacific oceans (Amaral et al. 2012). SST and salinity were significantly correlated with genetic diversity in bluefin tuna in the Mediterranean Sea (Riccioni et al. 2013). Genetic structure along an environmental gradient might suggest a preference towards certain mating and spawning habitat conditions (Riccioni et al. 2013). In Chapter 3, it was speculated that decreasing SST as the climate transitions from tropical (preferred by *P. h. homarus*) to sub-tropical conditions (preferred by *P. h. rubellus*), was partially responsible for the genetic diversity between these subspecies. The empirical tests confirmed that *P. h. homarus* and *P. h. rubellus* genetic variability was correlated with SST.

Based on the seascape genetics approach, genetic diversity between subspecies is driven predominantly by the complex physical oceanography of the Western Indian Ocean, such as the prevailing currents and mesoscale eddies. This finding supports the widely-held opinion that water movements are key to the dispersal of larvae during the planktonic stage (Masterson et al. 1997). Environmental factors such as temperature, turbidity and geography may have more subtle effects on the genetic variability in *P. homarus*, as these can influence larval mortality rates and determine the habitats of benthic juvenile and adult stages.

Importantly, the seascape genetics approach demonstrated that no one factor can explain observed genetic structure, and that it is more likely a result of interaction between several contributing factors. For example, environmental variables, spatial location and ocean circulation patterns all play a role in the genetic differentiation among the *P. homarus* subspecies. Over time, these factors led to speciation of *P. h. rubellus*.

4.4.5 Conclusion

To conclude, the seascape genetics approach highlighted the importance of linking population genetic information, ocean circulation patterns, and environmental variables when investigating connectivity and genetic differentiation in *P. homarus*. Adding larval behaviour, such as vertical positioning and directional swimming to the CBOCM is expected to improve its predictive utility, but it awaits the development of higher resolution particle models for the Western Indian Ocean.

4.5 References

Amaral, A. R., L. B. Beheregaray, K. Bilgmann, D. Boutov, L. Freitas, K. M. Robertson, M. Sequeira, K. A. Stockin, M. M. Coelho, and L. M. Möller. 2012. Seascape genetics of a globally distributed, highly mobile marine mammal: The short-beaked common dolphin (genus *Delphinus*). *PLoS ONE* **7**. DOI: 10.1371/journal.pone.0031482.

Ayre, D. J., and S. Dufty. 1994. Evidence for restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia's Great Barrier Reef. *Evolution* **48**: 1183-1201.

Benzie, J. A. H. 1999. Major genetic differences between crown-of-thorns starfish (*Acanthaster planci*) populations in the Indian and Pacific Oceans. *Evolution* **53**: 1782-1795.

Berry, P. F. 1971a. The biology of the spiny lobster *Panulirus homarus* (Linnaeus) off the east coast of southern Africa. *South African Association for Marine Biological Research, Investigational Report* **28**: 3-75.

Berry, P. F. 1971b. The spiny lobsters (Palinuridae) of the coast of southern Africa: distribution and ecological notes. *South African Association for Marine Biological Research, Investigational Report* **27**: 4-23.

Berry, P. F. 1974a. Palinurid and scyllarid lobster larvae of the Natal coast, South Africa. *South African Association for Marine Biological Research, Investigational Report* **34**: 3-44.

Berry, P. F. 1974b. A revision of the *Panulirus homarus*-group of spiny lobsters (Decapoda, Palinuridae). *Crustaceana* **27**: 31-42.

Blyton, M. D. J., and N. S. Flanagan. (n.d.). A comprehensive guide to: GenAlEx 6.5.

Booth, J. D. 2002. Early life history, recruitment processes and settlement of spiny lobsters. *Fisheries Science* **68**: 384-389.

Botsford, L. 2001. Physical influences on recruitment to California Current invertebrate populations on multiple scales. *ICES Journal of Marine Science* **58**: 1081-1091.

Bradford, R. W., B. D. Bruce, S. M. Chiswell, J. D. Booth, A. Jeffs, and S. Wotherspoon.

2005. Vertical distribution and diurnal migration patterns of *Jasus edwardsii* phyllosomas off the east coast of the North Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research* **39**: 593-604.

Bruce, J. G., D. R. Johnson, and J. C. Kindle. 1994. Evidence for eddy formation in the eastern Arabian Sea during the northeast monsoon. *Journal of Geophysical Research* **99**: 7651-7664.

Butler, M. J., C. B. Paris, J. S. Goldstein, H. Matsuda, and R. K. Cowen. 2011. Behavior constrains the dispersal of long-lived spiny lobster larvae. *Marine Ecology Progress Series* **422**: 223-237.

Canales-Aguirre, C. B., S. Ferrada-Fuentes, R. Galleguillos, and C. E. Hernández. 2016. Genetic structure in a small pelagic fish coincides with a marine protected area: seascape genetics in Patagonian Fjords. *PLoS ONE* **11**. DOI: 10.1371/journal.pone.0160670.

Caputi, N., W. Fletcher, A. Pearce, and C. Chubb. 1996. Effect of the Leeuwin Current on the recruitment of fish and invertebrates along the Western Australian Coast. *Marine and Freshwater Research* **47**: 147-155.

Carr, M. H., J. E. Neigel, J. A. Estes, S. Andelman, R. R. Warner, and J. L. Largier. 2003. Comparing marine and terrestrial ecosystems: implications for the design of coastal marine reserves. *Ecological Applications* **13**: 90-107.

Chelton, D., P. Bernal, and J. McGowan. 1982. Large-scale interannual physical and biological interaction in the California Current. *Journal of Marine Research* **40**: 1095-1125.

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

Chiswell, S. M., and J. D. Booth. 1999. Rock lobster *Jasus edwardsii* larval retention by the Wairarapa Eddy off New Zealand. *Marine Ecology Progress Series* **183**: 227-240.

Chiswell, S. M., J. Wilkin, J. D. Booth, and B. Stanton. 2003. Trans-Tasman Sea larval transport: Is Australia a source for New Zealand rock lobsters? *Marine Ecology Progress Series* **247**: 173-182.

Chiswell, S. M., and J. D. Booth. 2005. Distribution of mid- and late-stage *Jasus edwardsii* phyllosomas: Implications for larval recruitment processes. *New Zealand Journal of Marine and Freshwater Research* **39**: 1157-1170.

Chiswell, S. M., and J. D. Booth. 2008. Sources and sinks of larval settlement in *Jasus edwardsii* around New Zealand: Where do larvae come from and where do they go? *Marine Ecology Progress Series* **354**: 201-217.

Danielson, R., J. Johannessen, M.-H. Rio, F. Collard, C. Donlon, B. Chapron, and G. Quartly. 2017. An ocean surface current analysis (GlobCurrent) calibration and validation. 19th EGU General Assembly, EGU2017, proceedings from the conference held 23-28 April, 2017 in Vienna, Austria. p.15738 19:15738.

Dao, H. T., C. Smith-Keune, E. Wolanski, C. M. Jones, D. R. Jerry, and N. Suzuki. 2015. Oceanographic currents and local ecological knowledge indicate, and genetics does not refute, a contemporary pattern of larval dispersal for the ornate spiny lobster, *Panulirus ornatus* in the South-East Asian Archipelago. *PLoS ONE* **10**. DOI: 10.1371/journal.pone.0124568.

De Ruijter, W. P. M., H. M. van Aken, E. J. Beier, J. R. E. Lutjeharms, R. P. Matano, and M. W. Schouten. 2004. Eddies and dipoles around South Madagascar: Formation, pathways and large-scale impact. *Deep Sea Research Part I: Oceanographic Research Papers* **51**: 383-400.

Dubois, S., T. Comtet, C. Retiere, and E. Thiebaut. 2007. Distribution and retention of *Sabellaria alveolata* larvae (Polychaeta: Sabellariidae) in the Bay of Mont-Saint-Michel, France. *Marine Ecology Progress Series* **346**: 243-254.

Foll, M., and O. Gaggiotti. 2006. Identifying the environmental factors that determine the genetic structure of populations. *Genetics* **174**: 875-891.

Foster, N. L., C. B. Paris, J. T. Kool, I. B. Baums, J. R. Stevens, J. A. Sanchez, C. Bastidas, C. Agudelo, P. Bush, O. Day, R. Ferrari, P. Gonzalez, S. Gore, R. Guppy, M. A. McCartney, C. McCoy, J. Mendes, A. Srinivasan, S. Steiner, M. J. A. Vermeij, E. Weil, and P. J. Mumby. 2012. Connectivity of Caribbean coral populations: Complementary insights from empirical and modelled gene flow. *Molecular Ecology* **21**: 1143-1157.

François, O. 2016. Running Structure-like population genetic analyses with R. *R tutorials in*

population genetics, PDF, U. Grenoble-Alpes, 1-8, 2016.

George, R. W. 2005. Evolution of life cycles, including migration, in spiny lobsters (Palinuridae). *New Zealand Journal of Marine and Freshwater Research* **39**: 503-514.

Gilg, M. R., and T. J. Hilbish. 2008. The geography of marine larval dispersal: coupling genetics with fine-scale physical oceanography. *Ecology* **84**: 2989-2998.

Griffin, D. A., J. L. Wilkin, C. F. Chubb, A. F. Pearce, and N. Caputi. 2001. Ocean currents and the larval phase of Australian western rock lobster, *Panulirus cygnus*. *Marine Freshwater Research* **52**: 1187-1199.

Groeneveld J. C., S. von der Heyden, and C. A. Matthee. 2012. High connectivity and lack of mtDNA differentiation among two previously recognised spiny lobster species in the southern Atlantic and Indian Oceans. *Marine Biology Research* **8**: 764-770.

Gründlingh ML. 1974. A description of inshore current reversals off Richards Bay based on airborne radiation thermometry. *Deep-Sea Research* **21**: 47-55.

Guillot, G., F. Mortier, and A. Estoup. 2005. GENELAND: A computer package for landscape genetics. *Molecular Ecology Notes* **5**: 712-715.

Guillot, G. 2008. Inference of structure in subdivided populations at low levels of genetic differentiation—the correlated allele frequencies model revisited. *Bioinformatics* **24**: 2222-2228.

Hadfield, M., and V. Paul. 2001. Natural chemical cues for settlement and metamorphosis of marine-invertebrate larvae. *In: Marine Chemical Ecology*. CRC Press. Pages 431-461.

Halo, I., B. Malauene, and M. Ostrowski. 2017. Physical oceanography. *In: The RV Dr Fridtjof Nansen in the Western Indian Ocean: Voyages of marine research and capacity development*. J. C. Groeneveld and K. A. Koranteng (Eds). FAO, Rome, Italy. Pages 37–50.

Hart-Davis, M.G., B. C. Backeberg, I. Halo, E. van Sebille, and J. A. Johannessen. In Prep. Assessing the accuracy of satellite-derived ocean currents by comparing observed and virtual buoys in the greater Agulhas Region. *Submitted for Review to the Remote Sensing of Environment: Special Edition on the Advances in the Science of Surface Currents.*

Hinojosa, I. A., B. S. Green, C. Gardner, J. Hesse, J. A. Stanley, and A. G. Jeffs. 2016. Reef sound as an orientation cue for shoreward migration by pueruli of the rock lobster, *Jasus edwardsii*. *PLoS ONE* **11**. DOI: 10.1371/journal.pone.0157862.

Hoareau, T. B., E. Boissin, G. Paulay, and J. H. Bruggemann. 2013. The Southwestern Indian Ocean as a potential marine evolutionary hotspot: perspectives from comparative phylogeography of reef brittle-stars. *Journal of Biogeography* **40**: 2167-2179.

Holthuis, L. B. 1991. FAO Species Catalogue. Marine lobsters of the world. An annotated and illustrated catalogue of species of interest to fisheries known to date. *FAO Fisheries Synopsis* No. 125. Rome, Food and Agricultural Organization of the United Nations. Page 292.

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

Jeffs, A. G., J. C. Montgomery, and C. T. Tindle. 2005. How do spiny lobster post-larvae find the coast? *New Zealand Journal of Marine and Freshwater Research* **39**: 605-617.

Keenan, K., P. McGinnity, T. F. Cross, W. W. Crozier, and P. A. Prodöhl. 2013. diveRsity : An R package for the estimation and exploration of population genetics parameters and their associated errors. *Methods in Ecology and Evolution* **4**: 782-788.

Kimura, M., and G. H. Weiss. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics* **49**: 561-76.

Lange, M., and E. van Sebille. 2017. Parcels v0.9: prototyping a Lagrangian Ocean Analysis framework for the petascale age. *Geoscientific Model Development Discussions*: 1-20.

Legendre, P., and M. J. Anderson. 1999. Distance-Based Redundancy Analysis: testing multispecies responses in multifactorial ecological experiments. *Ecological Monographs* **69**: 1-24.

Liggins, L., E. A. Treml, and C. Riginos. 2013. Taking the plunge: an introduction to undertaking seascape genetic studies and using biophysical models. *Geography Compass* **7**: 173-196.

Lobe1, P. S. 1989. Ocean current variability and the spawning season of Hawaiian reef

fishes. *Environmental Biology of Fishes* **24**: 161-171.

Ludt, W. B., and L. A. Rocha. 2015. Shifting seas: the impacts of Pleistocene sea-level fluctuations on the evolution of tropical marine taxa. *Journal of Biogeography* **42**: 25-38.

Lutjeharms, J. R. E. 2006. The Agulhas Current. Springer-Verlag Berlin Heidelberg. 329 pages.

MacDonald, N.J., M. H. Davies, A. K. Zundel, J. D. Howlett, Z. Demirbilek, J. Z. Gailani, T. C. Lackey, and J. Smith. 2006. *PTM: Particle Tracking Model*. US Army Corps of Engineers: Washington DC.

Mace, A. J., and S. G. Morgan. 2006. Larval accumulation in the lee of a small headland: Implications for the design of marine reserves. *Marine Ecology Progress Series* **318**: 19-29.

Mann, K. and J. R. N. Lazier. 2006. Dynamics of marine ecosystems: biological-physical interactions in the oceans. Blackwell Publishing. 512 pages.

Marsac, F., R. Barlow, J. F. Ternon, F. Ménard, and M. Roberts. 2014. Ecosystem functioning in the Mozambique Channel: Synthesis and future research. *Deep-Sea Research Part II: Topical Studies in Oceanography* **100**: 212-220.

Masterson, C. F., B. S. Danilowicz, and P. F. Sale. 1997. Yearly and inter-island variation in the recruitment dynamics of the bluehead wrasse (*Thalassoma bifasciatum*, Bloch). *Journal of Experimental Marine Biology and Ecology* **214**: 149-166.

McCartney, M. A., G. Keller, and H. A. Lessios. 2000. Dispersal barriers in tropical oceans and speciation in Atlantic and eastern Pacific sea urchins of the genus *Echinometra*. *Molecular Ecology* **9**: 1391-1400.

McGaughran, A., K. Morgan, and R. J. Sommer. 2014. Environmental variables explain genetic structure in a beetle-associated nematode. *PLoS ONE* **9**. DOI: 10.1371/journal.pone.0087317.

Mendez, M., H. C. Rosenbaum, A. Subramaniam, C. Yackulic, and P. Bordino. 2010. Isolation by environmental distance in mobile marine species: Molecular ecology of franciscana dolphins at their southern range. *Molecular Ecology* **19**: 2212-2228.

Morgan, S. G., J. L. Fisher, and J. L. Largier. 2011. Larval retention, entrainment, and accumulation in the lee of a small headland: Recruitment hotspots along windy coasts. *Limnology and Oceanography* **56**: 161-178.

Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O' Hara, G. L. Simpson, P. Solymos, M. H. Stevens, H. Wagner. 2015. vegan: Community Ecology Package. Available at: http://CRAN.R-project.org/package=vegan.

Olson, D.B. and R. H. Evans. 1986. Rings of the Agulhas current. *Deep Sea Research Part A. Oceanographic Research Papers* **33**: 27-42.

Otwoma, L. M., and M. Kochzius. 2016. Genetic population structure of the Coral Reef Sea Star *Linckia laevigata* in the Western Indian Ocean and Indo-West Pacific. *PLoS ONE* **11**. DOI: 10.1371/journal.pone.0165552.

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

Paris, C. B., and R. K. Cowen. 2004. Direct evidence of a biophysical retention mechanism for coral reef fish larvae. *Limnology and Oceanography* **49**: 1964-1979.

Paris, C. B., L. M. Chérubin, and R. K. Cowen. 2007. Surfing, spinning, or diving from reef to reef: Effects on population connectivity. *Marine Ecology Progress Series* **347**: 285-300.

Peakall, R., and P. E. Smouse. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**: 288-295.

Phillips, B., and L. Olsen. 1975. Swimming behaviour of the puerulus larvae of the western rock lobster. *Marine and Freshwater Research* **26**: 415-417.

Phillips, B. F. 2013. Lobsters : Biology, Management, Aquaculture and Fisheries. Wiley-Blackwell.

Postaire, B., J. H. Bruggemann, H. Magalon, B. Faure, and T. Backeljau. 2014. Evolutionary Dynamics in the Southwest Indian Ocean Marine Biodiversity Hotspot: A Perspective from the Rocky Shore Gastropod Genus *Nerita*. *PLoS ONE* **9**. DOI: 10.1371/journal.pone.0095040.

R Development Core Team, R. 2011. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. 3-900051-07-0.

Rellstab, C., F. Gugerli, A. J. Eckert, A. M. Hancock, and R. Holderegger. 2015. A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology* **24**: 4348-4370.

Riccioni, G., M. Stagioni, M. Landi, G. Ferrara, G. Barbujani, and F. Tinti. 2013. Genetic structure of Bluefin Tuna in the Mediterranean Sea correlates with environmental variables. *PLoS ONE* **8**. DOI: 10.1371/journal.pone.0080105.

Ridderinkhof, W., D. Le Bars, A. S. von der Heydt, and W. P. M. de Ruijter. 2013. Dipoles of the South East Madagascar Current. *Geophysical Research Letters* **40**: 558-562.

Riginos, C., and L. Liggins. 2013. Seascape genetics: populations, individuals, and genes marooned and adrift. *Geography Compass* **7**: 197-216.

Rio, M.-H., S. Mulet, and N. Picot. 2014. Beyond GOCE for the ocean circulation estimate: Synergetic use of altimetry, gravimetry, and in situ data provides new insight into geostrophic and Ekman currents. *Geophysical Research Letters* **41**: 8918-8925.

Robins, P. E., S. P. Neill, L. Giménez, S. R. Jenkins, and S. K. Malham. 2013. Physical and biological controls on larval dispersal and connectivity in a highly energetic shelf sea. *Limnology and Oceanography* **58**: 505-524.

Rousset, F. 1997. Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. *Genetics* **145**: 1219–1228.

Saraceno, M., P. T. Strub, and P. M. Kosro. 2008. Estimates of sea surface height and near-surface alongshore coastal currents from combinations of altimeters and tide gauges. *Journal of Geophysical Research: Oceans (1978–2012)* **113**: C11013.

Schott, F. A., and J. P. McCreary. 2001. The monsoon circulation of the Indian Ocean. *Progress in Oceanography* **51**: 1-123.

Selkoe, K., K. T. Scribner, and H. M. Galindo. 2016. Waterscape genetics - applications of landscape genetics to rivers, lakes, and seas. *In: Landscape Genetics: Concepts, Methods, Applications*. N. Balkenhol, S. Cushman, A. Storfer, and L. Waits (Eds). First Edition. John Wiley & Sons. Pages 220-244.

Soria, G., A. Munguía-Vega, S. G. Marinone, M. Moreno-Báez, I. Martínez-Tovar, and R. Cudney-Bueno. 2012. Linking bio-oceanography and population genetics to assess larval connectivity. *Marine Ecology Progress Series* **463**: 159-175.

Steyn, E., and M. Schleyer. 2011. Movement patterns of the East Coast rock lobster *Panulirus homarus rubellus* on the coast of KwaZulu-Natal, South Africa. *New Zealand Journal of Marine and Freshwater Research* **45**: 85-101.

Storfer, A., M. A. Murphy, S. F. Spear, R. Holderegger, and L. P. Waits. 2010. Landscape genetics: Where are we now? *Molecular Ecology* **19**: 3496-3514.

Teske, P. R., I. Papadopoulos, B. K. Newman, P. C. Dworschak, C. D. McQuaid, and N. P. Barker. 2008. Oceanic dispersal barriers, adaptation and larval retention: an interdisciplinary assessment of potential factors maintaining a phylogeographic break between sister lineages of an African prawn. *BMC Evolutionary Biology* **8**: 341.

Teske, P. R., J. Sandoval-Castillo, E. van Sebille, J. Waters, and L. B. Beheregaray. 2015. On-shelf larval retention limits population connectivity in a coastal broadcast spawner. *Marine Ecology Progress Series* **532**: 1-12.

Teske, P. R., J. Sandoval-Castillo, E. van Sebille, J. Waters, and L. B. Beheregaray. 2016. Oceanography promotes self-recruitment in a planktonic larval disperser. *Scientific Reports* **6**: 34205.

Thomas, L. and J. J. Bell. 2013. Testing the consistency of connectivity patterns for a widely dispersing marine species. *Heredity* **111**: 345-354.

Truelove, N. K., A. S. Kough, D. C. Behringer, C. B. Paris, S. J. Box, R. F. Preziosi, and M. J. Butler. 2017. Biophysical connectivity explains population genetic structure in a highly dispersive marine species. *Coral Reefs* **36**: 233-244.

Villar, E., G. K. Farrant, M. Follows, L. Garczarek, S. Speich, S. Audic, L. Bittner, B. Blanke, J. R. Brum, C. Brunet and R. Casotti. 2015. Environmental characteristics of Agulhas rings affect interocean plankton transport. *Science* **348***:* 1261447.

Werner, F. E., R. C. Cowen, and C. B. Paris. 2007. Coupled biological and physical models: present capabilities and necessary developments for future studies of population connectivity. *Oceanography* **20**: 54-69.

Wiley, E. O. 1988. Vicariance Biogeography. *Annual Review of Ecology and Systematics* **19**: 513-542.

Wilkin, J. L., and A. G. Jeffs. 2011. Energetics of swimming to shore in the puerulus stage of a spiny lobster: Can a postlarval lobster afford the cost of crossing the continental shelf? *Limnology and Oceanography: Fluids and Environments* **1**: 163-175.

Williams, D. M., E. Wolanski, and J. C. Andrews. 1984. Transport mechanisms and the potential movement of planktonic larvae in the central region of the Great Barrier Reef. *Coral Reefs* **3**: 229-236.

Wright, S. 1943. Isolation by Distance. *Genetics* **28**: 114-38.

4.6 Supplementary information

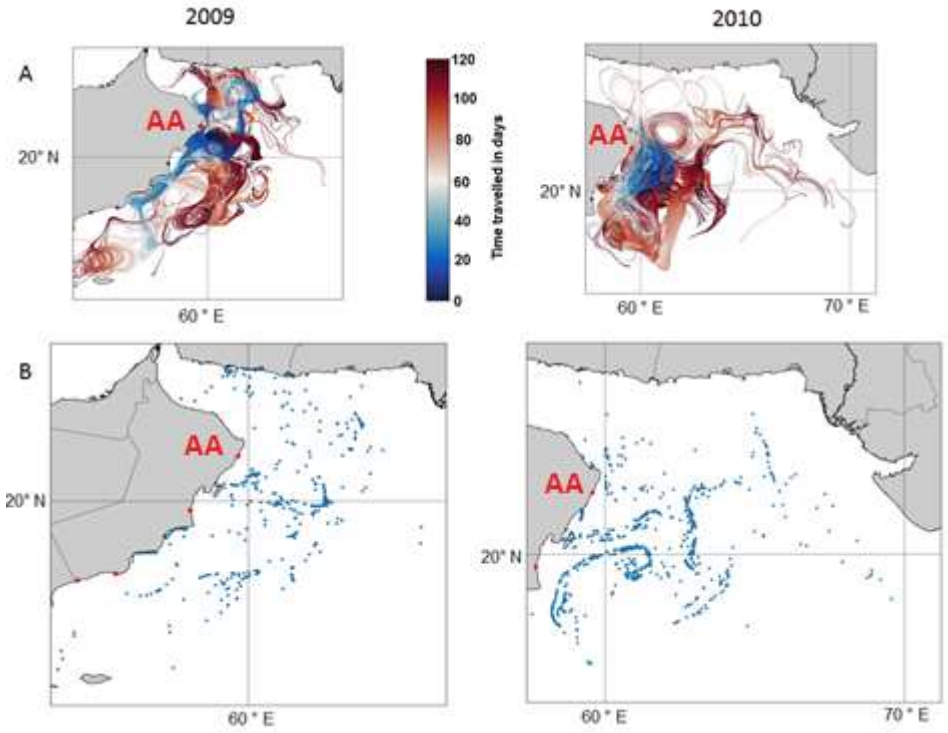


Figure S4.1 Al Ashkharah, Oman (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

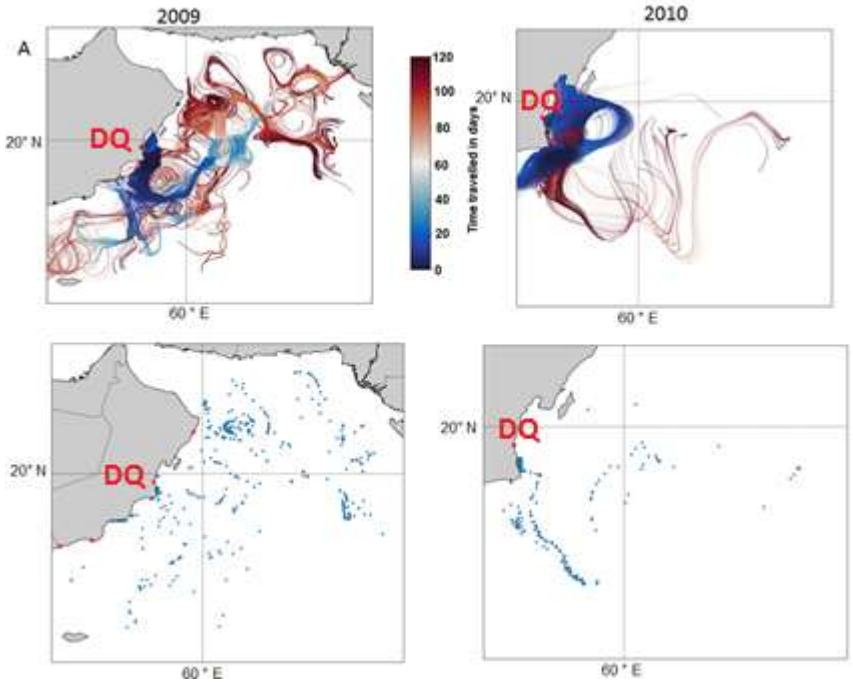


Figure S4.2 Duqm, Oman (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

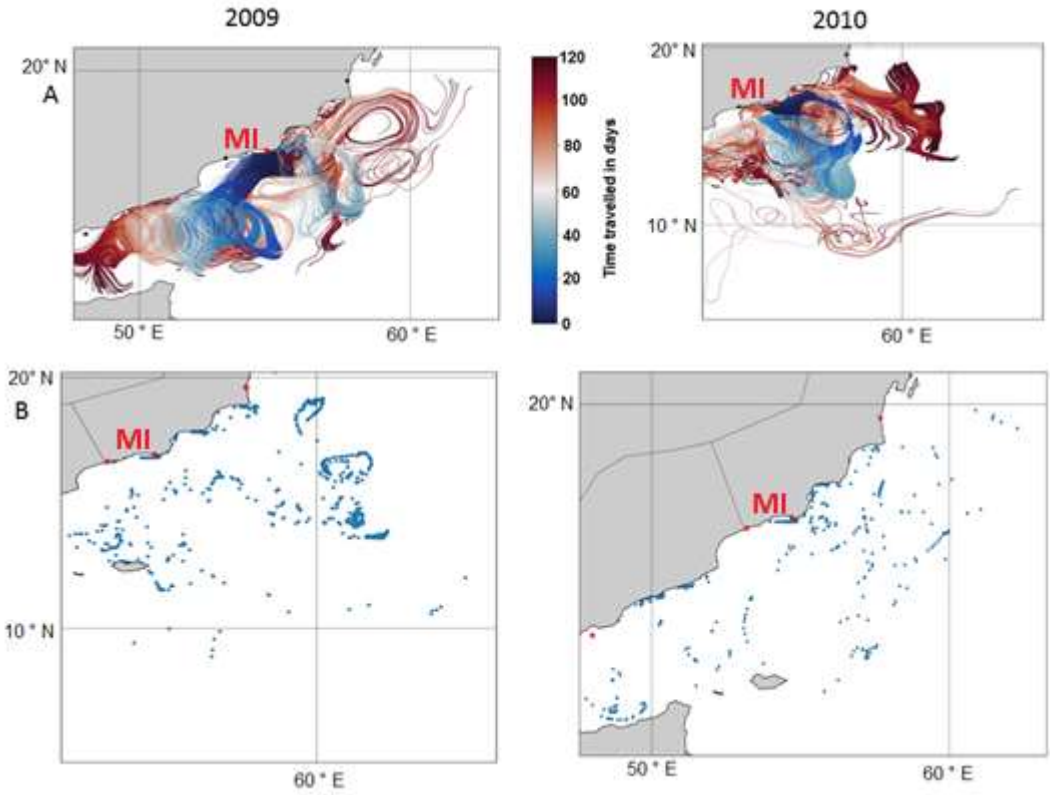
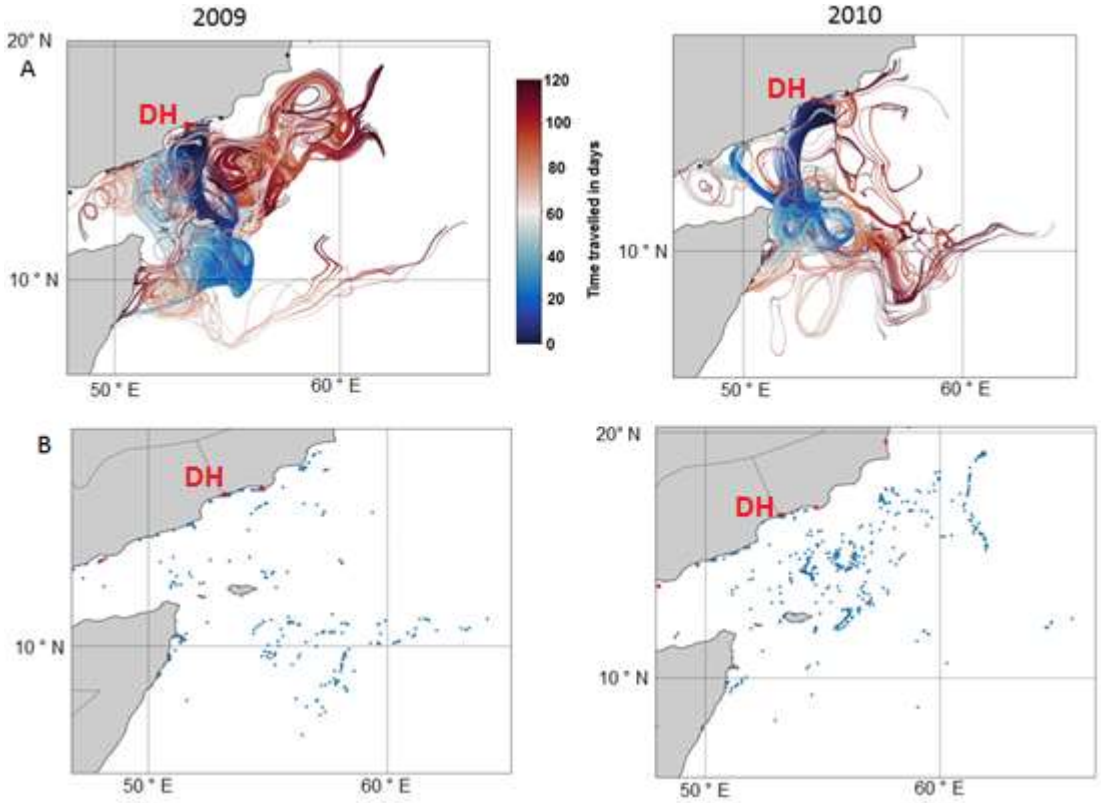


Figure S4.3 Mirbat, Oman (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.



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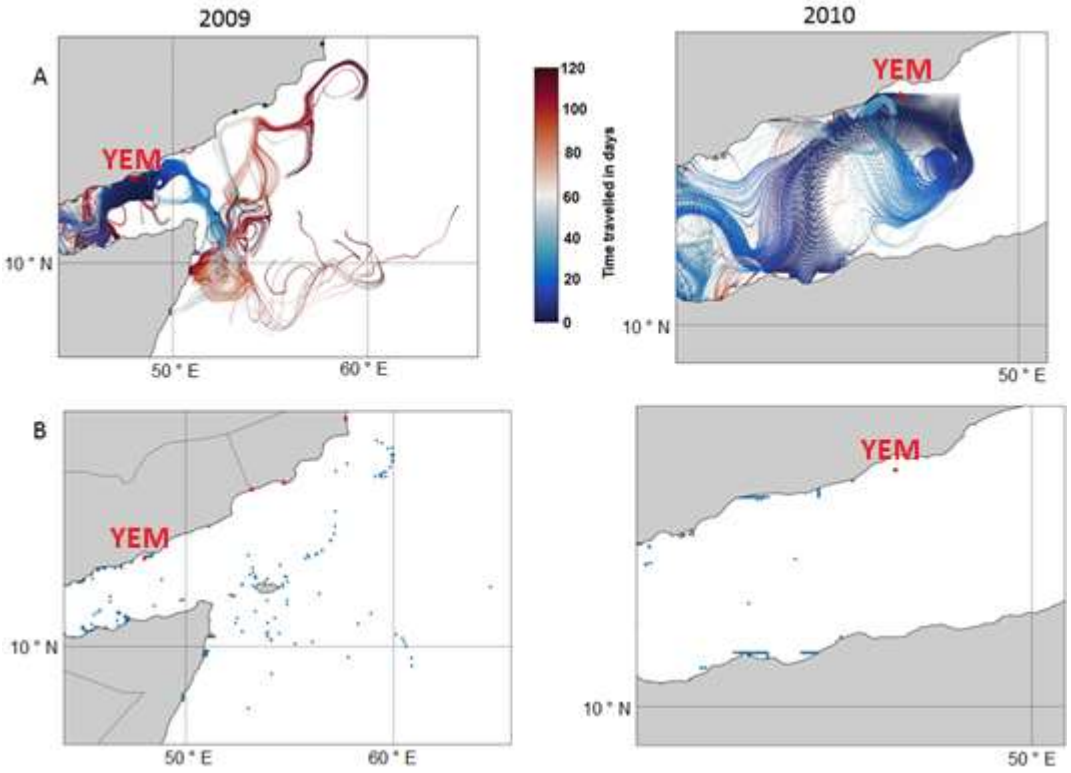

Figure S4.5 Yemen (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

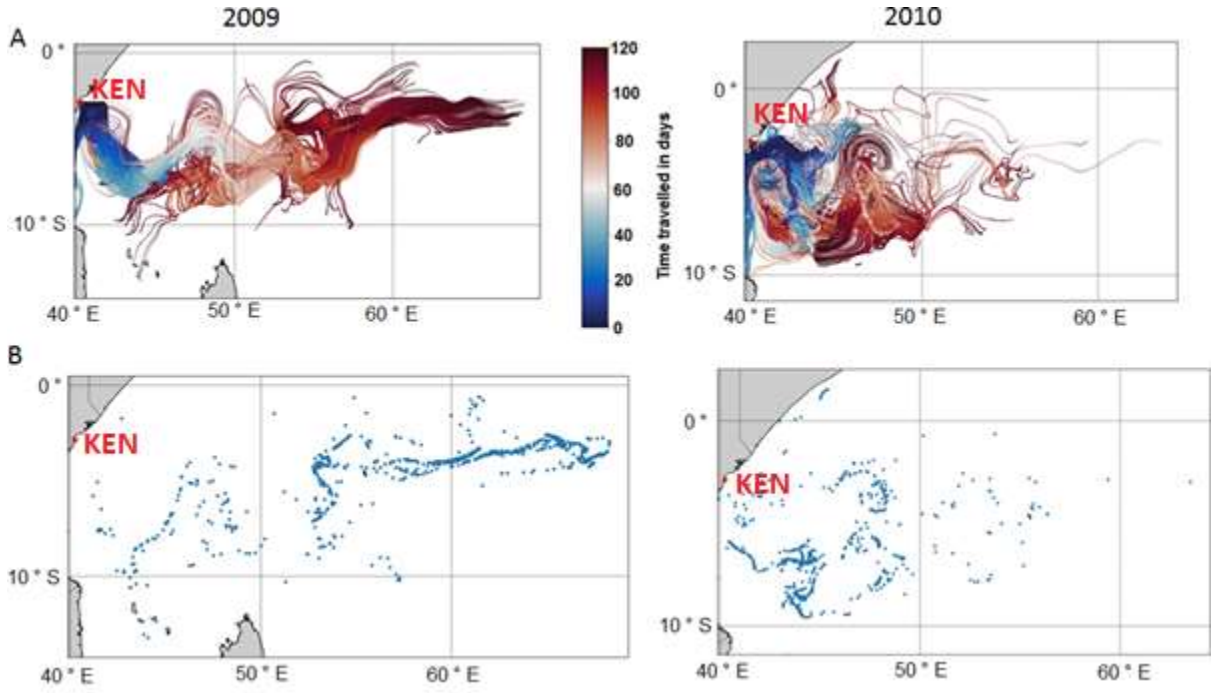

Figure S4.6 Kenya (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

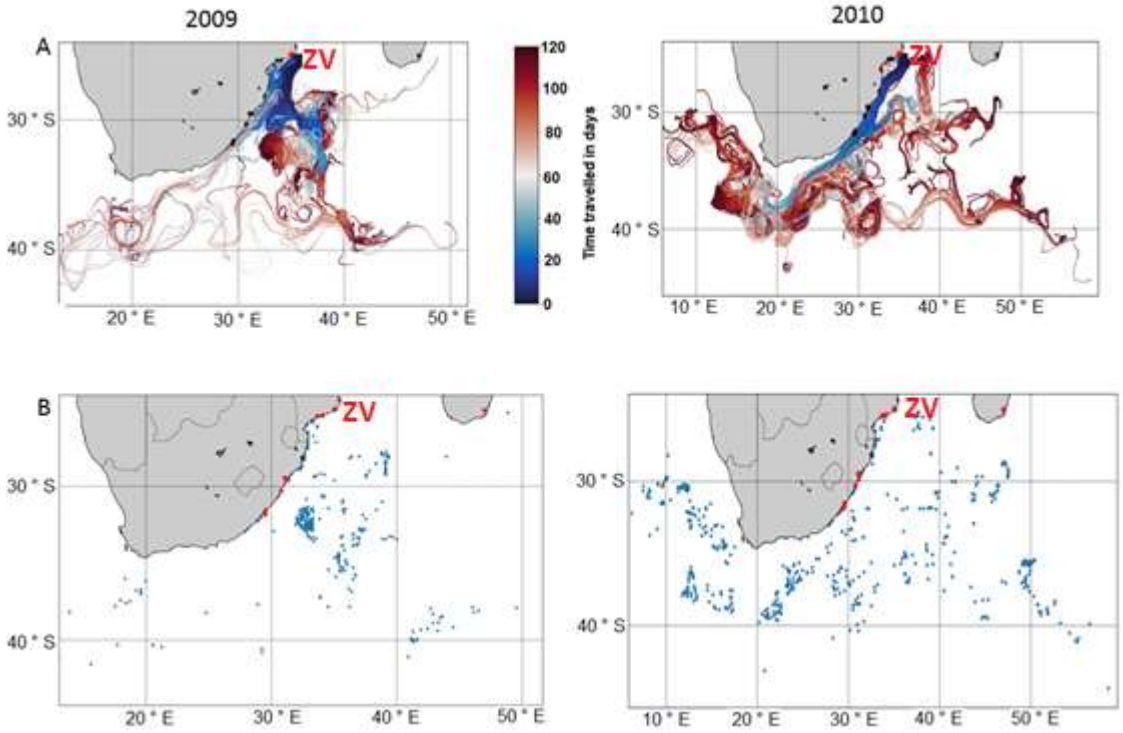

Figure S4.7 Zavora, Mozambique (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

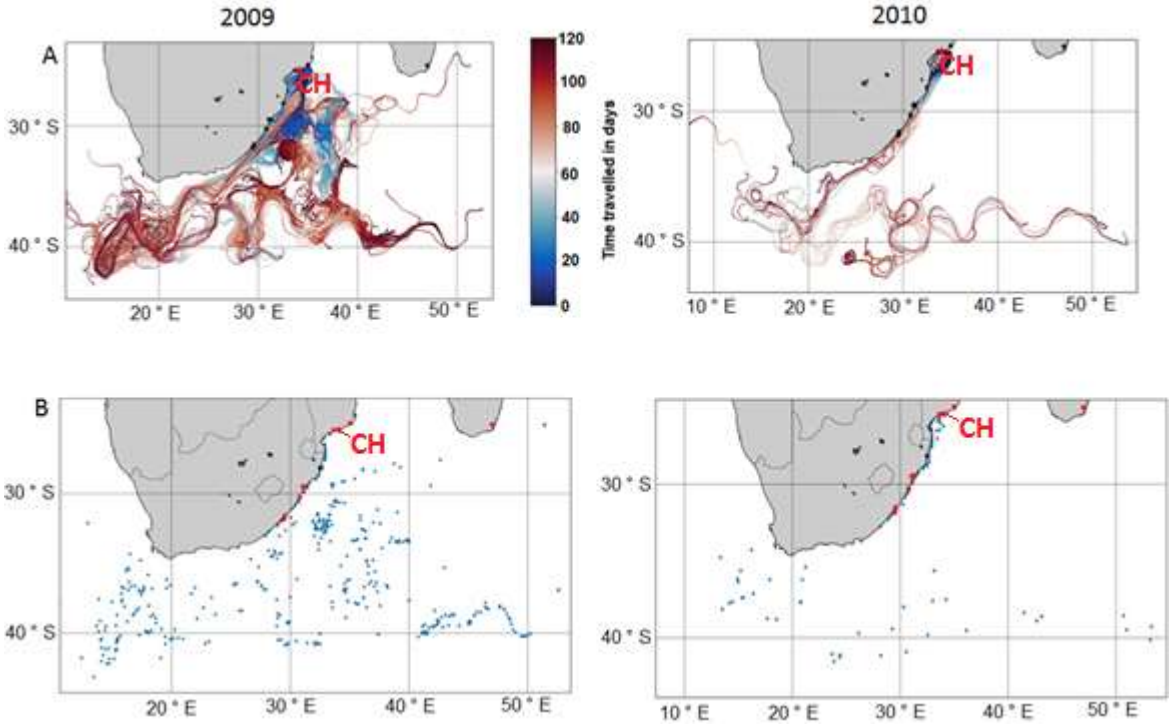

Figure S4.8 Chidenguele, Mozambique (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

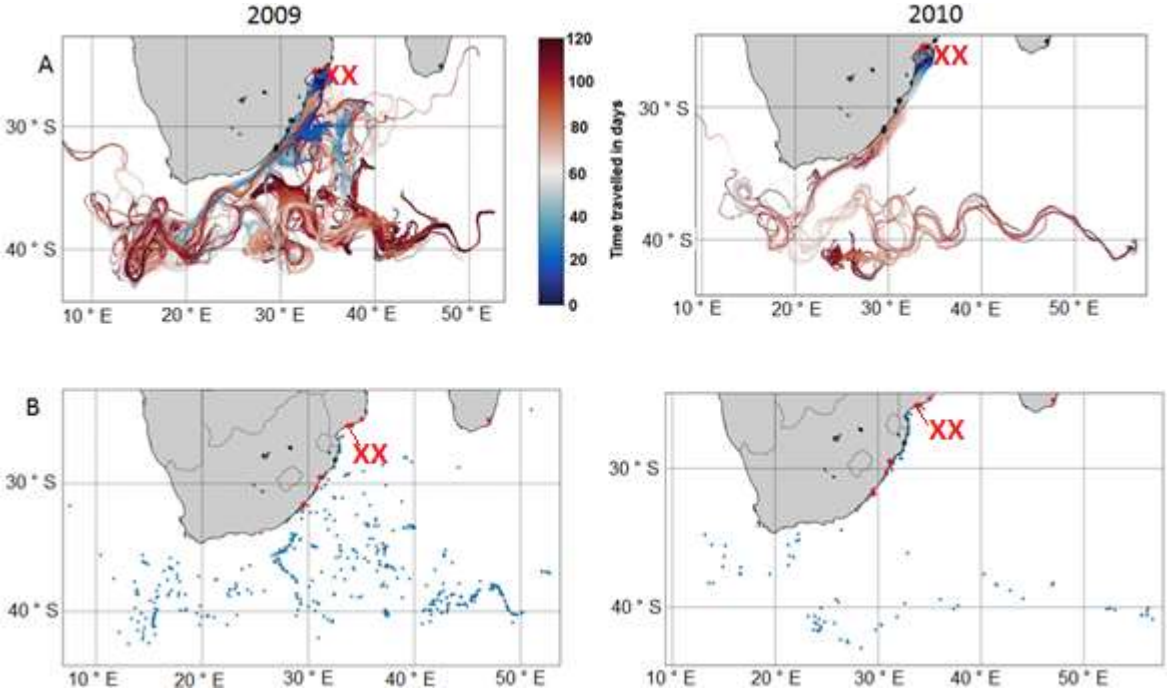

Figure S4.9 Xai Xai, Mozambique (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

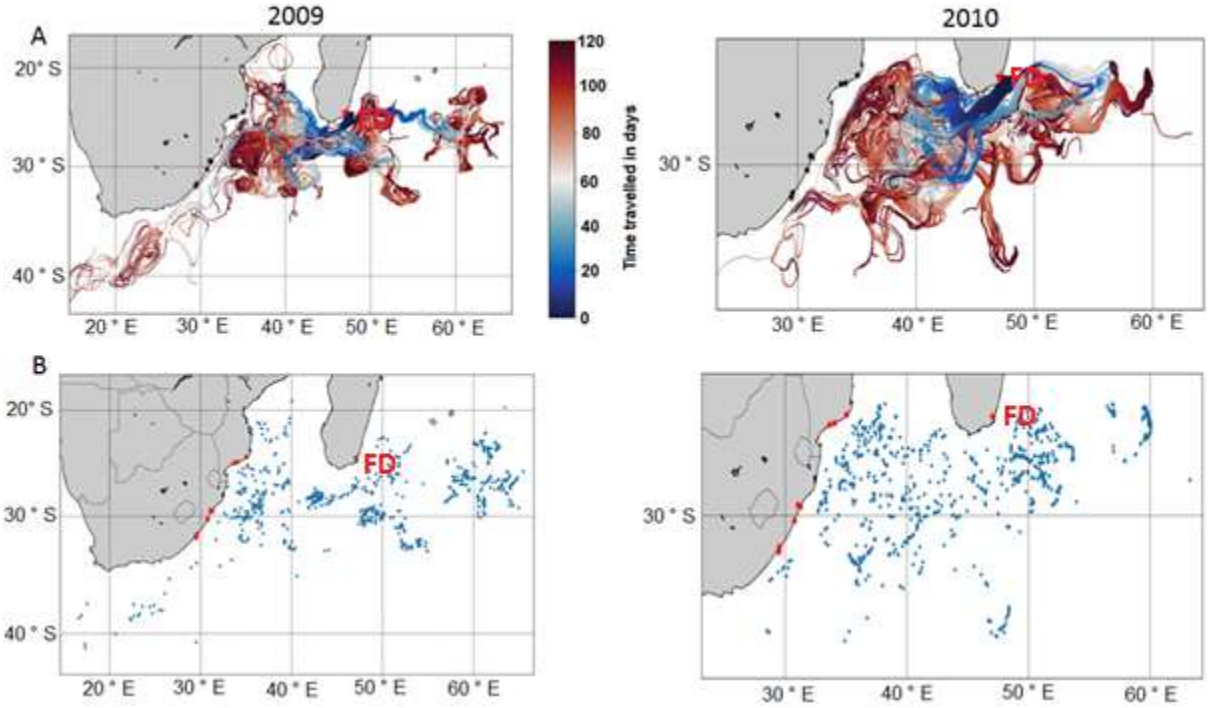

Figure S4.10 Fort Dauphin, Madagascar (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

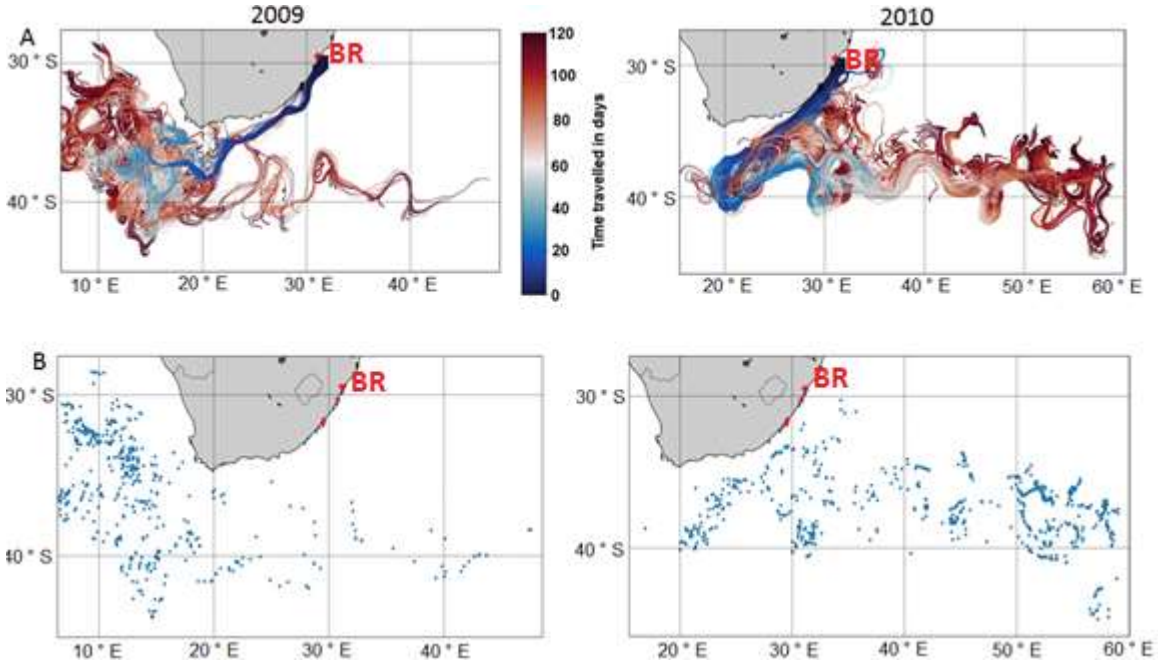

Figure S4.11 Blood Reef, South Africa (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

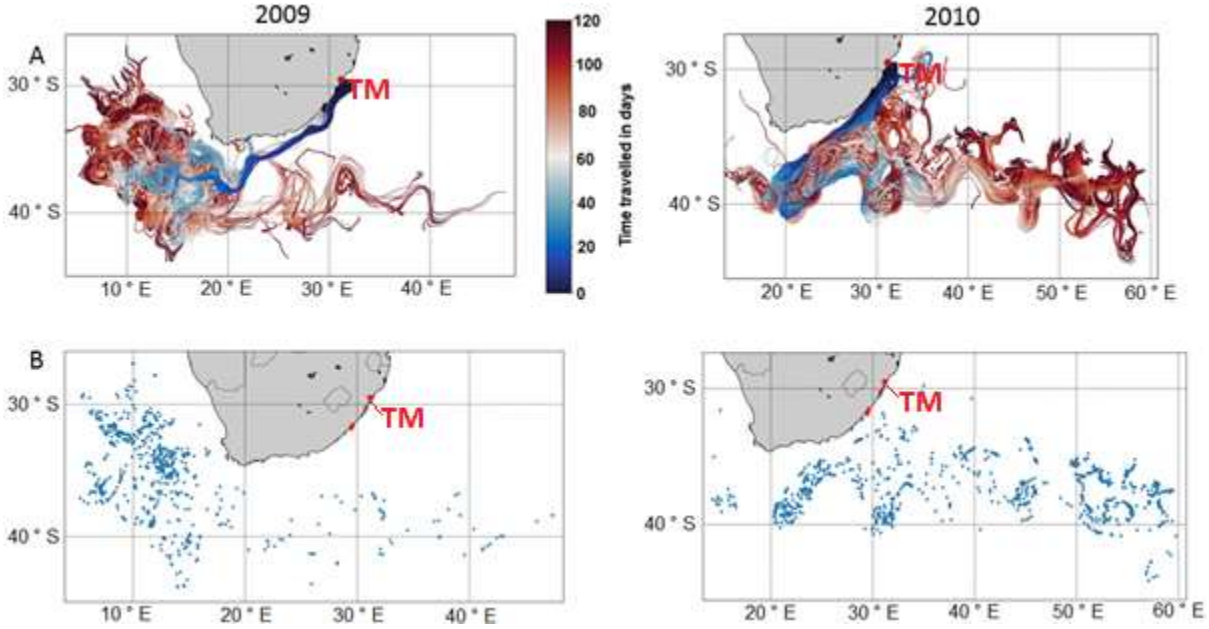

Figure S4.12 Tinley Manor, South Africa (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

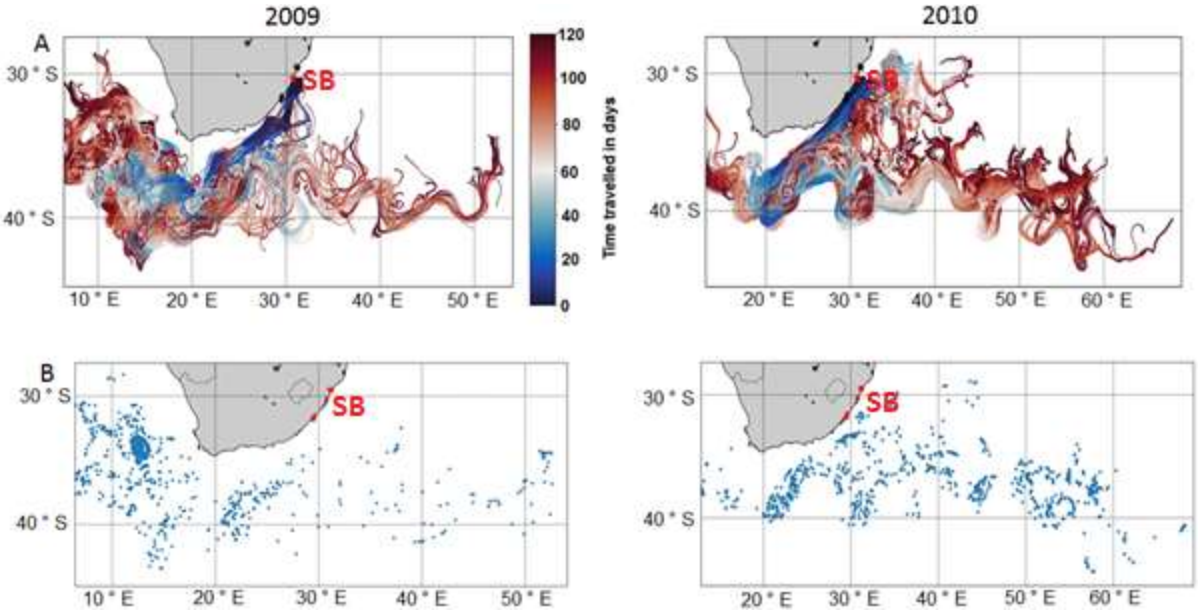

Figure S4.13 Scottburgh, South Africa (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

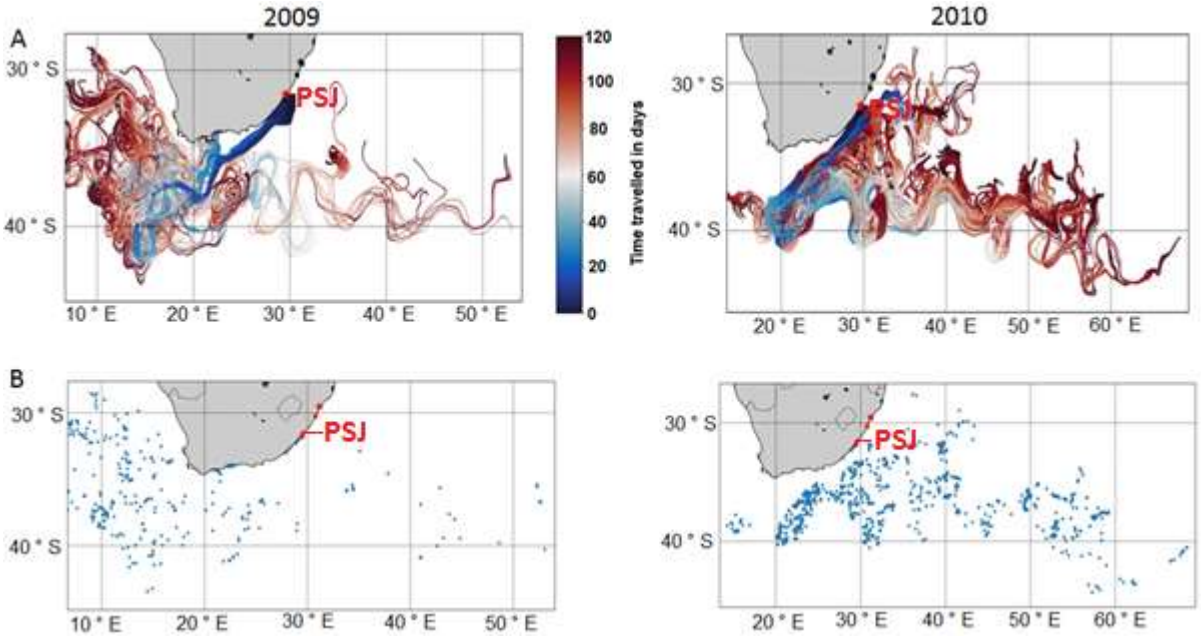

Figure S4.14 Port St Johns, South Africa (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

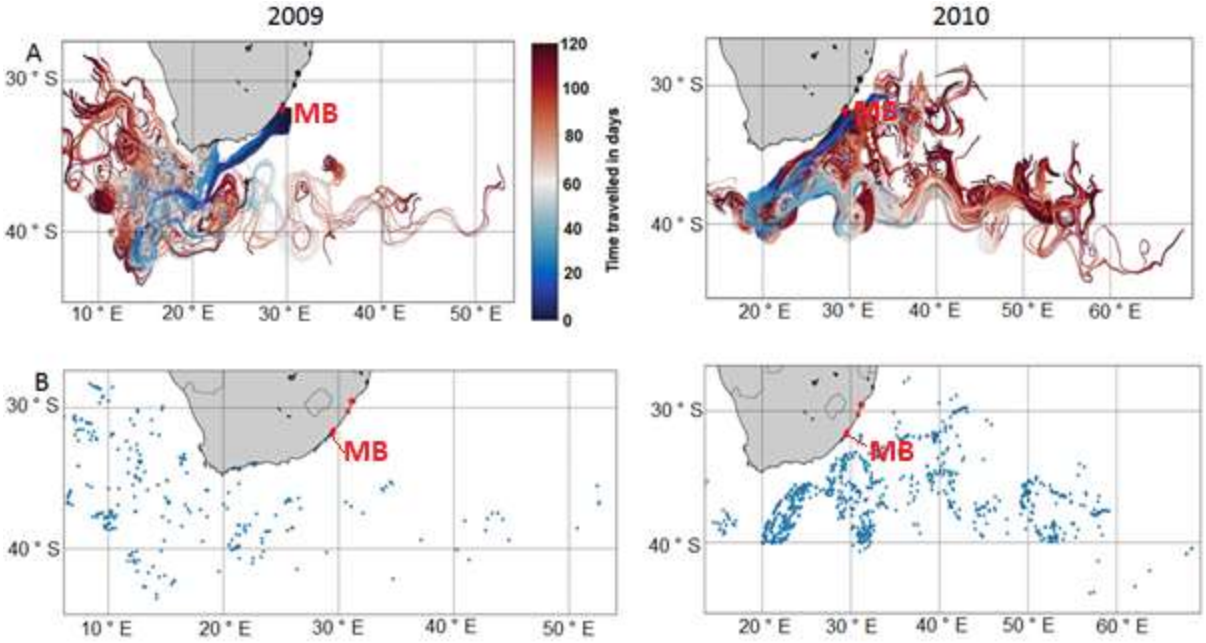

Figure S4.15 Mdumbi, South Africa (A) Particle dispersal trajectory after 120 days. (B) Final locations of particles after the simulations.

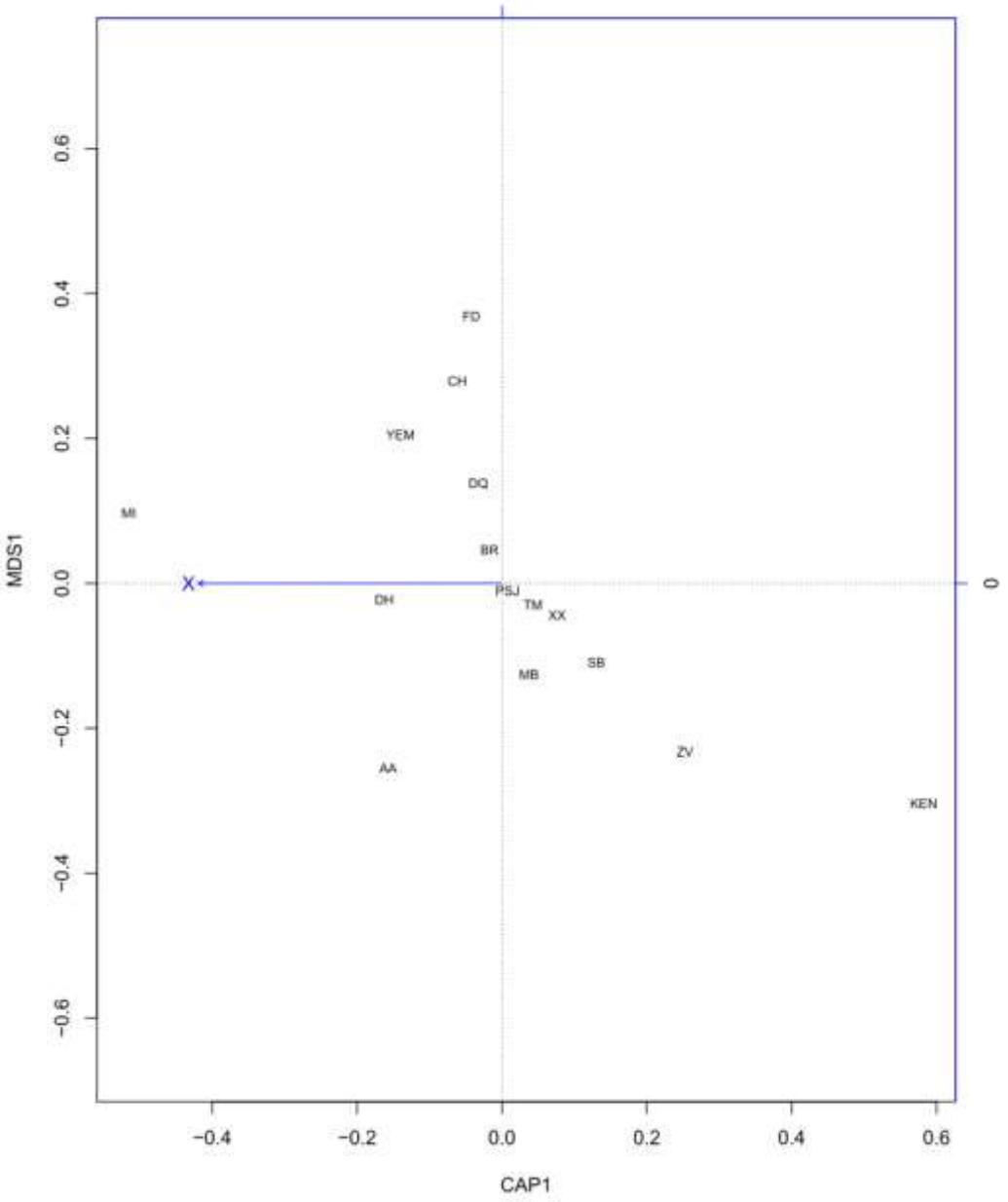

Figure S4.16 dbRDA plot of the results for model 2 with latitude conditioned on the environmental variables. The arrow indicates the direction of the maximum correlation and the length of the arrow represents the strength of the correlation. X = latitude.

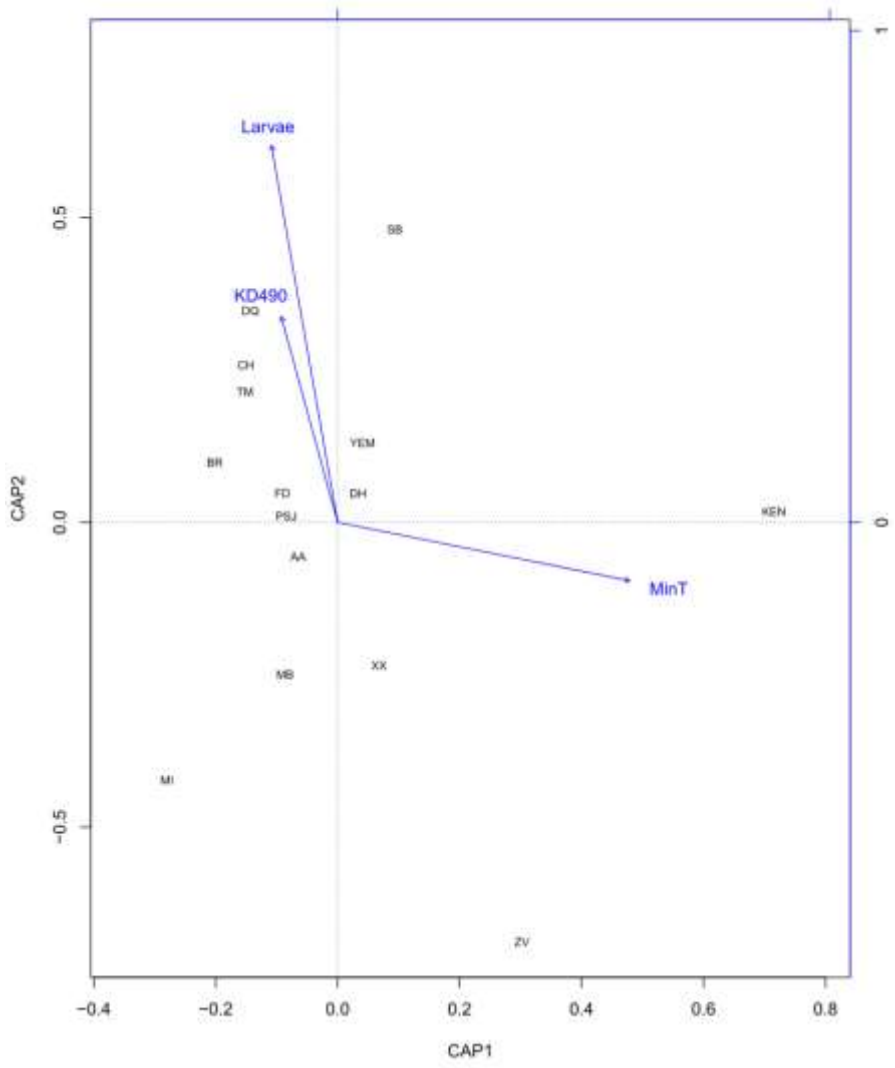

Figure S4.17 dbRDA plot of the results for model 3 with environmental variables conditioned on latitude. The arrow indicates the direction of the maximum correlation and the length of the arrow represents the strength of the correlation.

Chapter Five: General discussion and future recommendations

This PhD study presents a comprehensive genetic assessment of the scalloped spiny lobster, *Panulirus homarus* in the Western Indian Ocean, relative to the features of past and present ocean environments. It demonstrated how phylogenetics, phylogeography, population and seascape genetics can be complementary, by unravelling the historical and contemporary micro-evolutionary processes responsible for diversification. The study also shows how incorporating information from coupled-biophysical ocean circulation models (CBOCM's) and environmental data can enhance knowledge on the physical and environmental features that affect gene flow and genetic variation in marine organisms. The molecular markers used for this study were chosen to track evolutionary events across a broad temporal perspective, spanning from the Oligocene to more recent Pleistocene events, and to contemporary periods. Molecular markers evolve at different rates, and can therefore be used to discern genetic differentiation taking place during different epochs.

The sub-specific status of the three subspecies, especially *P. h. rubellus*, has come under scrutiny in recent studies (Farhadi et al. 2013 and 2017, Lavery et al. 2014, Reddy et al. 2014). Therefore, in Chapter 2, a panel of mitochondrial and nuclear intron DNA markers were used in conjunction with fossil data to examine the phylogenetic relationships between the subspecies, and to time their divergence. The results of Chapter 2 strongly supported the status of *P. h. rubellus* as a distinct species, for which the name *Panulirus rubellus* was suggested. Mitochondrial markers are considered to be ideal for detecting signatures of historical micro-evolutionary processes (Wang 2010), and were successfully used here, along with the nuclear introns, and with the divergence dating analysis to estimate that *P. h. homarus* + *P. h. megasculptus* and *P. h. rubellus* diverged from a common ancestor approximately 26 MYA, during the Oligocene. This epoch was characterized by a transition from warmer to cooler ocean temperatures, and the commencement of ocean circulation patterns observed today. The change in ocean circulation patterns may have promoted the speciation of these lineages. *P. h. rubellus* is estimated to have arisen between 10 – 16 MYA during the Miocene, possibly in response to the final closure of the Tethys Sea – another geological event which had a major impact on global ocean circulation.

A 120 m decline of the sea level during the Pleistocene had a major impact on populations of marine species that occur in shallow shelf waters, as it would have caused a reduction in the

amount of shelf habitats available and resulted in population declines. As the sea level rose when the temperature grew warmer after the glacial period, shelf habitats would have once again become available for re-colonization and subsequent population expansion (Otwoma and Kochzius 2016). Indeed, the demographic history analysis using COI and CR in Chapter 3 timed the event of a population bottleneck followed by a population expansion for *P. homarus* to have occurred within the last 100 000 years, during the Pleistocene. This epoch was noted to have had similar effects on the demographics of many other southern African spiny lobster species, including *P. gilchristi* (Tolley et al. 2005b), *P. delagoae* (Gopal et al. 2006), *J. tristani* (von der Heyden et al. 2007), *J. lalandii* (Matthee et al. 2007).

Nuclear microsatellites have a fast mutation rate which is about 10^4 times faster than mitochondrial DNA, making them useful for resolving contemporary and continuing micro-evolutionary processes (Wang 2010). Therefore, in Chapter 3, microsatellite data was used to reveal the recent past and present structure between the three subspecies of *P. homarus* and to examine connectivity between populations of *P. h. rubellus* from Mozambique, Madagascar and South Africa. Molecular genetic approaches can lead to valuable insights when coupled with other types of data and approaches, hence Chapter 4 examined the correlation of the empirical genetic data with a simple particle simulation model of larval dispersal, using a CBOCM.

Recent incipient genetic structure between *P. h. homarus* and *P. h. megasculptus* was observed with microsatellites, which was not detectible using the mitochondrial loci. This suggests that contemporary ocean circulation in the Arabian Sea – which is influenced by the seasonally reversing Somali Current and eddies along the coasts of Oman and Somalia – retains *P. h. megasculptus* larvae in that region. Particle simulations using the CBOCM supported the pattern observed in the genetic data.

Although Kenyan *P. h. homarus* lobsters were genetically indistinguishable from *P. h. homarus* in southern Mozambique (i.e. Zavora site), and gene flow analyses showed connectivity between these populations (Chapter 3), particle simulations could not reproduce a plausible oceanographic pathway linking these populations. It is possible that particles advected from unsampled locations further to the south, such as Tanzania or northern Mozambique, would be dispersed through the Mozambique Channel, to reach Zavora, or that particles seeded at a different season at the Kenyan site would be transported southwards and enter the Mozambique Channel.

In the southern hemisphere, oceanographic features are also responsible for the separation of *P. h. homarus* and *P. h. rubellus*, with the exception of a contact zone identified to occur in Mozambique. Spatially explicit genetic clustering methods using the microsatellite data identified a genetic discontinuity/ transition zone near Zavora in south-central Mozambique (Chapter 4). This contact zone may be facilitated by the Delagoa Bight lee eddy (Lutjeharms and Da Silva 1988, Lamont et al. 2010, Cossa et al. 2016) forming an intermittent barrier between *P. h. homarus* and *P. h. rubellus*, along with another seasonal anticyclonic eddy at the northern extreme of the Mozambique Channel (Donguy and Piton 1991) as identified in Chapter 3.

Mesoscale eddies are prominent ocean features that can affect genetic variability. In some cases, they have been identified as barriers to dispersal, for example, the Halmahera eddy in the Pacific Ocean forms a barrier to dispersal of stomatopod species (Barber et al. 2006). The Halmahera and Mindanao eddies restrict dispersal of coral reef sea stars *Linckia laevigata* from the Pacific Ocean to the Indo-Malay-Philippines Archipelago (Otwoma and Kochzius 2016). On the other hand, mesoscale eddies could serve as a habitat for drifting larvae, because they concentrate phyto- and zooplankton on which spiny lobster larvae can prey. These eddies are advantageous to the larvae that are entrained within them, and they are postulated to stimulate faster growth of larvae, increase their chance of survival and potentially transport larvae to suitable settlement habitats (Shulzitski et al. 2016). Another example of this is the Wairarapa eddy, which retains *J. edwardsii* larvae until they can migrate to the coast (Chiswell and Booth 1999, 2008). This could well be the case for eddies that are generated off the coast of southeast Madagascar. As detailed in Chapter 3, analysis of gene flow at all markers showed that gene flow from Madagascar to the African shelf is mainly in an east to west direction, supporting the notion that larvae are dispersed from the southeast coast of Madagascar, entrained in eddies which transport them to the southern coast of Mozambique. This eddy transport model was coined as "the suitcase hypothesis" (Marsac et al. 2014) – referring to larvae being packed into a metaphorical suitcase in Madagascar, transported in eddies across the Mozambique Channel, and then unpacked along the coasts of southern Mozambique and eastern South Africa. Genetic data and CBOCM larval dispersal simulations supported this hypothesis in the present study – showing both genetic connectivity and that larvae can be transported across the channel by water movements.

Overall, the results from Chapter 3 and 4 demonstrate that populations of *P. homarus* in the Western Indian Ocean are capable of self-recruitment and dispersal to neighbouring sites via

oceanic transport. Seascape genetic analyses of the effect of environmental variables on genetic differentiation showed that SST along a latitudinal gradient is responsible for some of the observed genetic differentiation in *P. homarus* (Chapter 4), and that it can partially explain the diversification into the subtropical *P. h. rubellus* lineage in the Southwest Indian Ocean and the more tropical *P. h. homarus* lineage occurring at lower latitudes.

5.1 Future possibilities

Marine protected areas (MPAs) are an essential component of fisheries management and marine conservation, because they can protect populations of species and the ecosystems that they inhabit (Palumbi 2003, Pujolar et al. 2013). Successful, sustainable MPAs need to account for contemporary larval dispersal – the rate at which larvae are retained in MPAs or transported to non-protected areas, and whether there is larval flow among MPA networks (Palumbi 2003, Botsford et al. 2009). This PhD study demonstrated that molecular genetic analyses can provide an indirect measure of connectivity between marine populations, and can infer exchange rates between populations through gene flow analyses (Chapter 3).

Designing and scaling marine reserves appropriately can also be informed by CBOCMs and Lagrangian particle tracking tools (Werner et al. 2007), and in combination with genetic information (as in this study), can be invaluable in designing MPAs. Other studies have also uncovered the benefits of using genetic analyses and particle tracking using CBOCMs, to assess how important MPAs are in sustaining populations of important marine species (Planes et al. 2009, Harrison et al. 2012).

Linking genetic information to fisheries management plans is still in its infancy, and so is implementing management plans based on the genetic information provided. These types of studies, such as seascape genetics, have the potential to lend answers to important questions and unknowns facing fisheries management and conservation, and also pose new and thought-provoking questions to investigate. As pointed out in Chapter 3, the Delagoa Bight, where the contact zone between *P. h. rubellus* and *P. h homarus* is located, provides an opportunity to investigate a potential biogeographic barrier.

The CBOCM showed high inter-annual variability in particle dispersal when comparing tracks of particles released in January 2009 and at the same time in 2010. Releasing particles

during other months or seasons will presumably add further seasonal variability to dispersal patterns. Hence, there is a need for more sophisticated, higher resolution CBOCM's, incorporating larval behaviour patterns (discussed in detail in Chapter 4) to be developed for the highly dynamic ocean circulation patterns of the Southwest Indian Ocean. Refined models can assist in clarifying subtle effects of larval dispersal on the genetic structure of African marine taxa. Ocean currents are highly variable on seasonal and inter-annual scales, and hence their larval dispersal patterns will depend on when and where they are released. Simulations therefore need to cover different seasons and years, to provide a more detailed assessment of potential larval dispersal routes.

5.2 Conclusion

The recurrent patterns emerging from all lines of evidence in this study (genetic, oceanography and environment) are that climatic fluctuations, which affect ocean currents and other oceanic variables, are in turn responsible for genetic connectivity or diversity. These observations can enhance knowledge of how these factors impact on adaptation and speciation, which can facilitate more informed predictions on how changing climates may impact the diversity of marine life.

5.3 References

Barber, P. H., M. V Erdmann, and S. R. Palumbi. 2006. Comparative phylogeography of three codistributed stomatopods: Origins and timing of regional lineage diversification in the Coral Triangle. *Evolution* **60**: 1825-1839.

Botsford, L. W., D. R. Brumbaugh, C. Grimes, J. B. Kellner, J. Largier, M. R. O 'Farrell, S. Ralston, E. Soulanille, and V. Wespestad. 2009. Connectivity, sustainability, and yield: bridging the gap between conventional fisheries management and marine protected areas. *Reviews in Fish Biology and Fisheries* **19**: 69-95.

Chiswell, S. M., and J. D. Booth. 1999. Rock lobster *Jasus edwardsii* larval retention by the Wairarapa Eddy off New Zealand. *Marine Ecology Progress Series* **183**: 227-240.

Chiswell, S. M., and J. D. Booth. 2008. Sources and sinks of larval settlement in *Jasus edwardsii* around New Zealand: Where do larvae come from and where do they go? *Marine Ecology Progress Series* **354**: 201-217.

Cossa, O., S. Pous, P. Penven, X. Capet, and C. J. C. Reason. 2016. Modelling cyclonic eddies in the Delagoa Bight region. *Continental Shelf Research* **119**: 14-29.

Donguy, J.-R., and B. Piton. 1991. The Mozambique Channel revisited. *Oceanologica Acta* **14**: 549-558.

Farhadi, A., H. Farahmand, M. A. Nematollahi, A. G. Jeffs, and S. D. Lavery. 2013. Mitochondrial DNA population structure of the scalloped lobster *Panulirus homarus* (Linnaeus 1758) from the West Indian Ocean. *ICES Journal of Marine Science* **70**: 1491-1498.

Farhadi, A., A. G. Jeffs, H. Farahmand, T. S. Rejiniemon, G. Smith and S. D. Lavery. 2017. Mechanisms of peripheral phylogeographic divergence in the Indo-Pacific: lessons from the spiny lobster *Panulirus homarus*. *BMC Evolutionary Biology* **17**: 195.

Gopal, K., K. A. Tolley, J. C. Groeneveld, and C. A. Matthee. 2006. Mitochondrial DNA variation in spiny lobster *Palinurus delagoae* suggests genetically structured populations in the southwestern Indian Ocean. *Marine Ecology Progress Series* **319**: 191-198.

Harrison, H. B., D. H. Williamson, R. D. Evans, G. R. Almany, S. R. Thorrold, G. R. Russ,

K. A. Feldheim, L. Van Herwerden, S. Planes, M. Srinivasan, M. L. Berumen, and G. P. Jones. 2012. Larval export from marine reserves and the recruitment benefit for fish and fisheries. *Current Biology* **22**: 1023-1028.

Lamont, T., M. J. Roberts, R. G. Barlow, T. Morris, and M. A. van den Berg. 2010. Circulation patterns in the Delagoa Bight, Mozambique, and the influence of deep ocean eddies. *African Journal of Marine Science* **32**: 553-562.

Lavery, S. D., A. Farhadi, H. Farahmand, T. Y. Chan, A. Azhdehakoshpour, V. Thakur, and A. G. Jeffs. 2014. Evolutionary divergence of geographic subspecies within the scalloped spiny lobster *Panulirus homarus* (Linnaeus 1758). *PLoS ONE* **9**. DOI: 10.1371/journal.pone.0097247.

Lutjeharms, J. R. E., and J. da Silva. 1988. The Delagoa Bight eddy. *Deep Sea Research II* **35**: 619-634.

Marsac, F., R. Barlow, J. F. Ternon, F. Ménard, and M. Roberts. 2014. Ecosystem functioning in the Mozambique Channel: Synthesis and future research. *Deep-Sea Research Part II: Topical Studies in Oceanography* **100**: 212-220.

Matthee, C. A., A. C. Cockcroft, K. Gopal, and S. von der Heyden. 2007. Mitochondrial DNA variation of the west-coast rock lobster, *Jasus lalandii*: marked genetic diversity differences among sampling sites. *Marine and Freshwater Research* **58**: 1130-1135.

Otwoma, L. M., and M. Kochzius. 2016. Genetic population structure of the Coral Reef Sea Star *Linckia laevigata* in the Western Indian Ocean and Indo-West Pacific. *PLoS ONE* **11**. DOI: 10.1371/journal.pone.0165552.

Palumbi, S. R. 2003. Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications* **13**: 146-158.

Planes, S., G. P. Jones, and S. R. Thorrold. 2009. Larval dispersal connects fish populations in a network of marine protected areas. *Proceedings of the National Academy of Sciences of the United States of America* **106**: 5693-5697.

Pujolar, J. M., M. Schiavina, A. Di Franco, P. Melià, P. Guidetti, M. Gatto, G. A. De Leo, and L. Zane. 2013. Understanding the effectiveness of marine protected areas using genetic connectivity patterns and Lagrangian simulations. *Diversity and Distributions*

19: 1531-1542.

Reddy, M. M., A. H. H. MacDonald, J. C. Groeneveld, and M. H. Schleyer. 2014. Phylogeography of the scalloped spiny-lobster *Panulirus homarus rubellus* in the Southwest Indian Ocean. *Journal of Crustacean Biology* **34**: 773-781.

Shulzitski, K., S. Sponaugle, M. Hauff, K. D. Walter, and R. K. Cowen. 2016. Encounter with mesoscale eddies enhances survival to settlement in larval coral reef fishes. *Proceedings of the National Academy of Sciences of the United States of America* **113**: 6928-6933.

Tolley, K. A., J. C. Groeneveld, K. Gopal, and C. A. Matthee. 2005. Mitochondrial DNA panmixia in spiny lobster *Palinurus gilchristi* suggests a population expansion. *Marine Ecology Progress Series* **297**: 225-231.

von der Heyden, S., J. Groeneveld, and C. Matthee. 2007. Long current to nowhere? — Genetic connectivity of *Jasus tristani* populations in the southern Atlantic Ocean. *African Journal of Marine Science* **29**: 491-497.

Wang, I. J. 2010. Recognizing the temporal distinctions between landscape genetics and phylogeography. *Molecular Ecology* **19**: 2605-2608.

Werner, F. E., R. C. Cowen, and C. B. Paris. 2007. Coupled biological and physical models: present capabilities and necessary developments for future studies of population connectivity. *Oceanography* **20**: 54-69.

Appendix 1