Genetic population structure of deep-water prawns *Haliporoides triarthrus* and langoustines *Metanephrops mozambicus* in the South West Indian Ocean: use of mitochondrial DNA to investigate metapopulation structure

By

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requirements for the degree of
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As the candidate's supervisor I have/have not approved this thesis/dissertation for submission.

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## 1.1 ABSTRACT

Deep-water prawns *Haliporoides triarthrus* and langoustines *Metanephrops mozambicus* are endemic to the South West Indian Ocean (SWIO) region and make up the largest proportion of deep-water crustacean trawl catches in Mozambique and South Africa. Despite their economic importance to these fisheries, little is known about their distribution, biology and genetic population structure.

The metapopulation genetic variation of *H. triarthrus* and *M. mozambicus* was assessed from 220 specimens per species collected from three sites in Mozambique (Bazaruto A, Boa Paz and Inhaca), two sites in western Madagascar (Morombe and Tulear) and one site in eastern South Africa (Durban). Two fragments of the mitochondrial region were amplified using universal primers ribosomal 16S subunit (16S) and mitochondrial cytochrome oxidase subunit I (COI). From *H. triarthrus,* fragments of 569 base pair (bp) (16S) and 1300 bp (COI) were amplified. A total of 207 sequences (16S) and 151 sequences (COI) were recovered, and 69 and 78 haplotypes identified, respectively. *Metanephrops mozambicus* 16S and COI genes produced similar fragment lengths, and 112 (16S) and 127 haplotypes (COI) were recovered.

Both species demonstrated high genetic diversity and significant population differentiation in the SWIO region. Two sister-species (or subspecies) of *H. triarthrus* were identified, one occurring along the African continental shelf and the other off western Madagascar. Furthermore, individual populations making up each lineage were genetically structured, as indicated by the absence of shared haplotypes, and should be recognized as demographically distinct subspecies. Both species have undergone recent population expansions, likely since the late Pleistocene.

The large anti-cyclonic and cyclonic eddies prevalent in the Mozambique Channel, and the boundary area between these eddies and upper Agulhas Current are likely factors driving larval retention or return process, thus giving rise to the observed genetically structured populations.

The findings from this study are unique for the SWIO region, and may lead to a paradigm shift in the way that deep-water crustacean stocks are perceived by fisheries managers – instead of single shared stocks, they comprise of many isolated ones, in spite of the dispersal potential of larvae in strong ocean current regimes. Thus stocks should be managed as small independent units.

## 1.2 PREFACE

The experimental work described in this dissertation was carried out in the School of Life Sciences, University of Natal, Durban, from August 2011 to April 2013, under the supervision of Dr. Angus MacDonald.

These studies represent original work by the author and have 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 acknowledged in the text.

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## 1.3 DECLARATION 1 - PLAGIARISM

I, Lourenço Zacarias declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

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3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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## 1.4 DECLARATION 2 – PUBLICATIONS

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Author contribution: L. Zacarias designed the study, performed the laboratory work for an aspect of the above presentation as did the other authors. A.H.H Macdonald developed the presentation and presented the final product and M.H. Schleyer, J.M. Lamb and J.C. Groeneveld co-authored and provided supervision.

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**Nhi nbongide**

**Nzi nbongile**

**Kanimanbo**

**Obrigado**

**Thanks**

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# Chapter One

## 1. Introduction

### *1.1 Background*

Deep-water crustaceans are valuable natural resources in the South West Indian Ocean (SWIO) region, where they are fished by bottom trawling at 100 - 700 m depth (Brinca *et al.* 1983; De Freitas 1985). Bottom trawl catches at these depths generally comprise a mixture of crustacean species (including the targeted species), as well as a bycatch of fish, sharks and cephalopods, which are either retained and sold or are discarded at sea if considered of low value (Fennessy & Groeneveld 1997). The distribution patterns of crustaceans overlap by depth and area, nevertheless, trawl skippers can target the most valuable species mix (in terms of quantity and value) by trawling at selected areas, depths and / or substrate types (Groeneveld & Melville-Smith 1995).

The most commonly caught crustacean species from the 100 – 700 m depth range in the SWIO are *Haliporoides triarthrus* (called knife prawns or pink prawns), *Aristaeomorpha folicea* (giant red shrimp), *Aristeus antennatus* (blue and red shrimp), *Aristeus virilis* (stout red shrimp), *Metanephrops mozambicus* (langoustine or African lobster), *Nephropsis stewarti* (Indian Ocean lobsterette), *Palinurus delagoae* (deep-water spiny lobster) and *Chaceon macphersoni* (deep-sea geryonid crab) (De Freitas 1985; Groeneveld & Melville-Smith 1995; Dias & Caramelo 2005, 2007). *Haliporoides triarthrus* and *M. mozambicus* make up most of the deep-water trawl catches in Mozambique and South Africa, and are endemic to the SWIO region (De Freitas 1985; Groeneveld 2012); they were therefore selected as study species.

### *1.2 Study species*

a) *Haliporoides triarthrus* (Stebbing 1914):

The genus *Haliporoides* (and the species *H. triarthrus*) was first described by Stebbing (1914), and prawns from this genus belong to the decapod family *Solenoceridae* (Farfante 1977; Holthuis 1980). De Freitas (1985) reported only three species from the genus: *H. triarthrus* from southern Africa and Madagascar see below (Fig. 1.1); *H. diomedea* from the Eastern Pacific; and *H. sibogae* from Eastern Australia (Baelde 1992, 1994). Further taxonomic study by Kensley *et al.* (1987) recognized at least six species and subspecies: *H. cristatus* (Eastern Australia); *H. diomedeae* (Chilean knife shrimp); *H. sibogae sibogae* (Jack-knife shrimp); *H. sibogae australiensis*; *H. sibogae madagascariensis*; and *H. triarthrus*. Two subspecies of *H. triarthrus* are presently recognized from the SWIO region, *H. triarthrus triarthrus* from South Africa and Mozambique, and *H. triarthrus vniroi* from Madagascar and Mozambique (Kensley *et al.* 1987; Pérez-Farfante & Kensley 1997; De Grave & Fransen 2011). First records of deep-water stocks of *H. triarthrus* in South Africa date from marine biological surveys undertaken during the early 1920s (Gilchrist 1920; Fennessy & Groeneveld 1997).

*Haliporoides triarthrus* occurs on the continental slope at depths of 300 to 800 m, but is more commonly fished between 400 and 600 m (Brinca *et al.* 1983; Torstensen & Pacule 1992; Groeneveld & Melville-Smith 1995; Dias & Caramelo 2005, 2007). Between 1990 and 2010, the majority (91%) of catches of *H. triarthrus* originated from Mozambique (26 153t) and South Africa contributed 9% (2 576t) to reported catches (Groeneveld 2012). Regular stock assessments of *H. triarthrus* in Mozambique have been carried out (Sobrino *et al.* 2007; Dias *et al.* 2008; Dias *et al.* 2009; Dias *et al.* 2011). Landings from South African fishing grounds have fluctuated between 50 and 300 t/y, and relative abundance trends over a 23 year period have recently been determined by Robey (2013).

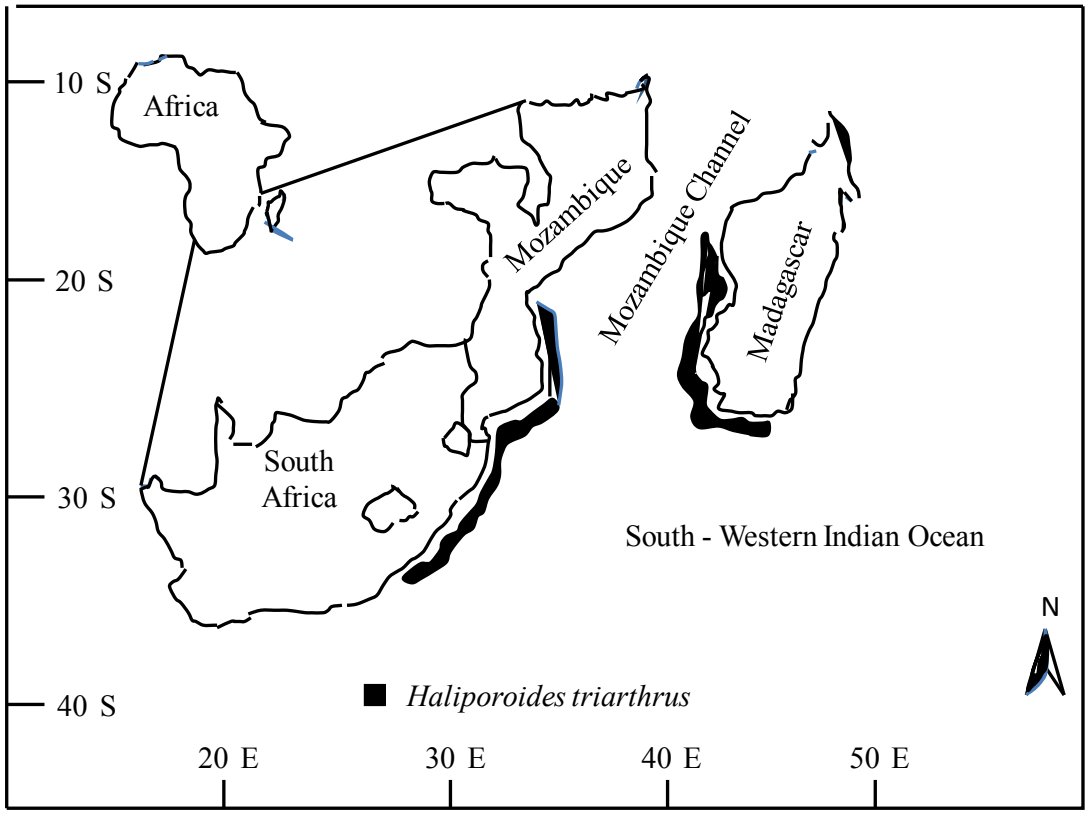


**Figure 1.1** The geographic distribution of *Haliporoides triarthrus* in the Western Indian Ocean. The black line shows the occurrence of *Haliporoides triarthrus*. Image modified from (Berry *et al.* 1975; Holthuis 1980; De Freitas 1985).

Despite the economic importance of *H. triarthrus* to deep-water trawl fisheries in the SWIO region, little is known about its biology and population dynamics (Groeneveld 2012). Females can achieve a maximum size of >60 mm carapace length (CL) and males >50 mm (Holthuis 1980; Brinca *et al.* 1983; Torstensen 1989; Robey 2013); total lengths of up to 150 mm have been reported (De Freitas 1985). Von Bertalanffy growth parameters ($L\infty$ and K) have been estimated for Mozambique (Dias *et al.* 2011) and South Africa (Robey 2013) and these authors have also provided length-weight parameters. Mozambique's smallest mature females measured 29 - 30 mm CL, and 50% maturity was estimated at 41 - 42 mm (Dias & Caramelo 2007; Dias *et al.* 2011). Yet, a much larger size at sexual maturity was described in South Africa; ranging between 37 and 38 mm CL, and 50% of females

matured at 49 - 50 mm (Berry *et al.* 1975; Robey *et al.* 2011). Males matured at approximately 31 - 32 mm CL (Dias *et al.* 2011; Robey *et al.* 2011).

Observations of ovarian activity suggested that females breed mostly in spring and early summer (August to December) (Berry *et al.* 1975; Groeneveld 2012); in Mozambique spawning occurred mainly in the last trimester of the year with peaks in November and December (Dias & Caramelo 2005, 2007). Abundant catches of small prawns (recruits) were reported in August and September and in January and February in Mozambique (Berry *et al.* 1975; Brinca *et al.* 1983). Most recruitment of *H. triarthrus* occurred in Bazaruto and Inhaca, and the highest proportions of mature females were found at Bazaruto, suggesting a spawning area (Berry *et al.* 1975; Brinca *et al.* 1983). Life span estimates ranged from 2 - 3 years (Berry *et al.* 1975; Holthuis 1980; De Freitas 1985; Dias & Caramelo 2005) to 5 - 6 years (Dias & Caramelo 2007; Dias *et al.* 2011).

b) *Metanephrops mozambicus* (Macpherson 1990)

The genus *Metanephrops* Jenkins (1972) has a cosmopolitan distribution and 17 living species are recognized (Griffin & Stoddart 1995; Tshudy *et al.* 2007). Holthuis (1991) decribed two species of importance to fisheries from the SWIO region: *Metanephrops andamanicus* from East Africa (Kenya and Tanzania) and *Metanephrops mozambicus* from Kenya to eastern South Africa, and off Madagascar (Fig. 1.2). The latter species inhabits the continental shelf and slope at depths of 200 to 750 m (mostly 400 to 500 m) (Berry 1969; Stentiford & Neil 2011). It is unclear whether, or where, the distributions of the two species overlap, because they are similar in appearance, and distinguishing between them during field sampling is problematic. *Metanephrops mozambicus* inhabits areas of sticky mud, in which it burrows (Macpherson 1990; Fennessy & Groeneveld 1997; Dias & Caramelo 2005, 2007). Males become slightly larger than females (88 mm versus 83 mm CL) with a maximum total length of 205 mm (Holthuis 1991; Groeneveld 2012).

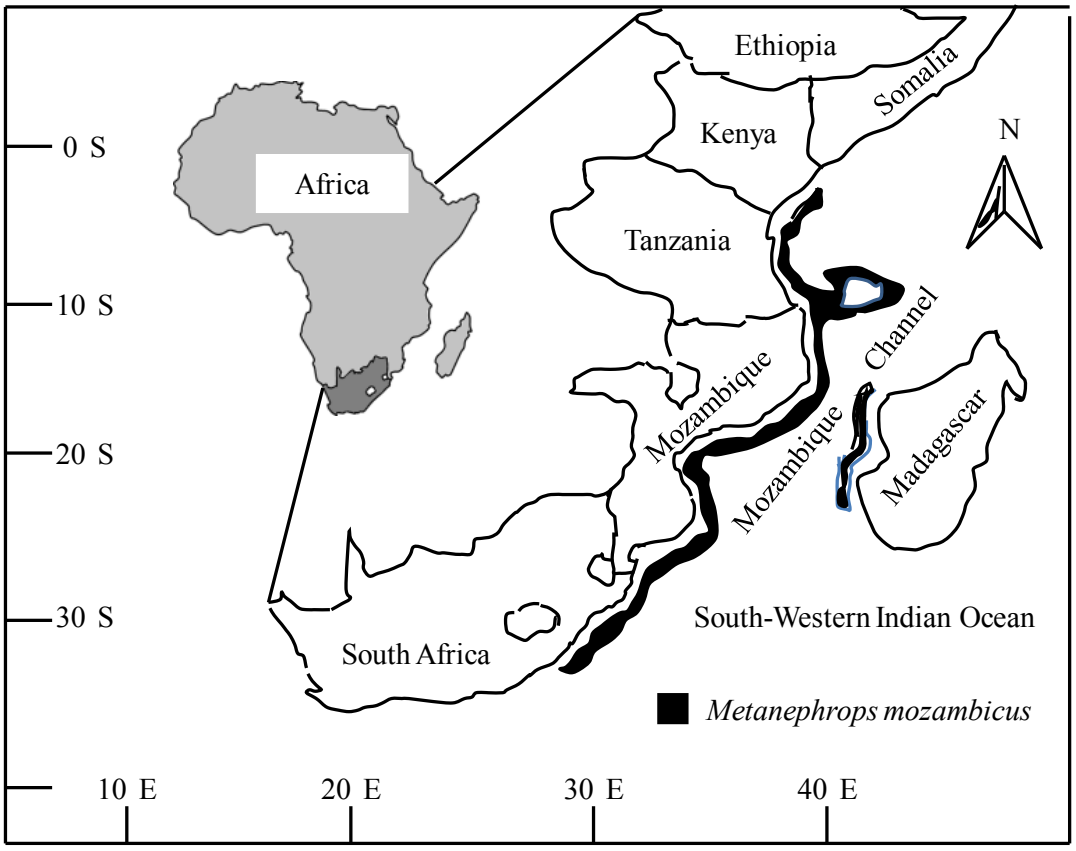


**Figure 1.2** The geographic distribution of *Metanephrops mozambicus* in the Western Indian Ocean. Records from the Comoros archipelago (mid-channel), Tanzania and Kenya may comprise both *M. mozambicus* and *Metanephrops andamanicus*. Image from Holthuis (1991).

Sexual maturity in males and females was achieved between 42 and 48 mm CL in Mozambique (Dias *et al.* 2009). Egg-bearing females occurred throughout the year (Berry 1969; Dias *et al.* 2009). More than 80% of mature females bore eggs between September and March, declining to 34 - 63% in April to July (Berry 1969; Dias *et al.* 2009). *Metanephrops mozambicus* females produced a single brood per year, which hatched after 9 - 10 months of incubation. Females produced between 600 and 1400 eggs per brood, and fecundity was proportional to size, except in the largest size class (Berry 1969). Larvae presumably hatched at an advanced stage of development and settle on the seafloor soon after hatching, because exopodites are not well adapted for swimming (Berry 1969).

Sexually immature males and females moulted several times per year (Schleyer *et al.* 1997). Males (> 47 mm CL) moulted once per year between December and March reach a peak in February (Schleyer *et al.* 1997). Most mature females moulted between May and July (Berry 1969). The mean CL, size frequency distribution and sex ratios of *M. mozambicus* remained constant over time, suggesting that populations were non-migratory (Berry 1969; Schleyer *et al.* 1997).

Although heavily fished, few formal stock assessments of *M. mozambicus* in Mozambique and South Africa have been undertaken. Biomass estimates based on survey data are available for Mozambique (Dias *et al.* 2008; Dias *et al.* 2009; Dias *et al.* 2011), and a standardized abundance index (1988 - 2010) has recently been developed for the South African stock (Robey 2013). Both nominal and standardized indices suggested a continual increase in abundance between 2003 and 2010 in South African waters, and the stock was therefore assumed to be healthy (Robey 2013).

### *1.3 Life history characteristics and larval dispersal*

The early life history of many marine taxa includes a drifting larval phase that may spend long periods in the open ocean, where the larvae are dispersed by marine currents (Matthee *et al.* 2007; Neethling *et al.* 2008; Goetze 2011; Gille 2012; McManus & Woodson 2012). In benthic crustaceans such as spiny lobsters, the drifting larval phase undergoes a metamorphosis into a settlement stage, which in some species is able to swim and select settlement areas (Cobb 1997). Newly settled individuals then moult into the first benthic juvenile stage, which is generally morphologically similar to adults (James-Pirri & Cobb 2000; Inoue *et al.* 2004; Saul 2004). After several moults, juveniles become sexually mature, and in some cases this process involves migrations to adult habitats (Luttikhuizen *et al.* 2008; Teske *et al.* 2008; Naro-Maciel *et al.* 2011). Larval dispersal by ocean currents and benthic migrations are therefore important in determining species distribution patterns, as well as stock identity (Smith & Jensen 2008; Rivera *et al.* 2010; Tsang *et al.* 2012).

The causes of the differentiation of populations of marine organisms are still not well understood. Biological traits to minimize larval loss and maintain adult distributions in

strong current regimes have been proposed for deep-water spiny lobsters (Sponaugle *et al.* 2002; Tolley *et al.* 2005; Groeneveld *et al.* 2007). As a matter of fact, several of these environmental factors, which can act as barriers to the dispersion among populations, may also be promoting a pattern of lack of genetic differentiation among other populations. Adaptations may include: inshore migrations of females (out of the strongest currents); carrying large eggs so that strong larvae in an advanced stage of development are released at hatching; a relatively short pelagic period before settlement on the seafloor; larvae remaining in benthic boundary layer where they are less affected by currents; and counter-current benthic juvenile migrations to redress downstream larval dispersal (Groeneveld 2002; Groeneveld & Branch 2002; Sekiguchi & Inoue 2002). Although proposed for deep-water spiny lobsters, some of these mechanisms may also operate for langoustines and deep-water prawns in strong current regimes, such as in the Mozambique Channel and upper Agulhas Current (Lutjeharms 1988; Ridderinkhof *et al.* 2001; Gopal *et al.* 2006).

### *1.4 Geographic scope and oceanographic features*

The large and meso scale oceanographic features of the Mozambique Channel and upper Agulhas Current dominate the marine environment off south-eastern Africa and western Madagascar (Quartly & Srokosz 2004; Lutjeharms 2006, 2007). The South Equatorial current diverges to the north of Madagascar to give rise to the Mozambique Channel waters which gradually move southwards through the channel, and to the East Madagascar Current (Lutjeharms 2006). Along the Mozambican coast, the circulation is characterized by the influence of three anti-cyclonic cells changing their position along the coast and some smaller cyclonic eddies (Saetre & da Silva 1984; De Ruijter *et al.* 2002; Sete *et al.* 2002; Lutjeharms 2006) (Fig. 1.3). An inshore northwards current seems to be present along most of the Mozambican coast, probably as a result of the presence of the cyclonic eddies (Lutjeharms & da Silva 1988; Paula *et al.* 2001; De Ruijter *et al.* 2002). The western seaboard of Madagascar is characterized by a zone of turbulence where current direction and strength is highly variable (Cooke *et al.* 2004; De Ruijter *et al.* 2004; Quartly & Srokosz 2004; Ansorge 2006). The turbulence is driven by changes in the wind regime,

tidal amplitude, the relief of the seabed, and the configurations of the opposing continental and island coastlines (Cooke *et al.* 2004; Quartly & Srokosz 2004).

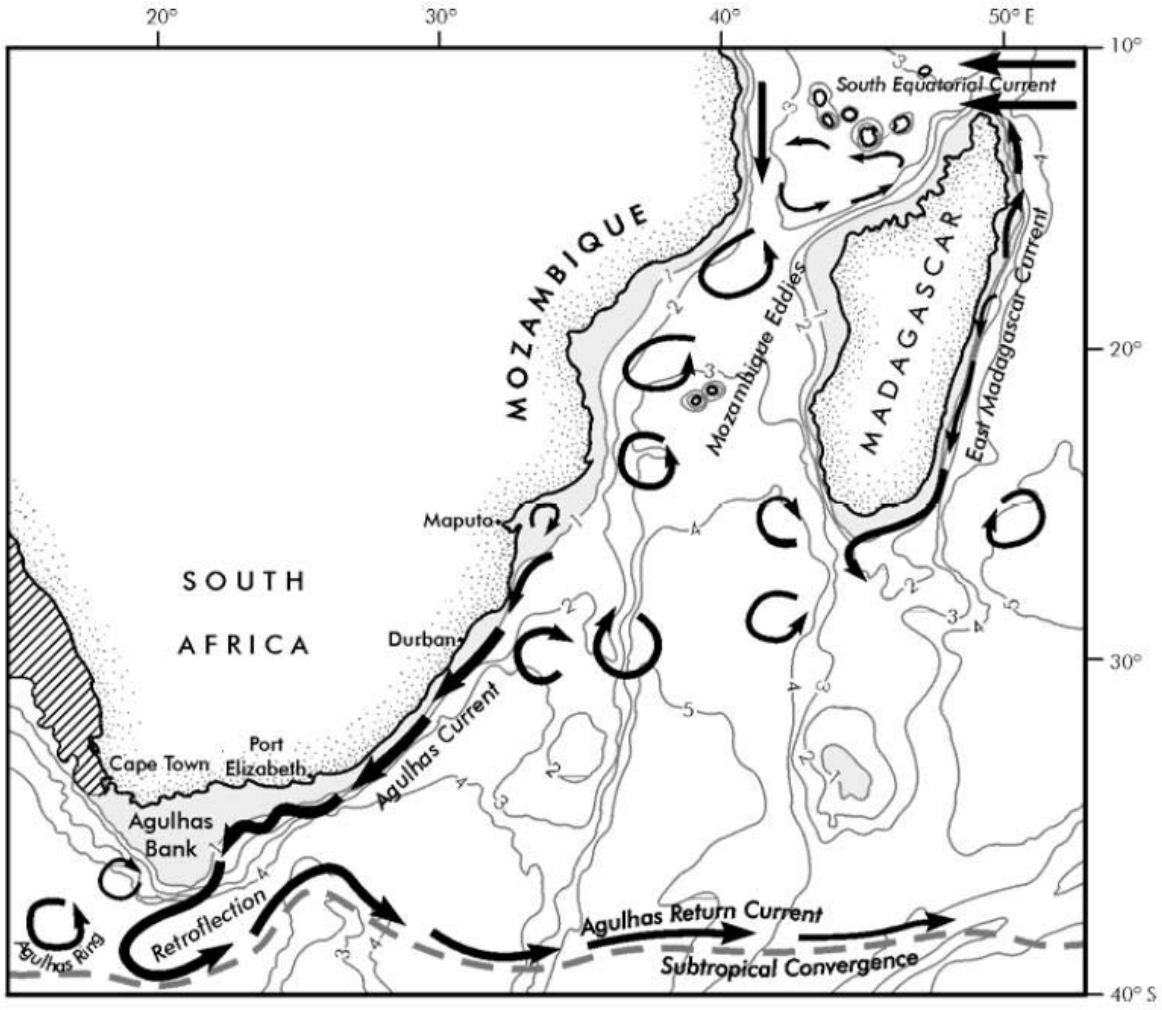


**Figure 1.3** The major ocean current features in the South West Indian Ocean (SWIO). The arrows show distinguishing current systems, and the circles show anti-cyclonic (anti-clockwise direction) and cyclonic (clockwise) eddies. Image from Lutjeharms (2006).

The Mozambique Channel waters and East Madagascar Current converge to form the upper Agulhas Current (Lutjeharms 1985; Gordon *et al.* 1987; Lutjeharms & da Silva 1988; Lutjeharms 2007). This current originates somewhere between 25°S (Southern Mozambique) and 30°S (Durban, South Africa) and flows in a south-westerly direction along the coast, roughly steered by the edge of the continental shelf. It reaches speeds of up to 2.6 $m.s^{-1}$ at the surface, with average surface speeds of between 1 and 2 $m.s^{-1}$, and its

polewards flow extends to a depth of over 2000 m (De Ruijter *et al.* 2002; Swart *et al.* 2010; Preu *et al.* 2011). The current moves further offshore at approximately 36°S, following the contours of the Agulhas Bank, and retroflects to form the Agulhas Return current which flows eastwards along the edge of the Subtropical Convergence (Gordon *et al.* 1987; De Ruijter *et al.* 2002; Lutjeharms 2006, 2007).

### *1.5 Mitochondrial DNA* (*mtDNA*)

The metazoan mitochondrial DNA (mtDNA) genome is a typically closed circular double stranded molecule that is highly variable in sequence but conservative in gene content (Wolstenholme 1992; Boore 1999; Minxiao *et al.* 2011) and order (Hickerson & Cunningham 2000; Avise 2009). Mitochondrial DNA is maternally inherited and has relatively fast evolutionary rates (Birky *et al.* 1989; Avise 2009), its haploid nature and limited recombination (Avise *et al.* 1987); also can be applicable for old even degraded samples (Bucklin & Allen 2004; Arif & Khan 2009). DNA fragments are widely used in many fields because universal primers have been developed that are relatively easy to use to amplify and sequence targed DNA (Frézal & Leblois 2008; Anker & Baeza 2012; Hui 2012). The mtDNA genes are regarded as powerful markers for genetic diversity analysis at family, genus and species levels (Meyer 2003; Arif & Khan 2009; Ahyong *et al.* 2011). Mitochondrial DNA is one of the most successful molecular markers used by many researchers in phylogenetics, population structure and for identification of stocks (Avise & Walker 2000; Froukh & Kochzius 2007; Palero *et al.* 2009; Shih *et al.* 2009).The mitochondrial large ribosomal subunit (16S) RNA and cytochrome c oxidase subunit I (COI) genes have been used for phylogeny and biogeography of crustaceans (Chu *et al.* 2003; Avise 2009; Dumont *et al.* 2009).

Several genetic population studies have been done on crustaceans in the Western Indian Ocean region (Teske *et al.* 2009a; Ragionieri *et al.* 2010; Penha-Lopes *et al.* 2011; Tsoi *et al.* 2011), however none of these have addressed the partially sympatric populations of *H. triarthrus* or *M. mozambicus.* Based on COI sequencing , *Scylla serrata* populations in the Red Sea, Mauritius and South Africa were genetically separate (Fratini & Vannini 2002). Fernandez *et al.* (2011b) showed that separate *Aristeus antennatus* populations occurred in

the Mediterranean Sea, Atlantic Ocean and Indian Ocean bioregions. No significant genetic differentiation could however be shown among populations of this species in the Mediterranean basin (Roldán *et al.* 2009), using control region, COI and 16S as markers. When there are no barriers to the dispersal of larvae and gene flow, populations that are thousands of kilometers apart may be connected through larval drift in ocean currents, as was recently shown for spiny lobsters *Jasus paulensis* and *J. tristani* in the South Atlantic and Southern Indian Ocean waters (Groeneveld *et al.* 2012).

In the Agulhas Current region off southern and south-eastern Africa, Tolley *et al.* (2005) showed that deep-water spiny lobster *Palinurus gilchristi* was panmictic across its distribution range, based on control region sequences. Conversely, Gopal *et al.* (2006) demonstrated a shallow genetic break among deep-water spiny lobster *Palinurus delagoae* populations occurring off South Africa and Mozambique. The genetic break was in the vicinity of the interface between the Mozambique Channel eddies and the upper Agulhas Current.

### *1.6 Objectives of the study*

The aims of the present study were to assess the metapopulation structure of *H. triarthrus* and *M. mozambicus* collected from deep-water trawl grounds off Mozambique, Madagascar and South Africa by sequencing mitochondrial DNA fragments. The two species occur in similar habitats, but their biology and behavior differ substantially. These differences may affect larval distribution patterns, and therefore population structure across the region. The results from this study are related to the oceanographic features of the region, and their likely effects on larval dispersal pathways. The genetic information is used to assess whether fished stocks are distributed across international boundaries, and are therefore shared among Mozambique, Madagascar and South Africa, or whether stocks are local. Fisheries management strategies at national and regional levels are discussed.

## Chapter Two

## 2. Materials and Methods

### *2.1 Field sampling*

A total of 220 whole specimens of *Haliporoides triarthrus* and *Metanephrops mozambicus*, respectively, were collected by onboard fisheries observers placed on commercial bottom trawlers between 2011 and 2012. Samples were collected at six sites, and grouped by country (or region) as follows: Mozambique, three sites at Bazaruto A, Boa Paz and Inhaca; Madagascar, two sites at Morombe and Tulear; and one site near Durban in South Africa (Table 2.1 and Fig. 2.1).

**Table 2.1** Sampling sites and coordinates for (a) *Haliporoides triarthrus* and (b) *Metanephrops mozambicus* collected for this study.

| Country (Region) | Sites | Species | Coordinates | |
|---|---|---|---|---|
| | | | Latitude (S) | Longitude (E) |
| Mozambique | Bazaruto A | a) | 21°53'21 | 35°41'36 |
| | | b) | 21°53'32 | 35°41'34 |
| | Boa Paz | a) | 24°55'66 | 35°30'39 |
| | | b) | 25°40'52 | 34°17'64 |
| | Inhaca | a) | 25°55'48 | 33°10'48 |
| | | b) | 25°51'07 | 33°10'09 |
| Madagascar | Tulear | a) and b) | 23°34'89 | 43°29'52 |
| | Morombe | a) and b) | 22°23'26 | 43°04'46 |
| South Africa | Durban | a) | 29°57'97 | 31°11'12 |
| | | b) | 29°53'17 | 31°22'71 |

a) *Haliporoides triarthrus* and b) *Metanephrops mozambicus*

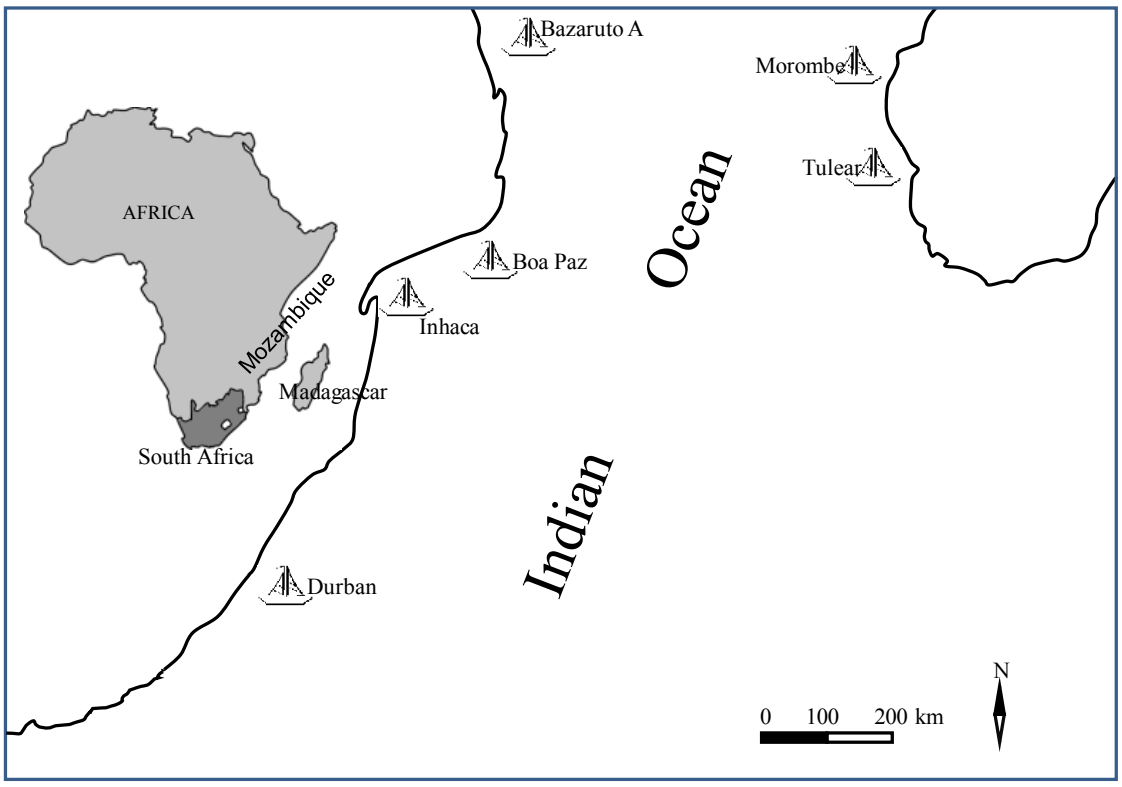


**Figure 2.1** Sampling sites for *Haliporoides triarthrus* and *Metanephrops mozambicus* at Bazaruto A (BA), Boa Paz (BP), Inhaca (IA) in Mozambique; Durban (DB) in South Africa and Morombe (MM) and Tulear (TR) in Madagascar.

The species were identified based on morphological characteristics (De Freitas 1985). About 40 specimens per species per site were numbered individually and digitally photographed. The carapace length (CL in millimeters, measured from the rear of the ocular indent on the rostrum to the posterior dorsal margin), sex, and weight (to the nearest 0.1 g) were recorded (see Sobrino & García 2007). Based on direct observations of female gonads (size and colouring), specimens were categorised into one of four maturity stages: virgin, developing, pre-spawning and spawning (De Freitas 2004; Sobrino *et al.* 2009). The first two stages were considered to represent immature females, and the last two mature females (Sobrino & García 2007; Sobrino *et al.* 2009). A small strip of tissue was excised from the abdominal muscle of each specimen and preserved in 95% ethanol. Specimens

were frozen onboard for later genetic analysis in the laboratory. Samples were transferred to the species conservation (CONSPEC) laboratory at the University of KwaZulu-Natal (UKZN) for subsequent analyses.

### *2.2 DNA extraction*

The DNA was extracted using a Zymo tissue kit (Zymo Research Catalog N$^{o}$ **6016**) with a slight modification from the manufacturers instructions. This method consists of separating organic products from nucleic acids. Compared with DNA classical methods of extraction this is relatively efficient and quick but provides less DNA extracted per sample (Popa *et al.* 2007; Wang *et al.* 2011; Wang *et al.* 2012). Approximately 50 mg tissue samples were added into the ZR Bashing Bead$^{TM}$ Tube, 750 µl of lysis solution and 15 µl of Proteinase K (20 mg/ml) and incubated overnight at 56$^{o}$ C. The following day the samples were centrifuged for 1 minute at 10000 x g to separate the supernatant from the sediments. Up to 400 µl of supernatant were transferred to a Zymo-Spin$^{TM}$ IV spin filter in a collection tube and centrifuged at 7000 x g for 1 minute. After centrifuging, 1200 µl of genomic lysis buffer was added to the filtrate in the collection tube and mixed by inversion. A volume of 800 µl of the mixture was transferred to a Zymo-Spin$^{TM}$ IIC column in a collection tube and centrifuged at 10000 x g for 1 minute. From the collection tube the flow through residual was discarded and the remaining mixture transferred to a Zymo-Spin$^{TM}$ IIC column and repeated. After the residual was removed, 200 µl of DNA Pre-wash buffer was added to the Zymo-Spin$^{TM}$ IIC column in a new collection tube and centrifuged at the same manner as previously. To improve the quality of DNA, the pre-wash elution step was repeated. The last step of DNA washing consisted of adding 500 µl of g-DNA wash Buffer to the Zymo-Spin$^{TM}$ IIC column and centrifuging at the previous conditions. The Zymo-Spin$^{TM}$ IIC column was transferred to a labeled clean 1.5 ml microcentrifuge tube and DNA eluted with 50 µl of DNA elution buffer added directly to the column matrix and spun at 10000 x g for 30 seconds and re-suspended. The DNA was stored at 4$^{o}$ C for further use.

To quantify the DNA extracted we used a spectrophotometer (Nanodrop technologies ND 1000). Extract quality was assessed using the absorbance A260/A280 ratio, in which 1.8-2.0 is considered to be appropriate (Hou *et al.* 2011; Wang *et al.* 2012). Above or below

this range, the extracted DNA is considered to be contaminated by RNA or protein (Moore *et al.* 2004; Wang *et al.* 2011). The Zymo kit extraction method resulted in <50 ng/µl per DNA extraction, sufficient to carry out PCR.

The quality of the DNA extract was assessed using electrophoretic gels. Fragments of DNA were visualized on a 1% agarose gel (General Purpose Agarose GP2) containing 10 mg/ml Ethidium Bromide. DNA templates (5 µl) as well as 3 µl of DNA ladder was loaded with a 1 µl mix of Bromophenol Blue/glycerol and loaded into the first well as size standard to the target fragment. The gel was run for 30 minutes at 100 volts using Bio-Rad agarose apparatus (Power Pac Basic), submerged in 1 x TAE buffer. After running the gels, BIORAD Geldoc and associated software version 2.0.1 were used to visualize and save the gel images on Image Lab™.

### *2.3 PCR amplification and sequencing*

Two mitochondrial fragments were amplified using universal primers ribosomal 16S subunit (16S) and mitochondrial cytochrome oxidase I gene (COI). The primer that amplified the 16S rRNA gene was designed by Roldán *et al.* (2009) specifically for another deep-water crustacean, *Aristeus antennatus*, while COI region primers were designed by Chow *et al*. (2006) to amplify spiny lobster (*Panulirus sp.*) DNA. In the thermal cycler (AB GeneAmp PCR System 2700), fragments of 569 bp (16S) and 1300 bp (COI) were amplified using the following primers: forward 16SARL (5'- TGC CTG TTT ATC AAA AAC AT - 3') and reverse 16SBRH (5'- CCG GTC TGA ACT CAA ATC ATG T - 3'), while for COI gene forward was CO165F1 (5'- GGA GCT TGA GCT GGA ATA GT - 3') and reverse CO1L342R1 (5'- GTG TAG GCG TCC TGG GTA GTC - 3'). For both species, the PCR reaction contained 5 µl of 10 x PCR buffer, 1.8 µl of MgCL in concentration at 25 mM, 0.84 µl of each primer in 10 µM, 0.15 µl of dNTP 40 mM, 0.2 µl of 0.5 Units/µl *Taq* DNA Polymerase (Supertherm), 1 µl of DNA template and was made up to a total volume of 25 µl with PCR grade water. Each reaction included a negative control to verify that there was no contamination during the preparation of the master mix.

a) *Haliporoides triarthrus*: PCR conditions for 16S were as follows: denaturation at 95°C for 5 minutes, followed by 35 cycles of a 30 second denaturation stage at 94°C, 30 second annealing set at 56.2°C and 60 second extension at 72°C and followed by final extension at 72°C for 10 minutes. The PCR for COI was performed using the following profile: denaturation at 95°C for 3 minutes, followed by 35 cycles of a 30 second denaturation stage at 94°C, 30 second annealing set at 52°C, and 90 second extension at 72°C and a final extension at 72°C for 5 minutes. The PCR cycles ended at 15°C, where after products were stored at 4°C in the freezer for further use.

b) *Metanephrops mozambicus*: The reaction mixture for 16S was preceded by denaturation at 95°C for 5 minutes, followed by 35 cycles of a 30 second denaturation stage at 94°C, 30 second annealing set at 53.7°C and 60 second extension at 72°C and followed by final extension at 72°C for 10 minutes. For the COI gene the PCR reactions were performed by denaturation at 95°C for 3 minutes, followed by 35 cycles of a 30 second denaturation stage at 94°C, 30 second annealing set at 55.4°C and followed by final extension at 72°C for 5 minutes. After confirmation of PCR product using gel electrophoresis, the amplified fragments were sequenced at the Central Analytical Facility (CAF), Stellenbosch University.

### *2.4 Data analysis*

The sequences were corrected manually, aligned and edited in Bioedit version 7.1.3 (Hall 1999). For population analysis, sequences were exported from Bioedit to ClustalX 2.1 (Larkin *et al.* 2007) and saved in file formats compatible to the software. The standard diversity indices, nucleotide and haplotype diversities were calculated in DnaSP program version 5.10.01 (Librado & Rozas 2009). A mismatch distribution (Harpending 1994) was calculated using DnaSP and 10000 permutations complemented by the raggedness index *r* (Harpending 1994; Rogers 1995) for the population growth model where post-hoc unimodal distributions imply that populations have undergone recent demographic expansion whereas multimodal distributions imply stable populations at long-term equilibrium (Froukh & Kochzius 2007; Xu *et al.* 2009b; Orsini *et al.* 2012). The software Arlequin version 3.5 (Excoffier & Lischer 2010) was used to calculate a number of different indices. Tajima's *D*

was used to compare estimates of the number of segregating sites and the mean pairwise difference between sequences (Tajima 1989) and Fu's *Fs* test (based on Ewens' sampling distribution and taking into account the number of different haplotypes in the sample) (Fu 1997) was used to examine neutrality deviation of the marker, with 10000 permutations. The formula $t = \tau / 2u$ was used to calculate approximate date of expansion (t): where tau ($\tau$) = the index of time since expansion, $u$ = the substitution rate per generation per sequence length (Rogers & Harpending 1992). The lack of calibrated mutation rates of *H triarthrus* and *M. mozambicus* led us to use mutation rates known for decapod crustaceans (Xu *et al.* 2009b; Daniels 2011). Thus, for the COI gene, mutation rates range from 1.40% to 2.60% (Schubart *et al.* 1998; Projecto-Garcia *et al.* 2010), and an intermediate value of 2% (Daniels 2011) divergence per million years was chosen for this study. For the 16S gene, mutation rates range from 0.64% to 1.42% per million years (Schubart *et al.* 1998; Klaus *et al.* 2010), and 1.02% (Daniels 2011) was chosen. Spreadsheet tools developed by Schenekar & Weiss (2011) and applied for divergence rates (http://www.uni-graz.at/zoowww/mismatchcalc/mmc1.php) were used to calculate demographic time of expansion for both species and genes. To further investigate the mismatch analysis the raggedness index *r* was examined (Harpending 1994); (Rogers 1995) where lower *r* values are expected under the population growth model. Gene flow (movement of genes between populations) is generally driven by larval dispersal and by migrations in benthic populations in the marine environment, and various parameters can be measured (Hellberg 2009; Allendorf *et al.* 2010; Ragionieri *et al.* 2010). Measuring and monitoring natural patterns of gene flow together with appropriate conservation measures can help to maintain viable populations (and metapopulations) in the face of changing environments and habitat fragmentation (Allendorf & Luikart 2007; Waples *et al.* 2008; Hickerson *et al.* 2010).

Haplotype / allele networks are often more informative than phylogenetic strict consensus trees to display intraspecific DNA sequence variation, both maximum parsimony and network-building algorithms are not guaranteed to find all most parsimonious phylograms for a set of sequences (Mardulyn 2012). However, if the data contained many loops the median joining networks may not very informative (Bandelt *et al.* 1999). Phylogenetic

analyses among haplotypes were carried out using PAUP version 4.0 (Swofford 2002) and the following methods of phylogenetic inference; Neighbor Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML). Bayesian analyses were constructed using Mr Bayes version 3.2.0 (Ronquist & Huelsenbeck 2003). Maximum-likelihood analysis was performed in GARLI program version 2.0 (Zwickl 2006). To visualize the above-mentioned phylogenetic tree, the software Treeview version 1.6.6 (Page 1996) was used. Out-group taxas sequences *Penaeus monodon* (AF217843.1), *Marsupenaeus japonicus* (AP006346.1), *Litopenaeus stylirostris* (EU517503.1), *Nephropsis stewarti* (U960861) and *Cherax destructor* (AY383557.2) were obtained from GenBank and added to the data set to root the molecular tree. To determine the best-fit model of DNA evolution, JModelTest version 2.2 (Posada 2008) was used. A haplotype network was constructed using Network program version 4.6.1.0 (Bandelt *et al.* 1999) for all individuals of each species. The genetic relationships among the haplotypes were inferred using the Median-Joining Network analysis (Bandelt *et al.* 1999) with Network version 4.6.1.0 (www.fluxus-engineering.com). The median-joining algorithm was used with the default parameters as recommended for multiple state data. Phylogeographical relationships among haplotypes were explored by haplotypic network analysis using the median-joining method, as implemented in NETWORK, version 4.6.1.0. For both NJ and MP analysis, the stability of the nodes was tested by 1000 bootstrap replicates. Median-Joining Networks (Bandelt, Forster & Röhl, 1995) were constructed using the software NETWORK, version 4.6.1.0 (Shareware Phylogenetic Network Software Web site; http://www.fluxus-engineering.com/sharenet.htm). The resulting network is a combination of minimum spanning trees, with median vectors (consensus sequences) added by a parsimony criterion (Roldán *et al.* 2009; Fernández *et al.* 2013).

Analysis of Molecular Variance (AMOVA) was performed in GenAlEx version 6.5 (Peakall & Smouse 2012) to investigate the genetic variation among region, and among and within populations. Both *H. triarthrus* and *M. mozambicus* are considered to be single or shared regional stocks across geopolitical boundaries, and in the present work, we set out to test whether stocks were genetically structured. To test this, AMOVA based on pairwise

differences was performed to determine the partitioning of genetic variance among sampling localities and among the regions defined by three *a priori* population groupings based on geopolitical boundaries. The variance components have been used to calculate a series of statistics called phi-statistics ($\Phi$), which summarize the degree of differentiation between populations, as they are analogous to F-statistics (Excoffier *et al.* 1992).

The AMOVA approaches were able to examine the genetic diversity in terms of distribution of variation ($\Phi_{PT}$), amongst regions ($\Phi_{RT}$) and populations within regions ($\Phi_{PR}$) (Breinholt *et al.* 2009). Values close to zero were interpreted as little differentiation among populations, whilst values close to one were an indication of high differentiation (Alfaya *et al.* 2012; Gille 2012). According to (Wright 1978), $\Phi_{PT}$ values ranging from 0 to 0.05 are considered low, 0.05 to 0.15 moderate, 0.15 to 0.25 large and 0.25 to 1.0 very large (Norton & Ashley 2004; De Oliveira *et al.* 2007; Baye 2011).

## Chapter Three

## 3. Results

a) *Haliporoides triarthrus*

### *3.1 Genetic diversity*

Most of the analysis for the two mitochondrial fragments 16S and COI were treated separately, but some were combined where appropriate. The final alignment of *H. triarthrus* using 16S rDNA obtained a fragment of 569 bp. From a total of 207 sequences 59 segregating sites were detected of which 31 were parsimony-informative with 69 haplotypes. Also, from the network, 43 haplotypes were considered missed or undetected haplotypes. Few haplotypes were shared among populations; between Bazaruto A and Durban haplotype two and haplotype four were shared (see Appendix I). Also, two haplotypes (haplotype 46 and haplotype 51) were exclusively shared between Madagascar sites, Morombe and Tulear and all others had unique haplotypes. The highest number of haplotypes were found in the Inhaca Area (22/69, 31.9%), and the lowest at Boa Paz (6/69, 8.6%). The haplotype diversity (*h*) within populations was high and ranged from 0.667 to 0.957 at Boa Paz and Inhaca sites. The lowest level of nucleotide diversity ($\pi$) was found at Bazaruto A, Boa Paz and Durban (0.002) and the highest at Inhaca 0.021 (Table 3.1).

**Table 3.1** *Haliporoides triarthrus* genetic diversity for the 16S gene from six sampling sites in the SWIO. Sites are Bazaruto A (BA), Boa Paz (BP), Durban (DB), Inhaca (IA), Morombe (MM) and Tulear (TR).

| Statistics | BA | BP | DB | IA | MM | TR | Total |
|---|---|---|---|---|---|---|---|
| Number of sequences, n: | 40 | 40 | 40 | 27 | 30 | 30 | 207 |
| Number of segregating sites, S: | 5 | 4 | 7 | 46 | 28 | 17 | 59 |
| Number of haplotypes, h | 8 | 6 | 9 | 22 | 12 | 16 | 69 |
| Haplotype diversity, *h*: | 0.765 | 0.667 | 0.755 | 0.957 | 0.844 | 0.862 | 0.946 |
| Nucleotide diversity, π: | 0.002 | 0.002 | 0.002 | 0.021 | 0.008 | 0.006 | 0.010 |

The mtDNA COI analysis revealed 151 sequences and 214 variable sites of which 128 were informative based on parsimony criteria, defining 78 haplotypes where 22 haplotypes were missed or undetected from the network haplotype. Most haplotypes were found at Inhaca (20/78, 25.6%) and Bazaruto A (19/78, 24.4%). There were no shared haplotypes among all populations (Appendix II). Total haplotype diversity was 0.956 and was lower among samples from Madagascar, Morombe (0.526) and Tulear (0.402). Nucleotide diversity within locations ranged from 0.001 to 0.019 (Table 3.2).

**Table 3.2** *Haliporoides triarthrus* genetic diversity for the COI gene from six sites in the SWIO. Sites are Bazaruto A (BA), Boa Paz (BP), Durban (DB), Inhaca (IA), Morombe (MM) and Tulear (TR).

| Statistics | BA | BP | DB | IA | MM | TR | Total |
|---|---|---|---|---|---|---|---|
| Number of sequences, n: | 27 | 22 | 27 | 25 | 23 | 27 | 151 |
| Number of segregating sites, S: | 70 | 84 | 30 | 45 | 101 | 9 | 214 |
| Number of haplotypes, h | 19 | 8 | 16 | 20 | 8 | 7 | 78 |
| Haplotype diversity, *h*: | 0.917 | 0.602 | 0.892 | 0.973 | 0.526 | 0.402 | 0.956 |
| Nucleotide diversity, π: | 0.019 | 0.016 | 0.005 | 0.012 | 0.014 | 0.001 | 0.043 |

### *3.2 Haplotype network*

The haplotype network shows few haplotypes shared among sites for 16S genes (Fig. 3.1). The most frequent haplotype occurred at Bazaruto A (35/207, 16.9%) followed by two common haplotypes at Boa Paz (17/207, 8.21% and 16/207, 7.73%). The majority of individual haplotypes (21) occurred at Inhaca.

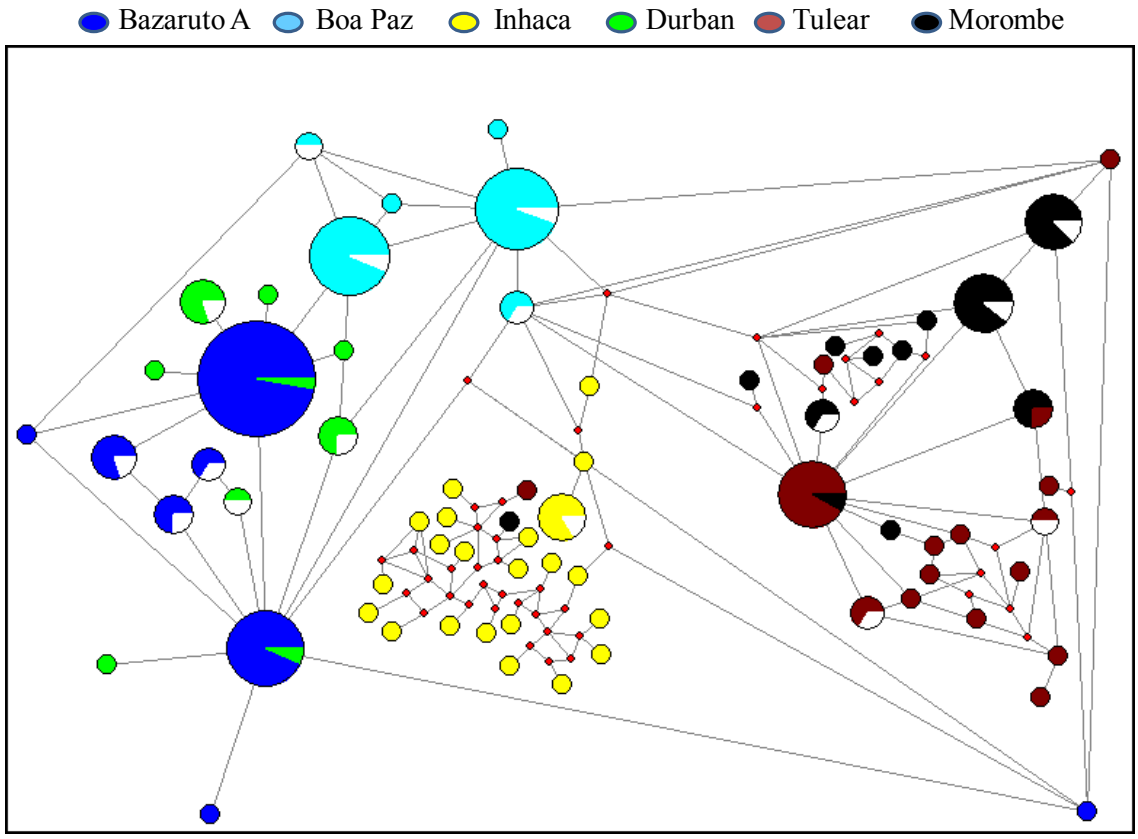


**Figure 3.1** Haplotype network for *Haliporoides triarthrus* among sampling sites from the SWIO for the 16S gene. Circles represent haplotypes. Circle diameter is proportional to frequency. The white colour wedges indicate the proportion of individuals from each sampling location with a given haplotype. Each line represents one mutational step, and the small red circles represent missing or undetected haplotypes.

Conversely, for 16S genes few haplotypes are shared among sites. The haplotype network based on COI sequences distinguished clearly among haplotypes from the African continental shelf (Bazaruto A, Boa Paz, Inhaca and Durban), and those from Madagascar

(Morombe and Tulear) (Fig. 3.2). The haplotype network suggested that populations were relatively isolated. The two sites from Madagascar shared the most common haplotypes (Tulear; 21/151, 13.9%: and Morombe; 16/151, 10.6%). The majority of shared haplotypes in continental shelf populations was observed at Boa Paz (14/151, 9.27%).

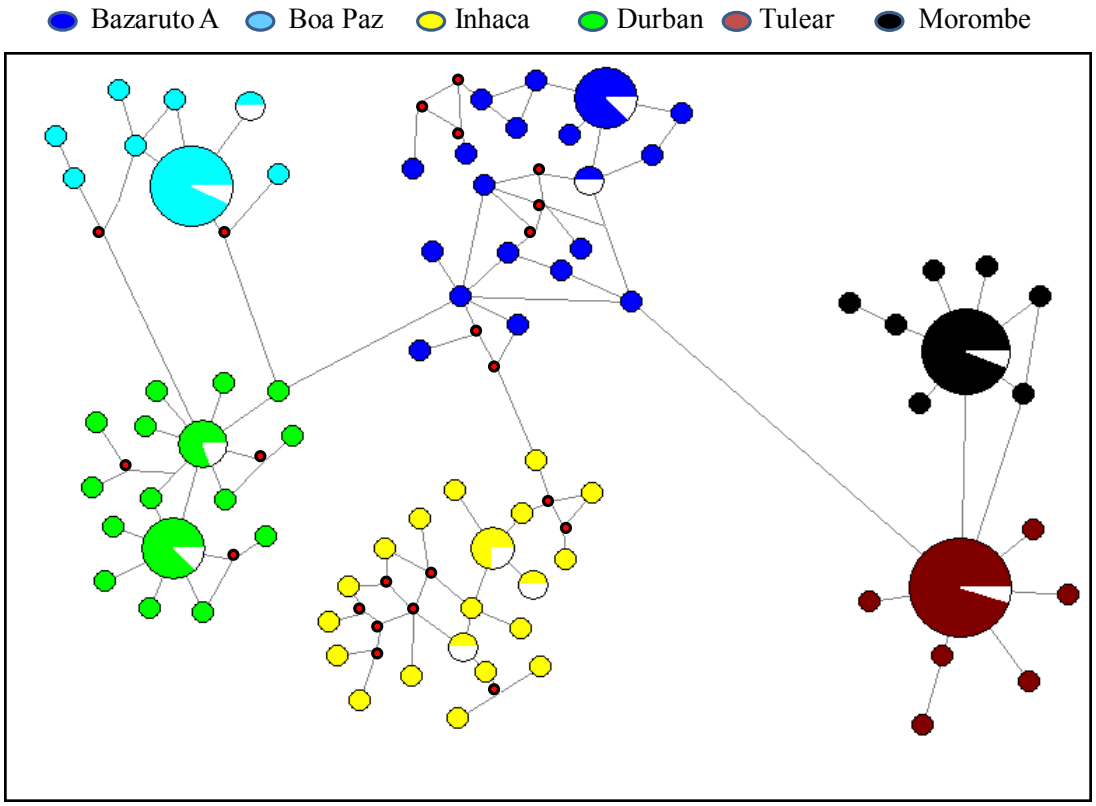


**Figure 3.2** Haplotype network for *Haliporoides triarthrus* among sampling six sites from the SWIO for the COI gene. Circles represent haplotypes. Circle diameter is proportional to frequency. The white colour wedges indicate the proportion of individuals from each sampling locations with a given haplotype. Each line represents one mutational step, and the small red circles represent missing or undetected haplotypes.

### *3.3 Population structure*

The AMOVA was performed on three regions to generate $\Phi$ statistic based on prior expectations concerning spatial population differentiation. The regions were based on geopolitical boundaries (Mozambique, South Africa and Madagascar). The analysis showed evidence of strong population structure within each level of spatial sampling. Significant

$\Phi_{PT}$ values were elucidated for 16S ($\Phi_{PT}$ = 0.454, $P$< 0.001) and COI ($\Phi_{PT}$ = 0.663, $P$< 0.001) indicating genetic differentiation among populations (Table 3.3). The results reflect differentiation among regions of 11% for 16S and 35% for COI. Further, this analysis revealed 55% differentiation for 16S and 34% for COI within populations (Fig. 3.3).

**Table 3.3** Estimation of fixation indices ($\Phi$) and $P$ - value for *Haliporoides triarthrus*. The indices represent correlation among region ($\Phi_{RT}$), among population ($\Phi_{PR}$) and within population ($\Phi_{PT}$). Bold values are significantly different to zero at the 0.001 % level.

| Stat | 16S | COI |
|---|---|---|
| $\Phi_{RT}$ | **0.114** | **0.350** |
| $\Phi_{PR}$ | **0.384** | **0.482** |
| $\Phi_{PT}$ | **0.454** | **0.663** |

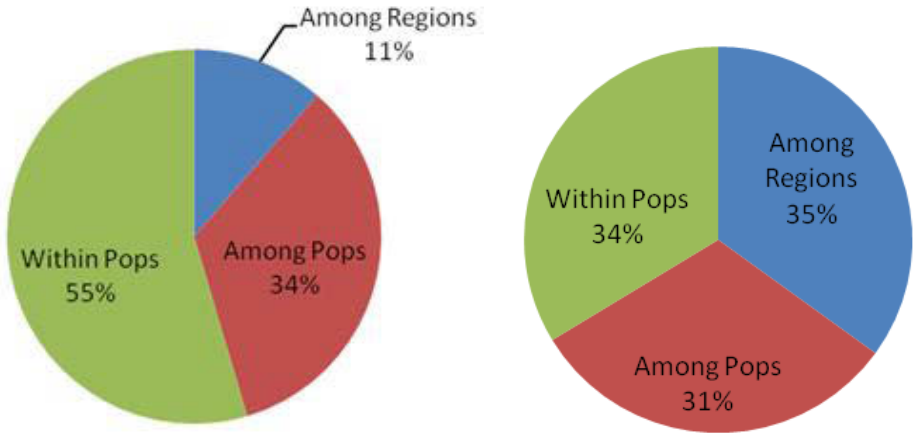


**Figure 3.3** Analysis of Molecular Variance within and among samples of *Haliporoides triarthrus* from three regions, Mozambique (BA+BP+IA), South Africa (DB) and Madagascar (MM and TR) using 16S (left) and COI (right).

Pairwise comparisons of all sampling sites showed significant differences ($P$ < 0.001) and for the 16S marker the highest values (0.632) occurred between Bazaruto A and Morombe and the lowest (0.251) between Inhaca and Durban (Table 3.4). For the COI marker, the highest values (0.762) occurred between Inhaca and Tulear and the lowest (0.244) between Madagascar sampling sites Tulear and Morombe.

**Table 3.4** Pairwise ($\Phi_{PT}$) values among sampling sites based on 16S gene (below diagonal) and COI gene (above diagonal) for *Haliporoides triarthrus* for each sampling site. All ($\Phi_{PT}$) values are significant at $P < 0.001$.

| | Bazaruto A | Boa Paz | Inhaca | Durban | Morombe | Tulear |
|---|---|---|---|---|---|---|
| Bazaruto A | - - - | 0.572 | 0.579 | 0.526 | 0.591 | 0.640 |
| Boa Paz | 0.565 | - - - | 0.397 | 0.585 | 0.704 | 0.759 |
| Inhaca | 0.281 | 0.556 | - - - | 0.610 | 0.710 | 0.762 |
| Durban | 0.308 | 0.354 | 0.251 | - - - | 0.657 | 0.713 |
| Morombe | 0.632 | 0.532 | 0.625 | 0.393 | - - - | 0.244 |
| Tulear | 0.529 | 0.388 | 0.529 | 0.301 | 0.365 | - - - |

$F_{ST}$ statistics were calculated for 16S and COI genes to estimate gene flow among populations (Table 3.5); both showed low levels of migration between populations ($N_em < 1$).

**Table 3.5** Gene flow estimates for *Haliporoides triarthrus* from the $F_{ST}$ parameter using 16S and COI genes.

| | 16S | | COI | |
|---|---|---|---|---|
| Reference | Statistics | Gene flow ($N_em$) | Statistics | Gene flow ($N_em$) |
| Hudson et al. (1992) | $F_{ST}$: 0.43682 | 0.32 | $F_{ST}$: 0.77163 | 0.07 |

### *3.4 Test of neutrality and mismatch distribution analyses*

The test statistic of selective neutrality (Tajima's D and Fu's) for all markers were negative, except for Tajima's D of 16S gene in Bazaruto A site and Fu's Fs of COI genes in Boa Paz and Morombe (Table 3.6). These results may imply natural selection or population expansion. For 16S gene, Tajima's D test significant *P*(F) values were observed for Morombe and Fu's test significant *P* (F) values were observed for Inhaca, Durban and Tulear. COI gene, for Tagima's D test significant *P*(F) values were observed for Boa Paz, Durban, Morombe and Tulear and also for Fu's test significant *P*(F) values were observed

for Inhaca, Durban and Tulear. Based on mismatch distribution analyses, the parameter Sum of Square Deviation (SSD) did not show significant differences between observed and expected distributions suggesting an expansion model, except for Boa Paz (16S and COI) and Morombe (COI)., The raggedness index also indicated that the observed mismatch distribution patterns did not significantly differ between observed and expected values supporting the ocurrence of recent population expansion (except for Boa Paz and Morombe for 16S).

**Table 3.6** Estimates of neutrality test Tajima's D and Fu's and mismatch distribution analyses, Sum of Squared Deviation (*SSD*) and raggedness index (*r*) of *Haliporoides triarthrus* for 16S and COI genes for each sampling site.

| | 16S | | | | COI | | | |
|---|---|---|---|---|---|---|---|---|
| Sites | D | F | *SSD* | *r* | D | F | *SSD* | *r* |
| Bazaruto A | 0.059 | -2.632 | 0.006 | 0.096 | -1.201 | -2.830 | 0.012 | 0.022 |
| Boa Paz | -0.311 | -1.902 | **0.040*** | **0.250***** | **-2.224**** | 4.185 | **0.356***** | 0.076 |
| Inhaca | -0.475 | **-7.729**** | 0.008 | 0.019 | -1.327 | **-8.348**** | 0.004 | 0.008 |
| Durban | -0.812 | **-3.880**** | 0.007 | 0.095 | **-2.193**** | **-8.654***** | 0.004 | 0.030 |
| Morombe | **-1.616*** | -1.994 | 0.043 | **0.139*** | **-2.644***** | 3.880 | **0.340***** | 0.171 |
| Tulear | -1.001 | **-8.062***** | 0.016 | 0.050 | **-2.182**** | **-4.120**** | 0.003 | 0.164 |
| Mean | -0.693 | -4.367 | 0.020 | 0.108 | **-1.962*** | -2.648 | 0.120 | 0.079 |

Statistically significant values are in bold face $0.01 < P < 0.05$ (*), $0.001 < P < 0.01$ (**) and $P < 0.001$ (***)

Figure 3.4 for *H. triarthrus* 16S (left panel) illustrates the unimodal mismatch distribution, suggesting a recent demographic expansion. Conversely the COI gene (right panel) demonstrates a multimodal distribution, generally a characteristic of population at demographic equilibrium. Nevertheless, these populations are not neccesarily stable, because the presence of two peak in the COI gene may suggest the presence of two distinct lineages.The coalescence estimate time of expansion was approximately 83 187 (64 542 - 116 982) years (yr) for 16S and 132 992 (108 812 - 170 989) yr for COI after ajustment and assuming a mean generation time of 3 years.

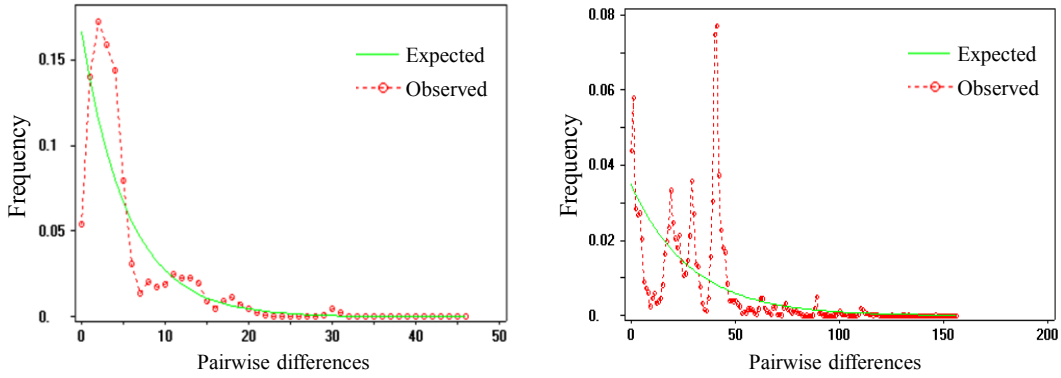


**Figure 3.4** Distributions of pairwise differences among sampling sites for 16S (left) and COI (right) genes of *Haliporoides triarthrus* in the SWIO.

### *3.5 Phylogenetic analysis*

The program JModeltest identified the General Time Reversible with gamma distributed rate (GTR+G) model selected using the Akaike Information Criterion (AIC, Akaike 1974) as the best model of nucleotide substitution. This included a gamma distribution shape parameter of 1.206 and the following base frequencies: Adenine (A = 37%), Cytosine (C = 21%), Guanine (G = 17%) and Thymine (T = 25%), showing an A-T bias typical for arthropods and mtDNA. Figure 3.5 shows the best fitting tree topology based on Bayesian analysis (BY) for the 16S sequences; with posterior probability values (BY) and bootstrap values of two methods (MP/ML) are shown for the most branches from all analyses. The tree was estimated using ML in Garli version 2. Maximum Parsimony and Bayesian analyses failed to recover significant branch support.

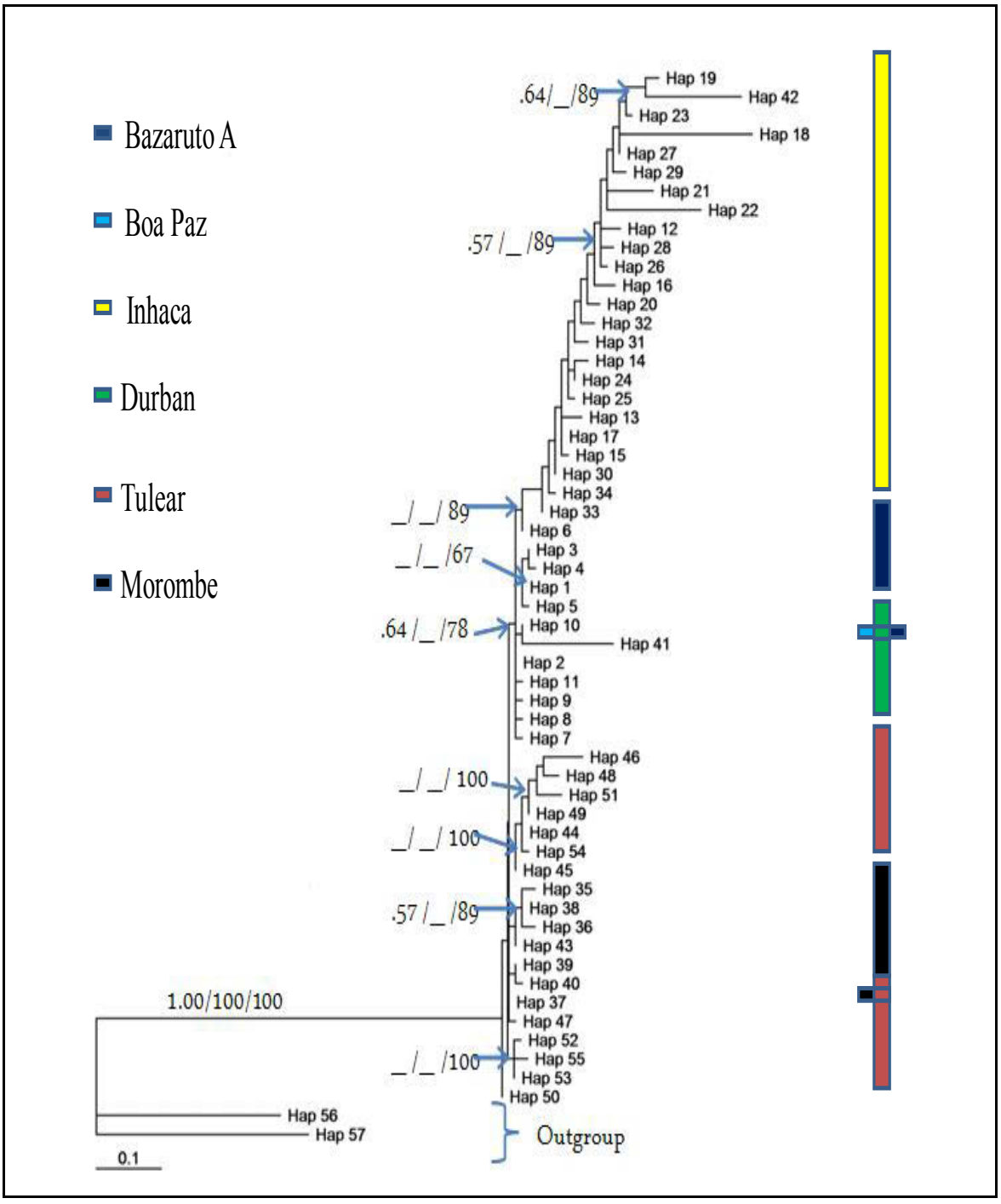


**Figure 3.5** Phylogenetic tree from Bayesian inferences reconstructed from mtDNA 16S sequences of *Haliporoides triarthrus*. Only values at or above 0.50 and 50% for posterior probability and bootstrap values, respectively, are reported. Outgroups (Hap 56 and Hap 57) were for *Penaeus monodon* (AF217843.1) and *Marsupenaeus japonicus* (AP006346.1).

Using JModeltest, the best-fit (lowest AIC) evolutionary model for *H. triarthrus* COI was the Transversion Parameter Model 3 with unequal frequencies and rate variation among sites (TPM3uf + G). The gamma distribution shape parameter was 4.766 and base frequencies were: A = 27%, C = 24%, G = 13% and T = 36%. An A-T bias was evident. Figure 3.6 shows the BY tree for the COI sequences with posterior probability values (BY) and bootstrap values of two methods (MP/ML) are shown for the most branches from all analyses. The strong differentiation and bootstrap-supported values suggested two distinct lineages, one from the Madagascar sites and the other from the African continental shelf sites. The two best supported lineages are separated by a sequence divergence of aproximately 2%.

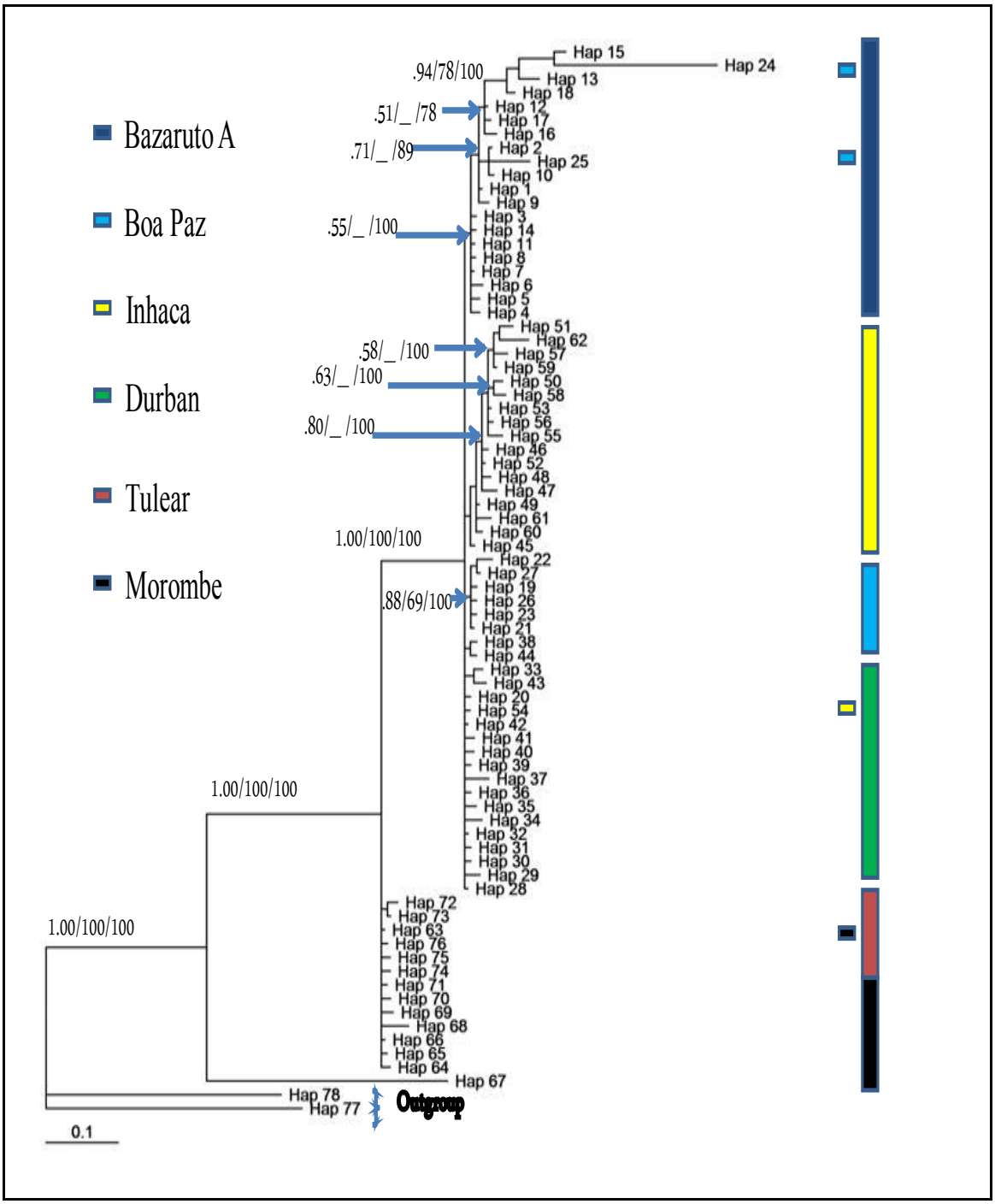


**Figure 3.6** Phylogenetic tree from Bayesian inferences reconstructed from mtDNA COI sequences of *Haliporoides triarthrus*. Only values at or above 0.50 and 50% for posterior probability and bootstrap values respectively, are reported. Outgroups (Hap 77 and Hap 78) were for *Litopenaeus stylirostris* (EU517503.1) and *Penaeus monodon* (AF217843.1).

*b) Metanephrops mozambicus*

### *3.6 Genetic diversity*

The fragment of 569 bp out of 218 aligned sequences of *M. mozambicus* using 16S rDNA revealed 112 haplotypes defined by 114 polymorphic sites, of which 62 were parsimony-informative. Also, from the network, 52 haplotypes were considered missed or undetected haplotypes. Most haplotypes were unique, and few were shared among sites (Appendix III). *M. mozambicus* exhibited high haplotype diversity at Boa Paz (0.982, range 0.871 - 0.985; Table 3.7). The lowest levels of nucleotide diversity were at Morombe ($\pi = 0.005$) and the highest at Durban ($\pi = 0.020$).

**Table 3.7** *Metanephrops mozambicus* genetic diversity for the 16S gene from six sampling sites in the SWIO. Sites are Bazaruto A (BA), Boa Paz (BP), Durban (DB), Inhaca (IA), Morombe (MM) and Tulear (TR).

| Statistics | BA | BP | DB | IA | MM | TR | Total |
|---|---|---|---|---|---|---|---|
| Number of sequences, n: | 40 | 40 | 38 | 40 | 30 | 30 | 218 |
| Number of segregating sites, S: | 22 | 49 | 46 | 58 | 25 | 19 | 114 |
| Number of haplotypes, h | 24 | 30 | 28 | 16 | 16 | 14 | 112 |
| Haplotype diversity, *h*: | 0.940 | 0.985 | 0.970 | 0.871 | 0.917 | 0.880 | 0.982 |
| Nucleotide diversity, $\pi$: | 0.006 | 0.013 | 0.020 | 0.009 | 0.005 | 0.008 | 0.014 |

The mtDNA sequences of approximately 1300 bp of COI gene were amplified and 161 sequences obtained. Of a total of 194 segregating sites, 92 were parsimony-informative and 127 were distinct haplotypes (Table 3.8). However, 71 haplotypes were missed or undetected from the network haplotype. There were no shared haplotypes among all populations (Appendix IV). Total haplotype diversity (*h*) was *0.993* (0.938 to 0.995) and the total nucleotide diversity ($\pi$) within locations was 0.017 (0.007 - 0.018).

**Table 3.8** *Metanephrops mozambicus* genetic diversity for the COI gene from six sites in the SWIO. Sites are Bazaruto A (BA), Boa Paz (BP), Durban (DB), Inhaca (IA), Morombe (MM) and Tulear (TR).

| Statistics | BA | BP | DB | IA | MM | TR | Total |
|---|---|---|---|---|---|---|---|
| Number of sequences, n: | 18 | 37 | 21 | 35 | 20 | 30 | 161 |
| Number of segregating sites, S: | 34 | 65 | 79 | 103 | 39 | 38 | 194 |
| Number of haplotypes, h | 17 | 28 | 16 | 23 | 19 | 24 | 127 |
| Haplotype diversity, *h*: | 0.993 | 0.944 | 0.967 | 0.938 | 0.995 | 0.979 | 0.993 |
| Nucleotide diversity, $\pi$: | 0.010 | 0.012 | 0.018 | 0.016 | 0.008 | 0.007 | 0.017 |

### *3.7 Haplotype network*

The mtDNA haplotype network for *M. mozambicus* was unclear for 16S (Fig. 3.7), with haplotypes shared among sites. Few haplotypes were shared among sampling sites and geographical partitioning of genetic diversity was uninformative. The two most common haplotypes were found at Inhaca and Morombe, with (14/217, 6.42%) each. Most unique haplotypes occurred at Durban (22 individuals). The rest of haplotypes were distributed in the network among each population, connected by a few mutational steps.

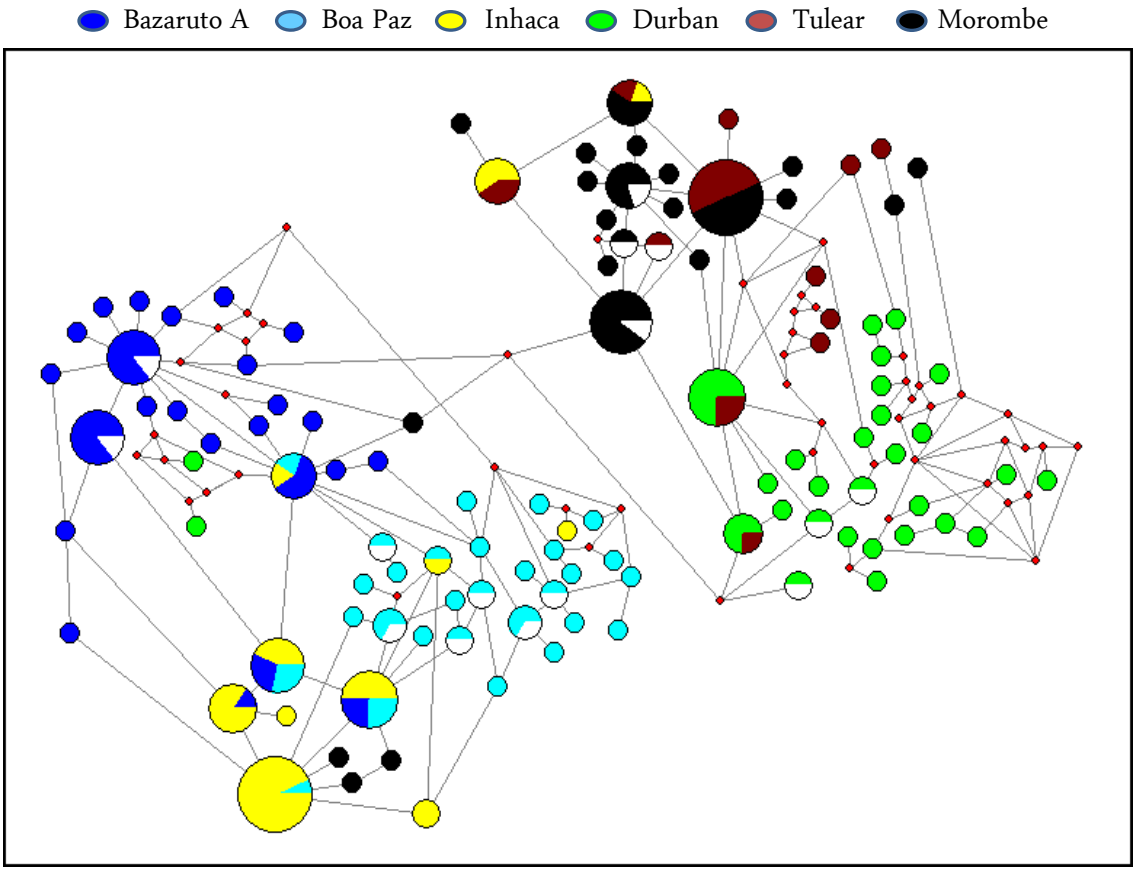


**Figure 3.7** Median-joining networks for *Metanephrops mozambicus* haplotypes from six sites in the SWIO for the 16S gene. Circles represent haplotypes. Diameter is proportional to frequency. The white colour wedges indicate the proportion of individuals from each sampling locations with a given haplotype. Each line represents one mutational step, and the small red circles represent missing or undetected haplotypes.

The haplotype network for the COI marker showed separate populations, except at Morombe, where some haplotypes from the continental shelf populations at Durban (two) and Bazaruto A (two) were present (Fig. 3.8). The most common haplotypes were encountered at Boa Paz (9/161, 5.59%) and Inhaca (8/161, 4.97%).

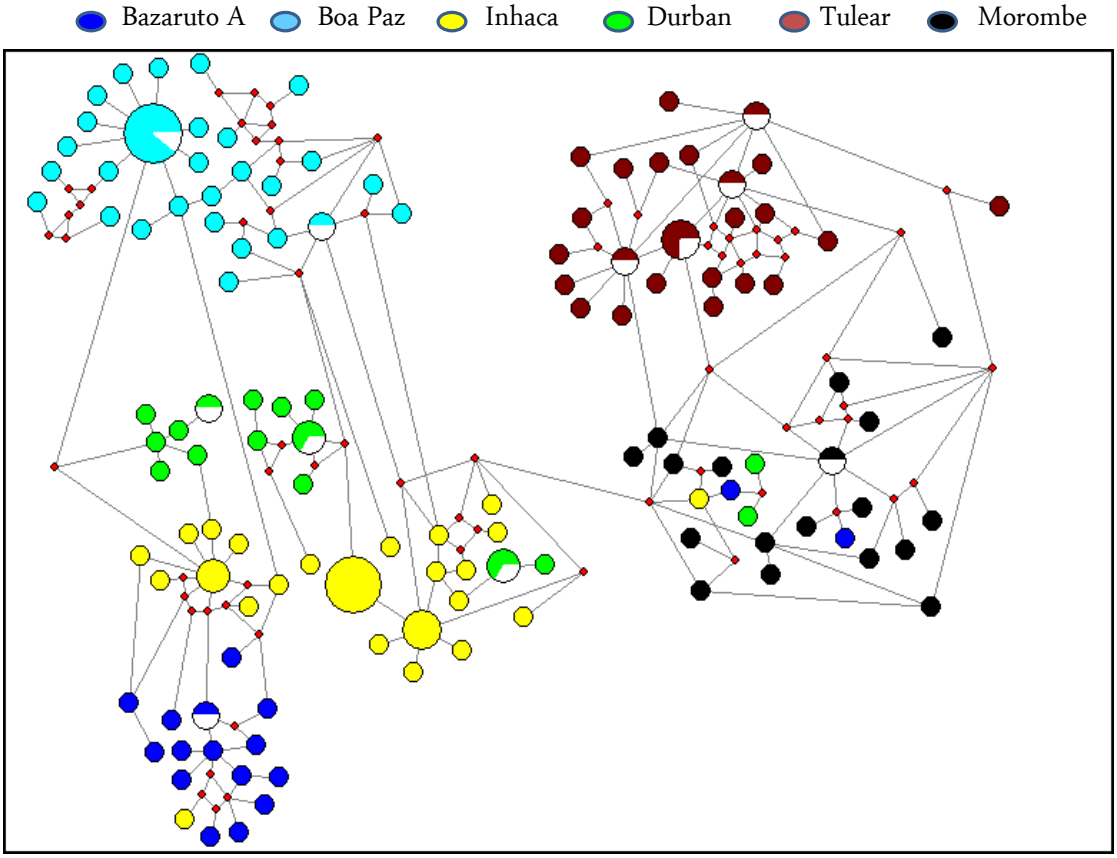


**Figure 3.8** Median-joining networks for *Metanephrops mozambicus* haplotypes from six sites in the SWIO for the COI gene. Circles represent haplotypes. Diameter is proportional to frequency. The white colour wedges indicate the proportion of individuals from each sampling locations with a given haplotype. Each line represents one mutational step, and the small red circles represent missing or undetected haplotypes.

### *3.7 Population structure*

The AMOVA was performed on three regions to generate $\Phi$ statistic based on prior expectations concerning spatial population differentiation. These regions were based on geopolitical boundaries (Mozambique, South Africa and Madagascar). AMOVA was conducted at all hierarchal levels (among regions, among populations and within populations). No differentiation among regions (Mozambique, South Africa and Madagascar) could be found using the 16S gene, although the COI gene indicated 14% differentiation among regions. The average $\Phi_{PT}$ values, 0.371 (16S) and 0.490 (COI) were

highly significant ($P< 0.001$) suggesting strong genetic variation between populations (Table 3.9). Haplotypes frequencies revealed that 61% (16S) and 51% (COI) of the genetic variation occurred within populations, and that 39% (16S) and 35% (COI) occurred among populations (Fig. 3.9).

**Table 3.9** Estimation of fixation indices ($\Phi$) and $P$ - value for *Metanephrops mozambicus* populations. The indices represent correlation among region ($\Phi_{RT}$), among population ($\Phi_{PR}$) and within population ($\Phi_{PT}$). Bold values are significantly different to zero at the 0.001% level.

| Stat | 16S | COI |
|---|---|---|
| $\Phi_{RT}$ | -0.032 | **0.143** |
| $\Phi_{PR}$ | **0.390** | **0.405** |
| $\Phi_{PT}$ | **0.371** | **0.490** |

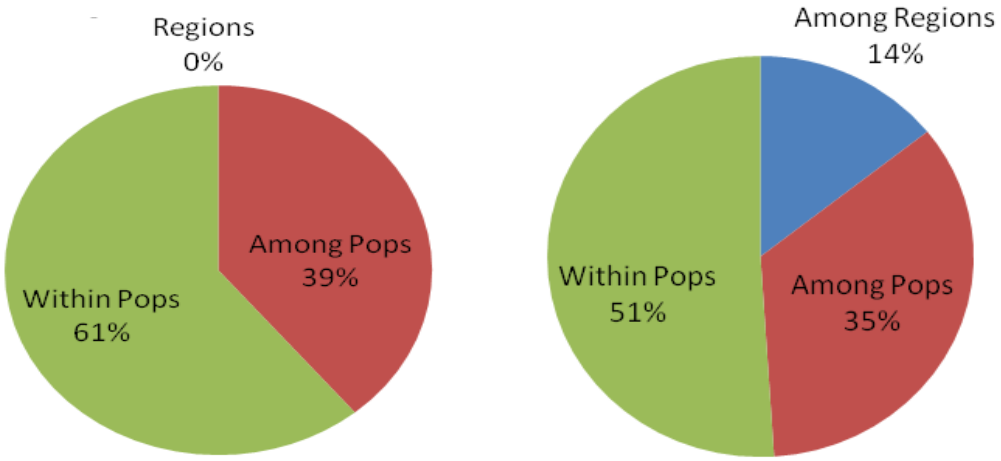


**Figure 3.9** Analysis of Molecular Variance within and among samples of *Metanephrops mozambicus* from three regions, Mozambique (BA+BP+IA), South Africa (DB) and Madagascar (MM and TR) using 16S (left) and COI (right).

Pairwise comparisons showed significant differences ($P < 0.001$) among sampling sites (Table 3.10). The highest value for the 16S marker (0.548) was between Morombe and Bazaruto A and the lowest (0.156) between Tulear and Inhaca. For COI, the highest value (0.599) occurred between Boa Paz and Tulear whilst the lowest (0.208) was between Inhaca and Durban.

**Table 3.10** Pairwises ($\Phi_{PT}$) values among sampling sites based on 16S gene (below diagonal) and COI gene (above diagonal) for *Metanephrops mozambicus* for each sampling site. All ($\Phi_{PT}$) values are significant at $P < 0.001$.

| | Bazaruto A | Boa Paz | Inhaca | Durban | Morombe | Tulear |
|---|---|---|---|---|---|---|
| Bazaruto A | - - - | 0.569 | 0.431 | 0.488 | 0.387 | 0.443 |
| Boa Paz | 0.440 | - - - | 0.326 | 0.473 | 0.583 | 0.599 |
| Inhaca | 0.396 | 0.350 | - - - | 0.208 | 0.432 | 0.488 |
| Durban | 0.507 | 0.277 | 0.331 | - - - | 0.502 | 0.547 |
| Morombe | 0.548 | 0.334 | 0.302 | 0.373 | - - - | 0.354 |
| Tulear | 0.479 | 0.386 | 0.156 | 0.313 | 0.290 | - - - |

Estimates of the expansion time for *M. mozambicus* populations ranged from 36 189 to 65 593 yr ago (16S) and 97 867 to 153 791 yr ago (COI). Figure 3.10 illustrates the unimodal mismatch distributions, which suggests that populations have undergone recent demographic expansion.

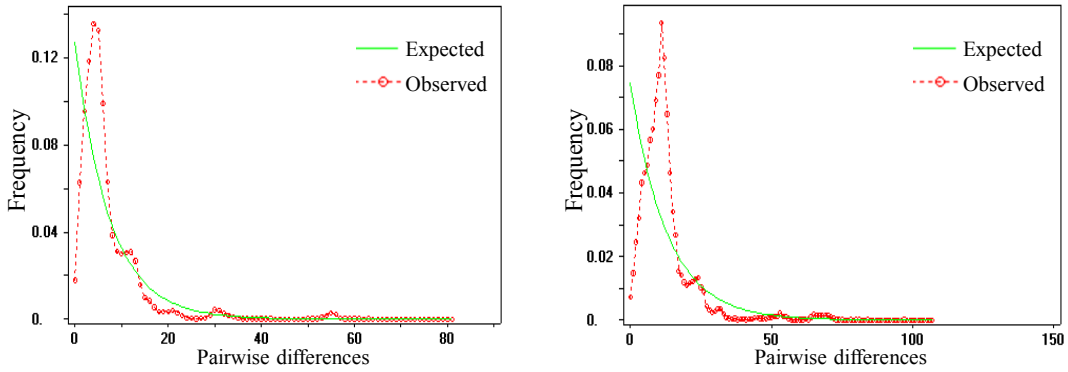


**Figure 3.10** Distributions of pairwise differences among sampling sites in the 16S (left) and COI (right) genes of *Metanephrops mozambicus* in the South West Indian Ocean.

To complement our study, the migration rate was estimated to test for gene flow among sites. The $F_{ST}$ statistics test showed low rates of migration ($N_em < 1$) (Table 3.11).

**Table 3.11** Gene flow estimates from $F_{ST}$ parameter for *Metanephrops mozambicus* for 16S and COI genes.

| | 16S | | COI | |
|---|---|---|---|---|
| Reference | Statistics | Gene flow ($N_em$) | Statistics | Gene flow ($N_em$) |
| Hudson et al. (1992) | $F_{ST}$: 0.30187 | 0.58 | $F_{ST}$: 0.32832 | 0.51 |

### *3.9 Analysis of historical demography*

The tests of selective neutrality (Tajima's D and Fu's Fs) among populations were negative (not all the values were signficant), except for Tajima's D of 16S gene in Durban and Tulear and Fu's Fs in Inhaca. Also the *P*- values were not significant for the COI gene in Bazaruto and Durban for Tajima's D and Fu's Fs. These results indicate a possibility of a natural selection or expansion of populations. Moreover, the Sum of Square Deviation and raggedness index from mismatch distribution patterns did not show significant differences *P*- value between the observed and expected distributions, implying a population historical event (except SSD for COI gene in Boa Paz and Durban) (Table 3.12).

**Table 3.12** Summary statistic of Tajima's D and Fu's Fs tests and mismatch distribution analyses; Sum of Squared Deviation (*SSD*) and raggedness index (*r*) of *Metanephrops mozambicus* for 16S and COI genes for each sampling site.

| | 16S | | | | COI | | | |
|---|---|---|---|---|---|---|---|---|
| Sites | D | F | *SSD* | *r* | D | F | *SSD* | *r* |
| Bazaruto A | **-1.564*** | **-20.820***** | 0.004 | 0.046 | -1.087 | **-9.802***** | 0.006 | 0.018 |
| Boa Paz | **-1.600*** | **-19.651***** | 0.002 | 0.013 | **-1.537*** | **-12.532**** | **0.534***** | 0.011 |
| Inhaca | **-2.387***** | -3.300 | 0.002 | 0.042 | **-1.996**** | **-4.250**** | 0.026 | 0.020 |
| Durban | -0.351 | **-10.777**** | 0.004 | 0.007 | **-1.591*** | -2.139 | **0.302***** | 0.033 |
| Morombe | **-2.110**** | **-9.540***** | 0.014 | 0.095 | **-1.754*** | **-13.657***** | 0.005 | 0.024 |
| Tulear | -0.809 | **-4.130*** | 0.021 | 0.061 | **-1.787*** | **-17.754***** | 0.002 | 0.016 |
| Mean | -1.469 | **-11.370*** | 0.008 | 0.044 | **-1.625*** | **-10.022*** | 0.146 | 0.020 |

Statistically significant values are in bold face $0.01 < P < 0.05$ (*), $0.001 < P < 0.01$ (**) and $P < 0.001$ (***).

### *3.10 Phylogenetic analysis*

Using the Jmodeltest programme, a Transversion Model with a gamma distributed rate (TVM+G) was selected (lowest AIC). The gamma distribution shape parameter was 1.0670 and base frequencies were A-T rich: Adenine (A = 32%), Cytosine (C = 17%), Guanine (G = 16) and Thymine (T= 35%). Figure 3.11 shows the best fitting tree topology based on Maximum-Likelihood analysis (ML) for the 16S sequences; with posterior probability values (BY) and bootstrap values of two methods (MP/ML). Bootstrap values below 50% are not shown. The tree was estimated using ML in Garli version 2.

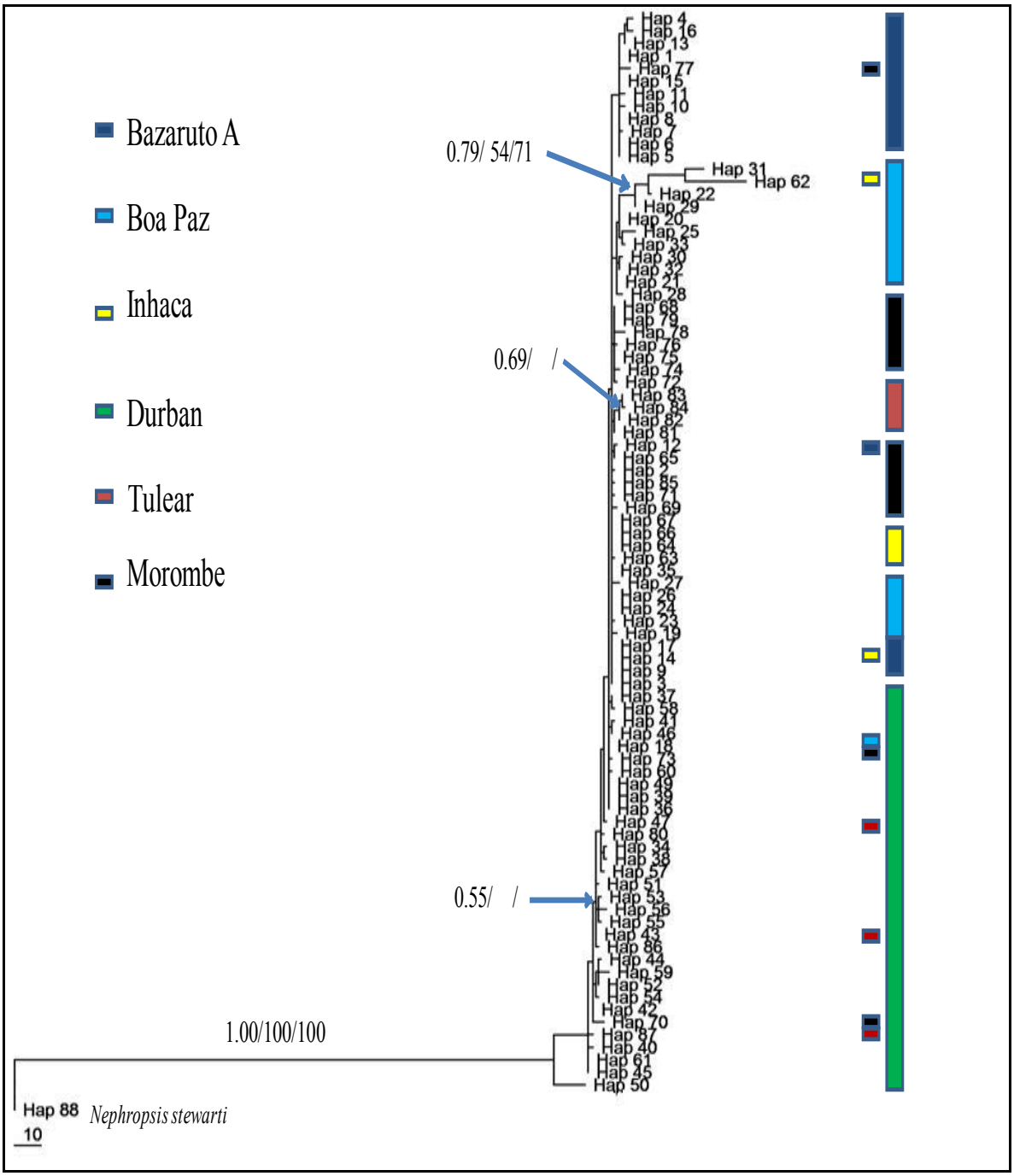


**Figure 3.11** Phylogenetic tree from Neighbour-joining reconstructed from mtDNA 16S sequences of *Metanephrops mozambicus*. Only values at or above 0.50 and 50% for posterior probability and bootstrap values, respectively, are reported. The outgroup (Hap 88) was for *Nephropsis stewarti* (U960861).

The best-fit evolutionary model for *M. mozambicus* using the COI marker was the Symmetrical Model characterized by equal base frequencies, and a symmetrical substitution matrix with rate variation among sites (SYM + G, Zharkikh, 1994) (Jmodeltest, lowest AIC). Nucleotides had a gamma distribution shape parameter of 1.9680 and the following substitution rates: A-C = 0.6187, A-G = 2.0245, A-T = 0.6982, C-G = 0.6456, C-T = 1.6266 and G-T = 1.000. Figure 3.12 shows the *M. mozambicus* Neighbour-joining tree for the COI gene.

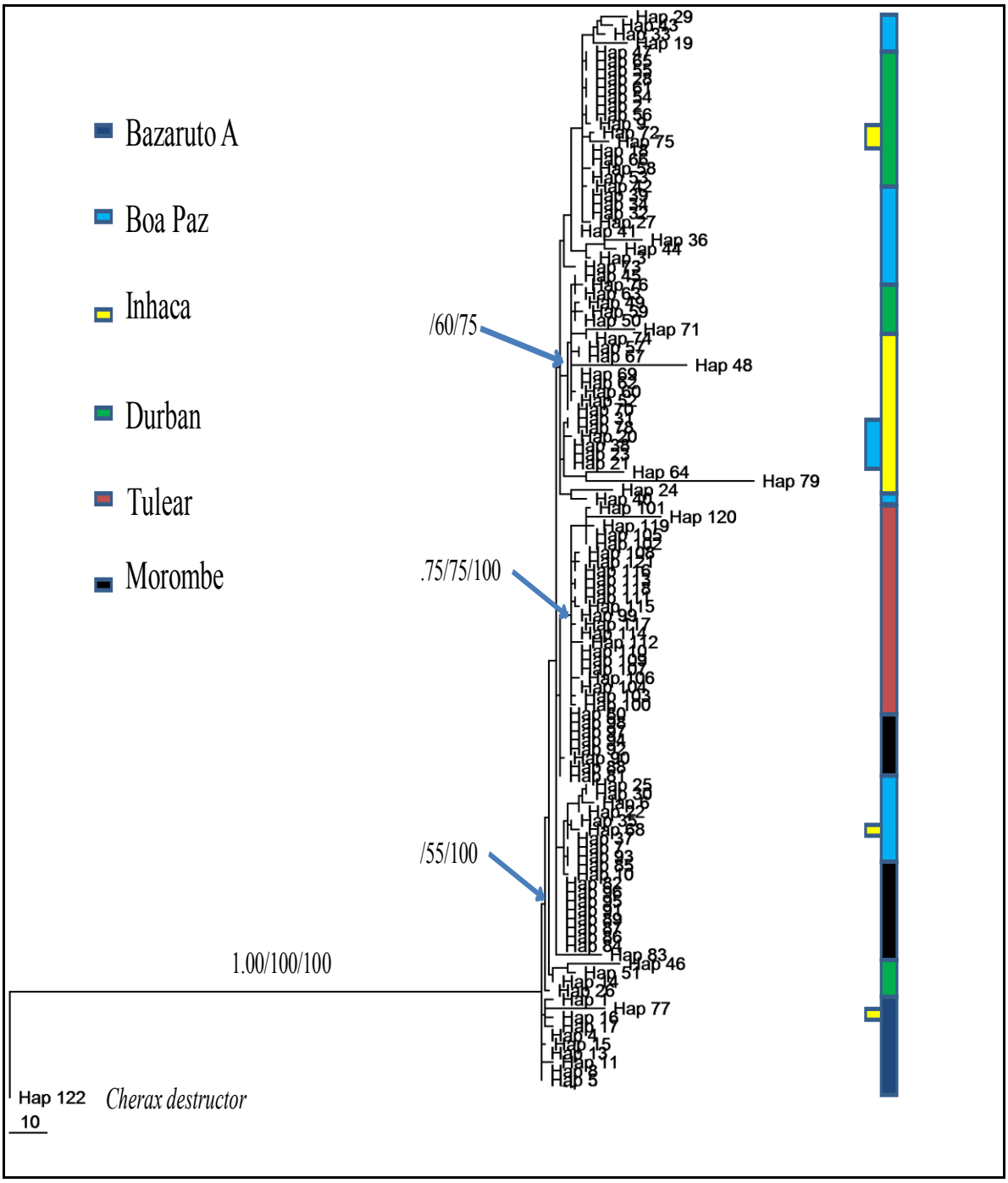


**Figure 3.12** Neighbour-joining phylogenetic tree reconstructed from mtDNA COI sequences of *Metanephrops mozambicus*. Only values at or above 0.50 and 50% for posterior probability and bootstrap values, respectively, are reported. The outgroup (Hap 122) was for *Cherax destructor* AY383557.2.

# Chapter IV

## 4. Discussion

### *4.1 General*

The mtDNA control region was used to examine genetic variability and metapopulation structure in the deep-water prawn *Haliporoides triarthrus* and langoustine *Metanephrops mozambicus* collected from six sites in the South West Indian Ocean (SWIO) region. To the best of our knowledge, this is the first reported work to assess the genetic population structure of *H. triarthrus* and *M. mozambicus*. The genetic diversity of the two mtDNA fragment sequenced (16S and COI) was moderately high compared to other deep-water crustaceans such as *Aristeus antennatus* (Roldán *et al.* 2009; Sardà *et al.* 2010; Cannas *et al.* 2011; Fernández *et al.* 2011a); *Aristaeomorpha foliacea* (Fernández *et al.* 2011b; Sacco 2011; Fernández *et al.* 2012); and spiny lobsters *Palinurus gilchristi* (Tolley *et al.* 2005)*, P. delagoae* (Gopal *et al.* 2006), *Jasus paulensis* and *J. tristani* (Groeneveld *et al.* 2012).

Comparing both data sets, from genetic diversity the total haplotype for *H. triarthrus* were slightly different 0.946 for 16S and 0.956 for COI whilst the nucleotide diversity were significantly different with 0.010 for 16S and 0.043 for COI. The total haplotype diversity for *M. mozambicus* were slightly different 0.982 for 16S and 0.993 for COI and the nucleotide diversity were moderately different 0.014 for 16S and 0.017 COI. However, the haplotype network reveled 43 (16S) and 22 (COI) missed or undetected haplotypes for *H. triarthrus*. Conversely, 52 (16S) and 71 (COI) missed or undetected haploypes for *M. mozambicus.* Also, COI is more useful in phylogenetic reconstruction for *H. triarthrus* and *M. mozambicus* compared with 16S. A sensible approach for tackling this problem was to use an appropriate nucleotide substitution model of evolution that incorporates multiple mutations at the same site for each gene, and to correct the observed distance for the multiple hits. The 16S gene was more conservative than the COI for both species as demonstrated by the polymorphism data and haplotype networks, although not significantly different.

Mismatch distribution analysis was used to calculate the frequency distribution of pairwise differences between sequences. Two different scenarios may be inferred from this analysis: a unimodal distribution characteristic of populations which have experienced a recent demographic expansion; otherwise unimodal distributions are formed by the accumulation of mutations with minimal lineage loss and indicate recent population expansion, whereas multimodal distributions are typically formed by mutations in demographic equilibrium with stochastic lineage loss and indicate the population has been constant in size (Cassone & Boulding 2006; Silva *et al.* 2010b; Shih *et al.* 2011). Our results demonstrated that, for both species, 16S and COI comprised a unimodal mismatch distribution (Fig. 3.8 and 3.12). This finding of a significant difference between the observed and expected distributions, is a possible consequence of recent demographic expansions. In most cases, Tajima's D and Fu's analyses resulted in significant negative values, supporting recent population expansions.

Expansion time for both species and markers ranged from 39 189 to 170 989 years which can be interpreted as a recent expansion after the Last Glacial Maximum at the end of the Pleistocene (Sotelo *et al.* 2009; Daniels 2011; Pulgarin & Burg 2012; Van der Plas *et al.* 2012). The study revealed previously unknown population history: the occurrence of two distinct lineages with apparently separate evolutionary trajectories followed by mixing and population expansion around the time of the Last Glacial Maximum (LGM). In other spiny lobsters of the Indian Ocean, genetic analysis detected signatures of population expansion potentially related to the Last Glacial Maximum (LGM) that could be attributed to colonizing of newly available habitat as glaciers melted and flooded previously terrestrial areas (*Palinurus delagoae*; Gopal *et al.* 2006, later split into *P. delagoae* and *P. barbarae*, Groeneveld *et al.* 2006). Analysis of *P. gilchristi* in South Africa suggested that current panmixia and levels of genetic diversity might be linked not only to ongoing larval dispersal, but also to recent expansion related to the LGM (Tolley *et al*. 2005). Many marine species were affected directly in their population distribution, diversity and demographic expansions as a consequence of the sea level and temperature fluctuations

during the Pleistocene-era (Peters *et al.* 2005; Li *et al.* 2009; Huang & Lin 2011; Shen *et al.* 2011; Van de Putte *et al.* 2012). Clemens *et al.* (1996) mentioned that during the Pleistocene, the Indian Monsoon winds changed intensity and phase with repercussions to the oceanic circulation in the Western Indian Ocean (Rai & Srinivasan 1994; Gupta & Thomas 1999; Ragionieri *et al.* 2010; Vogler *et al.* 2012). The effect of the sea level fluctuations during Pleistocene events appears to have completely interrupted gene flow of several marine taxa between the African mainland and Western Indian Ocean Islands (Daniels *et al.* 2002; Ragionieri *et al.* 2009; Daniels 2011). This may also have been the case for *H. triarthrus* and *M. mozambicus.*

Over a period of 20 years of deep-water trawl fishing in South Africa, the nominal catch rate (or catch-per-unit-effort, CPUE) of both *H. triarthrus* and *M. mozambicus* have remained relatively stable, with some increases measured since 2002 (Groeneveld 2012; Robey 2013) (Fig. 4.1). Off Mozambique, total catches of deep-water crustaceans have declined since the early 1990's but CPUE has increased, in concert with lower fishing effort (Groeneveld 2012). It remains unclear whether increases in CPUE are as a result of higher abundance, or of improvements in fishing gear and technology.

Effective population size can further impact on retention of genetic diversity which depends on high rates of multiple paternities thus increasing the reproductive rate (Harun 2013). Many features such as small population size, population which are slow to grow or are established in isolated regions can lead to a reduction in genetic diversity (Gregory *et al.* 2012). However, in the present study there appears to be large the haplotype and moderate nucleotide diversity.

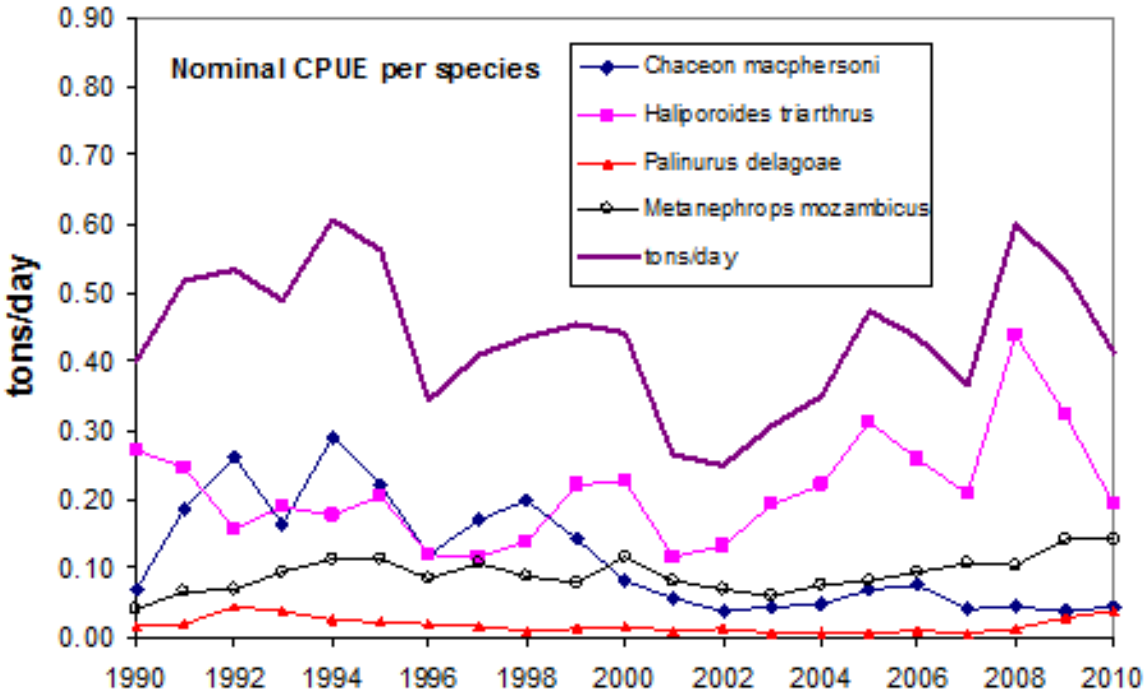


**Figure 4.1** Annual distributions of CPUE per species by tons/day from 1990 to 2010 for South African trawl grounds (from Groeneveld 2012).

### *4.1 Haliporoides triarthrus*

For *H. triarthrus*, 69 and 78 mtDNA haplotypes were recovered from the 16S and COI regions respectively, from six sampling sites (Table 3.1 and 3.2). The haplotypic diversity was (0.946 for 16S and 0.956 for COI) and nucleotide diversity (0.010 for 16S and 0.043 for COI). Similarly, an analysis of *A. antennatus* based on 16S sequences by (Fernández *et al.* 2011a) found 65 haplotypes from 506 individuals, with high haplotype diversity (0.259 - 0.666) and nucleotide diversity (0.0005 - 0.0017). The COI gene of *A. antennatus* demonstrated a congruent range for haplotype (0.378 - 0.978) and nucleotide diversity (0.0019 - 0.0072) (Fernández *et al.* 2013). These ranges are higher than for some other deep-water prawns, such as *A. foliacea* (Fernández *et al.* 2012): in this species the COI haplotype and nucleotide diversity ranged from 0.511 to 0.990 and 0.0011 to 0.0058, respectively. Therefore, *H. triarthrus* appear to have higher genetic diversity than many others deep-water prawn.

Analysis of Molecular Variance (AMOVA) revealed significant ($P < 0.001$) genetic differences among three regions (Mozambique, South Africa and Madagascar countries) ($\Phi_{RT}$ = 0.114 for 16S; 0.350 for COI), among populations ($\Phi_{PR}$ = 0.384; 0.482), as well as within populations sampled ($\Phi_{PT}$ = 0.454; 0.663) (Fig. 3.1; Table 3.4). Furthermore, the test showed genetic differentiation among populations of 34% for 16S and 31% for COI, respectively. A consistent genetic differentiation among populations of spiny lobster *Palinurus delagoae* from the Indian Ocean was shown by Gopal *et al.* (2006) based on COI. Most studies have been done in shallow water species, Gopurenko *et al.* (1999) used mtDNA control region sequences to show genetic differentiation among populations of mangrove crab *Scylla serrata* from the Red Sea, Mauritius and South Africa.

The genetic structure observed among *H. triarthrus* populations is most likely maintained by dynamic larval dispersal processes and pathways, formed by ocean currents, counter-currents and eddy systems in the Mozambique Channel and upper Agulhas Current along SWIO region. These water movements can either disperse larvae over long distances (see von der Heyden *et al.* 2008; Groeneveld *et al.* 2012), or return / retain them near their origin (see Chiswell & Booth 1999). Paula *et al.* (2001) suggested that eddy systems contribute to the retention of brachyuran larvae and restrict offshore dispersal, consequently preventing gene flow between southern and northern Mozambique. The gyre systems present along the Mozambique Channel can probably return drifting larvae of *H. triarthrus* to their origin, depending on larval behavior and duration of larval existence. This would be consistent with suggestions that a combination of life history attributes and behavior of larvae allow them to return a natal site in these systems (Sponaugle *et al.* 2002; Gopal 2007; Tilburg *et al.* 2012).

(Goetze 2011) reported genetic structure among populations of copepod *Pleuromomma xiphias* of the central and western Indian Ocean. (Silva *et al.* 2010b) studying crab *Uca annulipes* using control region mtDNA found no common haplotypes between southern and northern Mozambique. Therefore, recirculation within large-scale ocean features (such as subtropical gyres) may contribute to the retention of larvae and restrict offshore dispersal, as shown for various taxa with drifting larvae (Paula *et al.* 2001; Goetze 2011; Madeira *et*

*al.* 2012). Conversely, some deep-water crustaceans in the Mozambique Channel and Agulhas Current systems have shown panmixia across long stretches of coastline, for example deep-water lobsters *Palinurus gilchristi* (Tolley *et al.* 2005) and *Palinurus delagoae* (Gopal *et al.* 2006). In the present study, populations of *H. triarthrus* appeared to be completely separated from each other and characterized by no shared haplotypes (despite few shared haplotypes from the 16S gene) and low levels of migration, indicating reduced gene flow. It therefore appears that larvae of this deep-water species do not disperse widely, as is the case for deep-water lobsters (Tolley *et al.* 2005; Gopal *et al.* 2006), but that they rather remain, or are returned to natal sites.

Phylogenetic results show two distinct lineages/clades, which are supported by 100% bootstrap in Maximum Parsimony and Maximum Likelihood methods and 1.00 posterior probability values in Bayesian inference (Fig. 3.10). The first clade includes populations from Mozambique and South Africa (continental shelf) and the second clade includes Tulear and Morombe (Madagascar). These two clades differ by approximately 2% sequences divergence, suggesting that two sister species occur in the SWIO region, one along the African continental shelf, and the other along the west coast of Madagascar. Nucleotide sequence divergence permits one to ascertain the level of divergence between taxa (Hebert *et al.* 2003). Previous barcoding studies have indicated that interspecific divergences of only 2–3% should be sufficient for distinguishing invertebrate species (Naro-Maciel *et al.* 2011). A divergence of 2% per million years is the generally accepted rate of DNA divergence between lineages in the mtDNA gene (Hebert *et al.* 2004). The general consensus concerning approximately 2% sequence divergence has been used to delineate species but these estimates may increase for some species such as butterflies (3.6%) or decrease in others (Tsao & Yeh 2008). Nevertheless, levels of genetic differentiation between recognized different species of *Penaeus* ranged from 0.7% to 20.2% (Lavery *et al.* 2004). In the present study, nucleotide sequence divergence was approximately 2% when a General Time Reversible (Tavaré 1986) model was applied to the data set (Fig 3.10). This result supports earlier studies that recognized at least two subspecies from the region, *H. triarthrus triarthrus* from South Africa and Mozambique

and *H. triarthrus vniroi* from Madagascar and Mozambique (Kensley *et al.* 1987; Pérez-Farfante & Kensley 1997; De Grave & Fransen 2011). *Haliporoides triarthrus vniroi* was previously recognized from Madagascar by Crosnier and Jouannic (1973) and Crosnier (1978). A subspecies of the Indo West-Pacific congeneric, *Haliporoides sibogae (H. sibogae madagascariensis*) is also recognized from Madagascar (Crosnier 1978). Although the present genetic analysis supports the existence of the two subspecies in the region, it restricts their distribution ranges to the African continental shelf (presumably *H. triarthrus triarthrus*) and western Madagascar (*H. triarthrus vniroi*), respectively. This differs from earlier studies, where *H. triarthrus vniroi* apparently occurred along the coast of Mozambique as well. The earlier studies were, however, based on morphological differences between the two subspecies, which are difficult to distinguish during field sampling (see Kensley *et al.* 1987).

Gopal *et al.* (2006) suggested that larvae of deep-water spiny lobster *Palinurus delagoae* originating from the African shelf are unlikely to cross the Mozambique Channel to Madagascar, because they will be swept southwestwards along the African Coast by the Agulhas Current. Whether *H. triarthrus* larvae can cross the channel from Madagascar to the African shelf in anti-cyclonic eddies spinning off the East Madagascar Current around the southern tip of the island is yet unclear, and the subject of another study. Nevertheless, it is clear that oceanographic barriers and geological features can limit gene flow, and that in some cases ocean currents can isolate populations, rather than facilitate wide dispersal. Separation by ocean currents (i.e. the Mozambique Channel model) fits the data well, especially considering that haplotypes are not shared within these two clades, and that there is little evidence of connectivity.

The results showed that the African continental shelf population is genetically structured (few shared haplotypes), with little overlap between populations at neighbouring sites, even when they are relatively close to each other (a few hundred km). Few exceptions of shared haplotypes among populations along the African coast were observed (i.e. two 16S haplotypes were shared between Bazaruto A and Durban; two haplotypes were also common to Boa Paz and Bazaruto A; one haplotype shared between Inhaca and Durban). In

Madagascar, a haplotype common to Tulear also occurred at Morombe. Rates of mutation or deletion in this haplotype (individual) could be the reason for the mismatch. It is also possible that these exceptions were the result of mislabeling in the laboratory work, sequencing and data arrangement or post-processing stages of molecular analysis.

The large oceanic gyres derived from the Mozambique Channel in the WIO, are the most likely physical mechanisms that could act as barriers reducing dispersal for marine organisms (Madeira *et al.* 2012), and are thought to be key to speciation in other genera (i.e. *Palinurus;* Groeneveld *et al.* 2007). In this region abundant eddy recirculation occurs on a large-scale (Paula *et al.* 2001), characterized by nutrient richness (Luschi *et al.* 2006), and these could prevent gene flow between northern and southern regions. Chiswell and Booth (1999) and Matthee *et al.* (2007) have demonstrated that oceanic process such as eddies can return *Jasus* phyllosoma larvae to their coastal habitats after being dispersed far offshore. Groeneveld *et al*. (2007) has suggested that female deep-water lobster *Palinurus delagoae* females off Mozambique and eastern South Africa migrate shallower, out of the strongest currents, to release larvae. Furthermore, to retain populations in areas with strong currents, juveniles undertaking counter-current migrations, to upstream reproductive areas (Groeneveld 2002; Groeneveld & Branch 2002). Similar processes may also be at work in *H. triarthrus*, which inhabits the same depth range geographical area as *P. delagoae*. In this model, larval retention in eddies through a combination of life-history attributes and behaviour will eventually give rise to genetic partitioning among populations.

The present analysis suggests that *H. triarthrus* populations underwent a recent demographic expansion. Sardà *et al.* (2010) reported a similar expansion for deep-water shrimp *A. antennatus* in the Western Mediterranean (16S control region). Tolley *et al*. (2005) and Gopal *et al.* (2006) found similar demographic expansions in two deep-water lobster species, on the Agulhas Bank and in the Mozambique Channel, respectively. Likely recent demographic expansion of shallower water taxa, *Uca annulipes* (Silva *et al.* 2010b), *Perisesarma guttatum* (Silva *et al.* 2010a) and *Cerithidea decollate* (Madeira *et al.* 2012) between southern and northern Mozambique were suggested by the above mentioned

author when they found both Tajima's D and Fu's Fs tests were negative and statistically significant. These tests showed similar results in the present analysis.

Population expansion dates of *H. triarthrus* was estimated as 64 542 - 116 982 yr ago (16S) and 108 812 - 170 989 yr ago (COI). The estimates (64 542 - 170 989 yr ago) suggests that expansions occurred during the late Pleistocene (Xu *et al.* 2009a; Silva *et al.* 2010b; Duda *et al.* 2012), during a period of successive glaciations and sea-level changes. Similar results were obtained from other crustaceans taxa. Black tiger shrimp, *Penaeus monodon* began to expand around 19 527 - 164 705 yr ago (Waqairatu *et al.* 2012); kuruma shrimp *P. japonicus* expansions date from 75 669 - 129 052 yr (Shih *et al.* 2011); and fiddler crab *U. annulipes* expansions date from 15 000 to 275 000 yr (Silva *et al.* 2010b). Our results could be interpreted in a similar fashion, highlighting the recent demographic population expansion for *H. triarthrus* occurring since the late Pleistocene. The high genetic diversity, closely related haplotypes, and occurrence of two distinct lineages detected in this analysis were consistent with a history of isolation in two glacial refugia followed by population expansion and mixing around the time of the Last Glacial Maximum. The range in estimated time since expansion was wide, given uncertainty in mutation rates (Tolley *et al.* 2005; Gopal *et al.* 2006; Palero *et al.* 2008).

*4.1 Metanephrops mozambicus*

This study showed high levels of genetic diversity measured by haplotype (0.982 for 16S and 0.993 for COI) and nucleotide diversity (0.014 for 16S and 0.017 for COI) in populations of *M. mozambicus*. These levels were similar to those described in other crustaceans, for instance, an analysis based on COI of spiny lobster *Palinurus delagoae* found range for haplotype diversity (0.957 - 0.999) and nucleotide diversity (0.006 - 0.009) (Gopal *et al.* 2006); and deep-water spiny lobster *P. gilchristi* haplotype diversity ranged between (0.843 - 0.896) and nucleotide diversity (0.0038 - 0.0045) (Tolley *et al.* 2005). The high diversity in some marine species are indicative of either a long stable evolutionary history or secondary contact among differentiated lineages (Grant & Bowen 1998; Bayl *et al.* 2003).

Many factors such as historical events, anthropogenic activities, complex interaction of biology and geography, and low rates of mitochondrial evolution can influence genetic diversity and variability (Grant *et al.* 2006; Xiao *et al.* 2009; Shih *et al.* 2011; Xu *et al.* 2012). Our analysis of DNA sequences shows that the highest levels of genetic variation are within populations (61% for 16S and 51% for COI). The values of $\Phi_{PT}$ (0.371 and 0.490) and high and significant pairwise $\Phi_{ST}$ values between sampling sites is indicative of genetic differentiation within populations. Furthermore, the results show that few haplotypes are shared between populations at the different sampling, suggesting that effective gene flow between these locations is restricted. The $\Phi_{PT}$ statistics and haplotype networks suggest that these populations are genetically distinct and appear historically separated from each other. In addition, all pairwise estimates of $\Phi_{PT}$ were closer to one than zero, which may be considered an indication of isolation of populations (Holsinger & Weir 2009). Life-history patterns and pelagic larval duration are considered two of the many factors which result in population genetic structuring for marine organisms in southern Mozambique and South Africa (Evans *et al.* 2004; Teske *et al.* 2007).

The large negative Tajima's *D* and Fu's *Fs* values (1997) and the unimodal mismatch distribution observed in our study support the occurrence of recent population expansion in *M. mozambicus*. The expansion was dated at approximately 46 644 (36 189 - 65 593) yr ago for 16S and 119 615 (97 867 - 153 719) yr for COI, depending on the mutation rate and generation time assumed. Related mtDNA studies on other crustaceans species in Mozambique and South Africa have suggested similar recent population expansions (Tolley *et al.* 2005; Gopal *et al.* 2006; Neethling *et al.* 2008). Expansion of lobster *Jasus paulensis* populations among SWIO and South Atlantic islands and seamounts started between 14 000 - 118 000 yr ago (Groeneveld *et al.* 2012); *J. tristani* expansions at Tristan da Cunha archipelago in the South Atlantic between 12 000 - 99 000 yr ago (von der Heyden *et al.* 2007); deep-water spiny lobster *P. gilchristi* ranged between 5 300 - 10 600 yr ago (Tolley *et al.* 2005) and *P. delagoae* ranged between 9 000 - 40 000 yr ago (Gopal *et al.* 2006). It is likely during this time that *M. mozambicus* populations lost their habitats after sea level rise and recolonization of new area. Recently similar result has been reported in North-east

Atlantic a recent population expansion of *Nephrops norvegicus* occurred after the last glacial maximum (LGM) during Pleistocene era (Harun 2013).

Population structure has been reported for shallow water crustaceans such as the mud crab *Scylla serrata* (Gopurenko 2002); (Fratini & Vannini 2002); mussels of *Perna perna* and *Mytilus galloprovincialis* (Zardi *et al.* 2007); estuarine prawn *Callianassa kraussi* (Teske *et al.* 2009b) and mangrove crab *Perisesarma gluttatum* (Silva *et al.* 2010a). The physical / oceanographic mechanisms driving larval dispersal and thus genetic population structure is likely to differ greatly among coastal (shallow-water) and offshore (deep-water) benthic taxa.

Mitochondrial DNA analysis of deep-water spiny lobster *P. delagoae* in the SWIO showed a shallow genetic partitioning between populations off southern Mozambique and those off eastern South Africa (Gopal *et al.* 2006). This partitioning supported earlier morphological studies that suggested the occurrence of two populations along the southeast African coast (Berry & Plante 1973). The boundary between the two populations is consistent with the interface between the Mozambique Channel eddies and the upper Agulhas Current, in the vicinity of northern KwaZulu-Natal (South Africa) and southern Mozambique. Although some larvae doubtlessly disperse across this interface, others may be retained in the slow-moving anti-cyclonic eddies moving southwards along the Mozambique shelf-edge. In this case, the genetic partitioning was attributed to larval retention in these eddies, through a combination of life-history attributes and behaviour (Gopal *et al.* 2006). Genetic structure may have evolved, and are maintained in *M. mozambicus* populations in a similar way.

For *M. mozambicus*, egg-bearing females were present off South Africa and Mozambique - assuming larvae from the SA population are entrapped in the Durban eddy, and are returned to the area of origin (roughly), and that the same happens off Mozambique (i.e. larvae are trapped in the cyclonic/anticyclonic eddies and are eventually returned to their origin), then genetic structure might have evolved among subpopulations (Chiswell & Booth 1999; Matthee *et al.* 2007; Groeneveld *et al.* 2012).

De Ruijter *et al.* (2002) reported that the eddy systems must be enough to provide a significant connection between marine organisms. However, parental investment (production of larger eggs) could also be considered a strategy to establish and maintain stable populations (Cruz *et al.* 2006; Torres *et al.* 2007; Penha-Lopes *et al.* 2009). An alternative interpretation of isolation of *M. mozambicus* among sites, could be due to females bearing few eggs and larvae after hatching which are likely to settle immediately close to their natal source. This mechanism would counterbalance the high larvae mortality suffered by long-distance dispersal in ocean currents; species that disperse thus generally produce high numbers of small eggs (Pollock & Melville-Smith 1993). Short larvae periods and their retention near the natal population reduce larval encounters with predators (Pineda *et al.* 2007; Teske *et al.* 2008; Griffiths *et al.* 2010; Kimirei 2012; McManus & Woodson 2012).

## Chapter V

### 5. Conclusions and future research direction

In summary, we found high genetic diversity and significant differentiation in *H. triarthrus* and *M. mozambicus* in the SWIO region, using mtDNA 16S and COI genes.

The most important finding in this research is the support for two sister-species (or subspecies) of *H. triarthrus*, one occurring along the African continental shelf and other off western Madagascar. Furthermore, individual populations making up each lineage were genetically structured, as indicated by the absence of shared haplotypes, and should be recognized as being demographically distinct from each other.

Both species have undergone recent population expansions, likely since the late Pleistocene. Several other studies have suggested similar population expansions of SWIO taxa, both from species occurring in coastal (shallow) and offshore (deep) water habitats.

The large anti-cyclonic and cyclonic eddies prevalent in the Mozambique Channel, as well as the boundary area between these eddies and the upper Agulhas Current are likely factors driving larval retention or return processes, thus giving rise to genetically structured populations. Furthermore, water movements in the channel appear to restrict larval exchange (gene flow) between western Madagascar and Mozambique / eastern South Africa.

The strong signal of genetic differentiation is consistent across the sampling range, which comprised almost all of the entire known geographic distribution ranges of the two species. The finding of distinct metapopulations suggest that fisheries management strategies should be adapted to consider individual stock units, instead of treating the two species *H. triarthrus* and *M. mozambicus* as "single" stocks, even within each of the three countries. It also suggests that stocks are not necessarily shared by the three countries in which they are fished. Management of crustacean fisheries in the SWIO region takes place on a national level, and only a few fisheries are indeed managed actively (i.e., the larger industrial fisheries for prawns and lobsters in South Africa, Mozambique and Madagascar).

The findings from this study are unique for the SWIO region, and may lead to a paradigm shift in the way that deep-water crustacean stocks are perceived by fisheries managers - instead of single shared stocks, they comprise of many isolated ones, in spite of the dispersal potential of larvae in strong ocean current regimes. Thus they should be managed as smaller independent units.

Future studies need to: verify and expand on the genetic structure observed at various levels (site and region) by including nuclear markers and microsatellites; identify stock boundaries for fisheries management purposes; and address biological and ecological factors, such as fecundity, larval duration and behavior, and dispersal pathways to explain early life history in relation to environmental drivers.

## 6. References


Ahyong S.T., Andreakis N. & Taylor J. (2011) Mitochondrial phylogeny of the deep-sea squat lobsters, Munidopsidae (Galatheoidea). *Zoologischer Anzeiger - A Journal of Comparative Zoology* **250**, 367-77.

Alfaya J., Bigatti G. & Machordom A. (2012) Mitochondrial and nuclear markers reveal a lack of genetic structure in the entocommensal nemertean *Malacobdella arrokeana* in the Patagonian gulfs. *Helgoland Marine Research* 1-6.

Allendorf F.W., Hohenlohe P.A. & Luikart G. (2010) Genomics and the future of conservation genetics. *Nature Reviews Genetics* **11**, 697-709.

Allendorf F.W. & Luikart G.H. (2007) Conservation and the genetics of populations. Blackwell Publishing, Malden, Massachusetts.

Anker A. & Baeza J.A. (2012) Molecular and morphological phylogeny of hooded shrimps, genera *Betaeus* and *Betaeopsis* (Decapoda, Alpheidae): Testing the center of origin biogeographic model and evolution of life history traits. *Molecular Phylogenetics and Evolution* **64,** 401-415.

Ansorge I.J. (2006) A Review of Physical Oceanograph Research Undertaken in South Africa, 2003 -2006, pp. 11.

Arif I.A. & Khan H.A. (2009) Molecular markers for biodiversity analysis of wildlife animals: a brief review. *Animal Biodiversity and Conservation* **32**, 9-17.

Avise J.C. (2009) Phylogeography: retrospect and prospect. *Journal of Biogeography* **36**, 3-15.

Avise J.C., Arnold J., Ball R.M., Bermingham E., Lamb T., Neigell J.E., Reeb C.A. & Saunder N.C. (1987) Intraspecific Phylogeography: The Mitochondrial DNA Bridge Between Population Genetics and Systematics. *Annual Review of Ecology and Systematics* **18**, 489-522.

Avise J.C. & Walker D. (2000) Abandon all species concepts? A response. *Conservation Genetics* **1**, 77-80.

Baelde P. (1992) Marine Biology Reproductive biology of commercially exploited deep-water royal red prawns (*Haliporoides sibogae,* Solenoceridae) in south-east Australia. *Marine Biology* **113**, 447-56.

Baelde P. (1994) Growth, mortality and yield-per-recruit of deep-water royal red prawns (*Haliporoides sibogae*) off eastern Australia, using the length-based MULTIFAN method. *Marine Biology* **118**, 617-25.

Bandelt H.J., Foster P. & Rohl A. (1999) Median-Joining Networks for Inferring Intraspecific Phylogenies. *Molecular Biology and Evolution* **16**, 37-48.

Baye T.M. (2011) Inter-chromosomal variation in the pattern of human population genetic structure. *Human Genomics* **5,** 220-240.

Bayl L.K., Choat J.H., van Herwerdeni L. & Robertson D.R. (2003) High genetic diversities and complex genetic structure in an Indo-Pacific tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past? *Marine Biology*, 31.

Berry P.F. (1969) The Biology of *Nephrops andamanicus* Wood-Mason (Decapoda, Reptantia). In: *Investigational Report of the Oceanographic Research Institute of South Africa,* (22): 1-55.

Berry P.F., Heydorn A.E.F. & Alletson D.J. (1975) The biology of the knife prawn, *Hymenopenaeus triarthrus* off the Natalcoast. ORI Unpublished Reports, Oceanographic Research Institute. 75: 23.

Berry P.F. & Plante R. (1973) Revision of the spiny lobster genus Palinurus in the South-West Indian Ocean. *Transactions of the Royal Society South Africa* **40**, 373–80.

Birky C.W., Fuerst P. & Maruyama T. (1989) Organelle gene diversity under migration, mutation and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells and comparison to nuclear genes. *Genetics* **121**, 613–27.

Boore J.L. (1999) Animal mitochondrial genomes. *Nucleic Acids Research* **27**, 1767-80.

Breinholt J.W., Van Buren R., Kopp O.R. & Stephen C.L. (2009) Population genetic structure of an endangered Utah endemic, *Astragalus ampullarioides* (Fabaceae). *American Journal of Botany* **96,** 661-7.

Brinca L., Cristo M. & Silva C. (1983) Camarao de profundidade. Relatòrio dos Cruzeiros realizados com o N/I "Ernst Haeckel". In: *Revista de Investigacao Pesqueira* (5): 1-99.

Bucklin A. & Allen L.D. (2004) MtDNA sequencing from zooplankton after long-term preservation in buffered formalin. *Molecular Phylogenetics and Evolution* **30,** 879-882.

Cannas R., Sacco F., Follesa M.C., Sabatini A., Arculeo M., Lo Brutto S., Maggio T., Deiana A.M. & Cau A. (2011) Genetic variability of the blue and red shrimp *Aristeus antennatus* in the Western Mediterranean Sea inferred by DNA microsatellite loci. *Marine Ecology*, 1-14.

Cassone B.J. & Boulding E.G. (2006) Genetic structure and phylogeography of the lined shore crab, *Pachygrapsus crassipes*, along the northeastern and western Pacific coasts. *Marine Biology* **149**, 213-26.

Chiswell S.M. & Booth J.D. (1999) Rock lobster *Jasus edwardsii* larval retention by the Wairarapa Eddy off New Zealand. *Marine Ecology Progress Series* **183**, 227-40.

Chow S., Suzuki N., Imai H. & Yoshimura T. (2006) Molecular species identification of spiny lobster phyllosoma larvae of the genus *Panulirus* from the Northwestern Pacific. *Marine Biotechnology (NY)* **8**, 260-7.

Chu K.H., Li C.P., Tam Y.K. & Lavery S. (2003) Application of mitochondrial control region in population genetic studies of the shrimp *Penaeus*. *Molecular Ecology Notes* **3**, 120-2.

Clemens S.C., Murray D.W. & Prell W.L. (1996) Nonstationary phase of the Plio-Pleistocene Asian Monsoon. *Science* **274**, 943–8.

Cobb J.S. (1997) Oceanic processes affecting lobster larvae: report from a workshop. *Marine Freshwater Research* **48**, 771–5.

Cooke A., Lutjeharms J.R.E. & Vasseur P. (2004) Marine and coastal ecosystems. In: *The Natural History of Madagascar*, editors: Goodman, S. M. and Benstead, J. P. The University of Chicago Press, Chicago, 179-209.

Crosnier A. (1978) Crustacés Décapodes pénéides Aristeidae (Benthesicyminae, Aristeinae, Solenocerinae).— Faune de Madagascar 46: 1-197.

Crosnier A. & Jouannic C. (1973) Note d'information sur les prospections de la pente continentale Malgache effectuees par le N.O. Vauban (Bathymetrie – Sedimentologie – Peche au chalut). ORSTROM Doc no 42. Dec. 1973. pp. 36.

Cruz R., Lalana R., Perera E., Báez-Hidalgo M. & Adriano R. (2006) Large Scale Assessment of Recruitment for the Spiny Lobster, *Panulirus argus*, Aquaculture Industry. *Crustaceana* **79**, 1071-96.

Daniels S.R. (2011) Reconstructing the colonisation and diversification history of the endemic freshwater crab (*Seychellum alluaudi*) in the granitic and volcanic Seychelles Archipelago. *Molecular Phylogenetics and Evolution* **61,** 534-542.

Daniels S.R., Stewart B.A., Gouws G., Cunningham M. & Matthee C.A. (2002) Phylogenetic relationships of the southern African freshwater crab fauna (Decapoda: Potamonautidae: Potamonautes) derived from multiple data sets reveal biogeographic patterning. *Molecular Phylogenetics and Evolution* **25,** 511-523.

De Freitas A.J. (1985) The penaeoidea of southeast Africa. II. The families *Aristeidae* and *Solenoceridae*. Oceanographic Research Institute. Investigational Report 57. pp. 1-69.

De Freitas A.J. (2004) The Penaeoidea of southeast Africa IV – The Family Penaeidae: Genus Penaeus. Oceanographic Research Institute. *Investigational Report of Oceanographic Research Institute*, (59): 1 – 112.

De Grave S. & Fransen C.H.J.M. (2011) Carideorum Catalogus: the recent species of the dendrobranchiate, stenopodidean, procaridideanand caridean shrimps (Crustacea: Decapoda). Zoologische Mededelingen, 85(9). NCB Naturalis: Leiden. 195-588 pp.

De Oliveira J.D., De Paiva Igarashi M.L.S., Machado T.M.M., Miretti M.M., Ferro J.A. & Contel E.P.B. (2007) Structure and genetic relationships between Brazilian naturalized and exotic purebred goat domestic goat (*Capra hircus)* breeds based on microsatellites. *Genetics and Molecular Biology* **30**, 356-63.

De Ruijter W.P.M., Ridderinkhof H., Lutjeharms J.R.E., Schouten M.W. & Veth C. (2002) Observations of the flow in the Mozambique Channel. *Geophysical Research Letters* **29,** 141-143.

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

Dias N. & Caramelo A.M. (2005) Avaliação do estado dos stocks de gambas em Moçambique. In: *Instituto Nacional de Investigação Pesqueira, Maputo, 15 de Novembro de 2005*, pp. 25.

Dias N. & Caramelo A.M. (2007) Avaliação do estado do stock de *Haliporoides triarthrus* e o estado de exploração da pescaria de gamba. In: *Instituto Nacional de Investigação Pesqueira, Maputo, Janeiro 2007*, pp. 16.

Dias N., Sobrino I., Balguerias E., Palha de Sousa L., Burgos C. & Varela D. (2008) Relatório do cruzeiro de investigação de recursos de profundidade realizado a bordo do b/o Vizconde de Eza de 13 de Março a 9 de Abril 2008. In: *Instituto Nacional de Investigação Pesqueira, Maputo, 11 Agosto 2008*, pp. 116.

Dias N., Sobrino I., Garcia E., Silva L., Burgos C. & Muñoz I. (2009) Relatório do cruzeiro de investigação de recursos de profundidade realizado a bordo do b/o Vizconde de Eza de 13 de Março a 9 de Abril 2009. In: I*nstituto Nacional de Investigação Pesqueira, Maputo, 15 de Outubro 2009*, pp. 148.

Dias N., Zacarias L. & Caramelo A.M. (2011) Análise de pescaria de gamba e perspectivas de gestáo. In: *Instituto Nacional de Investigação Pesqueira*, pp. 15.

Duda J.T.F., Terbio M., Chen G., Phillips S., Olenzek A.M., Chang D. & Morris D.W. (2012) Patterns of population structure and historical demography of *Conus* species in the tropical Pacific. *American Malacological Bulletin* **30**, 175-87.

Dumont L.F.C., Hwang G. & Maclean N. (2009) The mtDNA Control Region of the Barba-ruça Shrimp *Artemesia longinaris* (Decapoda:Penaeidae) and its Potential use as a Marker for Population Analysis. *Atlantica* **31**, 199-207.

Evans B.S., Sweijd N.A., Bowie R.C.K., Cook P.A. & Elliott N.G. (2004) Population genetic structure of the perlemoen *Haliotis midae* in South Africa: evidence of range expansion and founder events. *Marine Ecology Progress Series* **270**, 163–72.

Excoffier L. & Lischer H.E. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564-7.

Excoffier L., Smouse P.E. & Quattro J.M. (1992) Analysis of Molecular Variance Inferred From Metric Distances Among DNA Haplotypes: Application to Human Mitochondrial DNA Restriction Data. *Genetics* **131**, 479–91.

Farfante I.P. (1977) American Solenocerid Shrimps of the Genera *Hymenopenaeus*, *Haliporoides*, *Pleoticus*, *Hadropenaeus* new Genus and *Mesopenaeus* new Genus. *Fisheries Bulletin* **75**, 261-346.

Fennessy S.T. & Groeneveld J.C. (1997) A review of the offshore trawl fishery for crustaceans on the east coast of South Africa. *Fisheries Management and Ecology* **4**, 135-47.

Fernández M.V., Heras S., Maltagliati F. & Roldán M.I. (2012) Deep genetic divergence in giant red shrimp *Aristaeomorpha foliacea* (Risso, 1827) across a wide distributional range. *Journal of Sea Research*.

Fernández M.V., Heras S., Maltagliati F., Turco A. & Roldán M.I. (2011a) Genetic structure in the blue and red shrimp *Aristeus antennatus* and the role played by hydrographical and oceanographical barriers. *Marine Ecology Progress Series* **421**, 163-71.

Fernández M.V., Heras S., Vinas J., Maltagliati F. & Roldán M.I. (2013) Multilocus Comparative Phylogeography of Two Aristeid Shrimps of High Commercial Interest (*Aristeus antennatus* and *Aristaeomorpha foliacea*) Reveals Different Responses to Past Environmental Changes. *Plos One* **8**, 1-10.

Fernández M.V., Maltagliati F., Pannacciulli F.G. & Roldán M.I. (2011b) Analysis of genetic variability in *Aristaeomorpha foliacea* (crustacea, aristeidae) using DNA-ISSR (Inter Simple Sequence Repeats) markers. *Comptes Rendus Biologies* **334**, 705-12.

Fratini S. & Vannini M. (2002) Genetic differentiation in the mud crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean. *Jornal of Experimental Marine Biology and Ecology* **272**, 103-16.

Frézal L. & Leblois R. (2008) Four years of DNA barcoding: Current advances and prospects. *Infection, Genetics and Evolution* **8**, 727-36.

Froukh T. & Kochzius M. (2007) Genetic population structure of the endemic fourline wrasse (*Larabicus quadrilineatus*) suggests limited larval dispersal distances in the Red Sea. *Molecular Ecology* **16,** 1359-67.

Fu Y.-X. (1997) Statistical Tests of Neutrality of Mutations Against Population Growth, Hitchhiking and Background Selection. *Genetics Society of America* **147**, 915-25.

Gille D.A. (2012) Genetic Population Structure and Cryptic Speciation of Ghost Shrimp (*Neotrypaea californiensis*) in North American West Coast Estuaries. MSc Thesis, San Jose State University, USA pp. 61.

Goetze E. (2011) Population differentiation in the open sea: insights from the pelagic copepod *Pleuromamma xiphias. Integrative and Comparative Biology* **51**, 580-97.

Gopal K. (2007) Genetic Population Structure of spiny lobster *Palinurus delagoae* in the south-western Indian Ocean and the Evolutionary History of *Palinurus*. MSc Thesis, University of Stellenbosch, South Africa, pp. 85.

Gopal K., Tolley K.A., Groeneveld J.C. & Matthee C.A. (2006) Mitochondrial DNA variation in spiny lobster *Palinurus delagoae* suggests genetically structured populations in the Southwestern Indian Ocean. *Marine Ecology Progress Series* **319**, 191-8.

Gopurenko D. (2002) Genetic structure within the distribution of the Indo-West Pacific mud crab *Scylla serrata* (Forskal, 1775). PhD thesis, Griffith University, Queensland, Australia, pp. 146.

Gopurenko D., Hughes J.M. & Keenan C.P. (1999) Mitochondrial DNA evidence for rapid colonisation of the Indo-West Pacific by the mudcrab *Scylla serrata*. *Marine Biology* **134**, 227–33.

Gordon A.L., Lutjeharms J.R.E. & Grundlingh M.L. (1987) Stratification and circulation at the Agulhas Retroflection. *Deep Sea Research* **34**, 565-99.

Grant W.S. & Bowen B.W. (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Heredity* **89**, 415-26.

Grant W.S., Spies I.B. & Canino M.F. (2006) Biogeographic evidence for selection on mitochondrial DNA in North Pacific Walleye Pollock *Theragra chalcogramma*. *Journal of Heredity* **97**, 571–80.

Gregory A.J., Kaler R.S.A., Prebyl T.J., Sandercock B.K. & Wisely S.M. (2012) Influence of translocation strategy and mating system on the genetic structure of a newly established population of island ptarmigan. *Conservation Genetics* **13**, 465-74.

Griffin D.J.G. & Stoddart H.E. (1995) Deep-water Decapod Crustacea from Eastern Australia: Lobsters of the Families Nephropidae, Palinuridae, Polychelidae and Scyllaridae. *Records of the Australian Museum* **47**, 231-63.

Griffiths C.L., Robinson T.B., Lange L. & Mead A. (2010) Marine Biodiversity in South Africa: An Evaluation of Current States of Knowledge. *Plos One* **5**, 13.

Groeneveld J.C. (2002) Long-distance migration of the rock lobster *Palinurus delagoae* off South Africa and Mozambique. *African Journal of Marine Science* **24**, 395-400.

Groeneveld J.C. (2012) Retrospective Analysis of existing data on deep-water trawl-fisheries for crustaceans in the South West Indian Ocean. Specialist Report prepared for the South West Indian Ocean Project. SWIOFP RMU, KMFRI, Mombasa, Kenya, pp. 65.

Groeneveld J.C. & Branch G.M. (2002) Long-distance migration of South African deep-water rock lobster *Palinurus gilchristi*. *Marine Ecology Progress Series* **232**, 225–38.

Groeneveld J.C., Gopal K., George R.W. & Matthee C.A. (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.

Groeneveld J.C. & Melville-Smith R. (1995) Spatial and Temporal Availability in the Multispecies Crustacean Trawl Fishery Along the East Coast of South Africa and Southern Moçambique, 1988-1993. *African Journal of Marine Science* **15**, 123-36.

Groeneveld J.C., von der Heyden S. & Matthee C.A. (2012) High connectivity and lack of mtDNA differentiation among two previously recognized spiny lobster species in the Southern Atlantic and Indian Oceans. *Marine Biology Research* **8**, 764-70.

Gupta A.K. & Thomas E. (1999) Latest Miocene-Pleistocene Productivity and deep-sea Ventilation in the Northwestern Indian Ocean (Deep Sea Drilling Project Site 219). *Paleoceanography* **14**, 62-73.

Hall T.A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* **41**, 95-8.

Harpending H.C. (1994) Signature of Ancient Population Growth in a Low-Resoluation Mitochondrial DNA Mismatch Distribution. *Human Biology* **66**, 591-600.

Harun H.B.C. (2013) Molecular Ecology of Two Commercially Important Crustacean Species, *Nephrops norvegicus* and *Macrobrachium rosenbergii*: Implications for the Management of Fisheries and Aquaculture. PhD Thesis, University of Glasgow, Scotland, pp. 160.

Hebert P.D., Cywinska A., Ball S.L. & de Waard J.R. (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society London Series B*, **270**: 313-321.

Hebert P.D., Penton E.H., Burns J.M., Janzen D.H. & Hallwachs W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Science*, **101**: 14812-14817.

Hellberg M.E. (2009) Gene Flow and Isolation among Populations of Marine Animals. *Annual Review Ecology and Evolution Systematic* **40**, 291–310.

Hickerson M.J., Carstens B.C., Cavender-Bares J., Crandall K.A., Graham C.H., Johnson J.B., Rissler L., Victoriano P.F. & Yoder A.D. (2010) Phylogeography's past, present, and future: 10 years after. *Molecular Phylogenetic and Evolution* **54**, 291-301.

Hickerson M.J. & Cunningham C.W. (2000) Dramatic Mitochondrial Gene Rearrangements in the Hermit Crab *Pagurus longicarpus* (Crustacea, Anomura). *Molecular Biology and Evolution* **17**, 639-44.

Holsinger K.E. & Weir B.S. (2009) Genetics in geographically structured populations defining, estimating and interpreting F(ST), pp. 30.

Holthuis L.B. (1980) FAO species catalogue. Vol. 1. Shrimps and prawns of the world. An annotated catalogue of species of interest to fisheries. FAO *Fisheries Synopsis*, 125, 261 pp. Rome, FAO.

Holthuis L.B. (1991) FAO species catalogue. Vol. 13. Marine lobsters of the world. An annotated and illustrated catalogue of species of interest to fisheries known to date. FAO *Fisheries Synopsis*, 125, 292 pp. Rome, FAO.

Hou P., Xie Z., Zhang L., Song Z., Mi J., He Y. & Li Y. (2011) Comparison of three different methods for total RNAextraction from *Fritillaria unibracteata*: A rare Chinese medicinal plant. *Journal of Medicinal Plants Research* **5**, 2834-8.

Huang J.P. & Lin C.P. (2011) Lineage-specific late pleistocene expansion of an endemic subtropical gossamer-wing damselfly, *Euphaea formosa*, in Taiwan. *Bio Med Central Evolutionary Biology* **11**, 1-14.

Hudson R.R., Slatkin M. & Maddison W.P. (1992) Estimation of levels of gene flow from DNA sequence data. *Genetics* **132**, 583–9.

Hui M. (2012) Connectivity and Evolution of Giant Clams (*Tridacnidae)*: A Molecular Genetic Approach. PhD thesis, University of Bremen, Germany, pp. 85.

Inoue N., Minami H. & Sekiguchi H. (2004) Distribution of Phyllosoma Larvae (Crustacea: Decapoda: Palinuridae, Scyllaridae and Synaxidae) in the Western North Pacific. *Journal of Oceanography* **60**, 963-76.

James-Pirri M.-J. & Cobb J.S. (2000) Influence of size and delayed settlement on the recapture rate of newly settled American lobsters *Homarus americanus*. *Marine Ecology Progress Series* **208**, 197–203.

Jenkins R.J.F. (1972) *Metanephrops*, a New Genus of Late Pliocene to Recent Lobsters (Decapoda, Nephropidae). *Crustaceana* **22**, 161-77.

Kensley B., Tranter H.A. & Griffin D.J.G. (1987) Deepwater decapod Crustacea from eastern Australia (Penaeidea and Caridea). *Records of the Australian Museum* **39**, 263-331.

Kimirei I.A. (2012) Importance of mangroves and seagrass beds as nurseries for coral reef fishes in Tanzania. PhD Thesis, Faculty of Science, Radboud University Nijmegen, The Netherlands. pp. 204.

Klaus S., Schubart C.D., Streit B. & Pfenninger M. (2010) When Indian crabs were not yet Asian--biogeographic evidence for Eocene proximity of India and Southeast Asia. *Bio Med Central Evolutionary Biology* **10**, 287.

Larkin M.A., Blackshields G., Brown N.P., Chenna R., McGettigan P.A., McWilliam H., Valentin F., Wallace I.M., Wilm A., Lopez R., Thompson J.D., Gibson T.J. & Higgins D.G. (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* **23**, 2947-8.

Lavery S., Chan T.Y., Tam Y.K. & Chu K.H. (2004) Phylogenetic relationships and evolutionary history of the shrimp genus *Penaeus* s.l. derived from mitochondrial DNA. *Molecular Phylogenetics and Evolution* **31,** 39-49.

Li Y.L., Kong X.Y., Yu Z.N., Kong J., Ma S. & Chen L.M. (2009) Genetic diversity and historical demography of Chinese shrimp *Feneropenaeus chinensis* in Yellow Sea and Bohai Sea based on mitochondrial DNA analysis. *African Journal of Biotechnology* **8**, 1193-202.

Librado P. & Rozas J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**, 1451-2.

Luschi P., Lutjeharms J.R.E., Lambardi P., Mencacci R., Hughes G.R. & Hays G.C. (2006) A review of migratory behaviour of sea turtles off Southeastern Africa. *South African Journal of Science* **102**, 51-8.

Lutjeharms J.R.E. (1985) *The physical oceanology of the Southern Ocean South of Africa: a bibliography*. Physical Oceanography Division, National Research Institute for Oceanology, Council for Scientific and Industrial Research, Stellenbosch, South Africa.

Lutjeharms J.R.E. (1988) On the role of the East Madagascar current as a source of the Agulhas current. *African Journal of Marine Science* **84**, 236–8.

Lutjeharms J.R.E. (2006) The Coastal Oceans of South-Eastern Africa. *In The Sea* **14B**, 783-834.

Lutjeharms J.R.E. (2007) Three decades of research on the greater Agulhas Current. *Ocean Science* **3**, 129-47.

Lutjeharms J.R.E. & da Silva A.J. (1988) The Delagoa Bight eddy. *Deep Sea Research* **35**, 619-34.

Luttikhuizen P.C., Campos J., Bleijswijk J.v., Peijnenburg K.T.C.A. & van der Veer H.W. (2008) Phylogeography of the common shrimp, Crangon crangon (L.) across its distribution range. *Molecular Phylogenetics and Evolution* **46,** 1015-1030.

Macpherson E. (1990) Crustacea Decapoda : On a collection of Nephropidae from the Indian Ocean and Western Pacific. *Museum Historia Nature* **6**, 289-328.

Madeira C., Alves M.J., Mesquita N., Silva S.E. & Paula J. (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*, 1-17.

Mardulyn P. (2012) Trees and ⁄ or networks to display intraspecific DNA sequence variation? *Molecular Ecology* **21**, 3385–3390.

Matthee C.A., Cockcroft A.C., Gopal K. & von der Heyden S. (2007) Mitochondrial DNA variation of the west-coast rock lobster, *Jasus lalandii:* Marked genetic diversity differences among sampling sites. *Marine and Freshwater Research* **58**, 1130-5.

McManus M.A. & Woodson C.B. (2012) Plankton distribution and ocean dispersal. *Journal of Experimental Biology* **215**, 1008-16.

Meyer C.P. (2003) Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biological Journal of the Linnean Society* **79**, 401-59.

Minxiao W., Song S., Chaolun L. & Xin S. (2011) Distinctive mitochondrial genome of Calanoid copepod *Calanus sinicus* with multiple large non-coding regions and reshuffled gene order: useful molecular markers for phylogenetic and population studies. *Bio Med Central Genomics* **12**, 73.

Moore E., Arnscheidt A., Kruger A., Strompl C. & Mau M. (2004) Simplified protocols for the preparation of genomic DNA from bacterial cultures. *Molecular Microbial Ecology*, 96.

Naro-Maciel E., Reid B., Holmes K.E., Brumbaugh D.R., Martin M. & DeSalle R. (2011) Mitochondrial DNA sequence variation in spiny lobsters: population expansion, panmixia, and divergence. *Marine Biology* **158**, 2027-41.

Neethling M., Matthee C.A., Bowie R.C. & von der Heyden S. (2008) Evidence for panmixia despite barriers to gene flow in the southern African endemic, Caffrogobius caffer (Teleostei: Gobiidae). *Bio Med Central Evolutionary Biology* **8**, 325.

Norton J.E. & Ashley M.V. (2004) Genetic Variability and Population Differentiation in Captive Baird's Tapirs (*Tapirus bairdii*). *Zoo Biology* **23**, 521–31.

Orsini L., Mergeay J., Vanoverbeke J. & De Meester L. (2012) The role of selection in driving landscape genomic structure of the waterflea *Daphnia magna. Molecular Ecology*, 1-19.

Page R.D.M. (1996) TreeView: An application to display phylogenetic trees on personal computers. *Computers Application Bioscience* **12**, 357–8.

Palero F., Crandall K.A., Abello P., Macpherson E. & Pascual M. (2009) Phylogenetic relationships between spiny, slipper and coral lobsters (Crustacea, Decapoda, Achelata). *Molecular Phylogenetics and Evolution* **50,** 152-62.

Paula J., Dray T. & Queiroga H. (2001) Interaction of offshore and inshore processes controlling settlement of brachyuran megalopae in Saco mangrove creek, Inhaca Island (South Mozambique). *Marine Ecology Progress Series* **215**, 251–60.

Peakall R. & Smouse P. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* **28**, 2537-9.

Penha-Lopes G., Torres P., Cannicci S., Narciso L. & Paula J. (2011) Monitoring anthropogenic sewage pollution on mangrove creeks in southern Mozambique: A test of *Palaemon concinnus* Dana, 1852 (Palaemonidae) as a biological indicator. *Environmental Pollution* **159**, 636-45.

Penha-Lopes G., Torres P., Narciso L., Cannicci S. & Paula J. (2009) Comparison of fecundity, embryo loss and fatty acid composition of mangrove crab species in sewage contaminated and pristine mangrove habitats in Mozambique. *Journal of Experimental Marine Biology and Ecology* **381**, 25-32.

Pérez-Farfante I. & Kensley B. (1997) Penaeoid and sergestoid shrimps and prawns of the world. Keys and diagnoses for the families and genera. *Mémoires du Muséum national d'Histoire naturelle* **175**.

Peters J.L., Gretes W. & Omland K.E. (2005) Late Pleistocene divergence between eastern and western populations of wood ducks (*Aix sponsa*) inferred by the 'isolation with migration' coalescent method. *Molecular Ecology* **14**, 3407-18.

Pineda J., Hare J.A. & Sponaugle S. (2007) Larval Transport and Dispersal in the Coastal Ocean and Consequences for Population Connectivity. *In: Marine Population Connectivity* **20** (3): 22-39.

Pollock D.E. & Melville-Smith R. (1993) Decapod life histories and reproductive dynamics in relation to oceanography off Southern Africa. *South African Journal of Marine Science* **13**, 205-12.

Popa O.P., Murariu D. & Popa L.O. (2007) Comparison of four DNA Extraction Methods from Invasive Freshwater Bivalve Species (Mollusca: Bivalvia) in Romanian Fauna. *Travaux du Muséum National d'Histoire Naturelle «Grigore Antipa»* **1**, 527-36.

Posada D. (2008) JModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* **25**, 1253-6.

Preu B., Spieß V., Schwenk T. & Schneider R. (2011) Evidence for current-controlled sedimentation along the southern Mozambique continental margin since Early Miocene times. *Geo-Marine Letters* **31**, 427-35.

Projecto-Garcia J., Cabral H. & Schubart C.D. (2010) High regional differentiation in a North American crab species throughout its native range and invaded European waters: a phylogeographic analysis. *Biology Invansions* **12**, 253-63.

Pulgarin R.P.C. & Burg T.M. (2012) Genetic Signals of Demographic Expansion in Downy Woodpecker (*Picoides pubescens*) after the Last North American Glacial Maximum. *Plos One* **7**, 1-14.

Quartly G.D. & Srokosz M.A. (2004) Eddies in the southern Mozambique Channel. *Deep Sea Research* **51**, 69-83.

Ragionieri L., Cannicci S., Schubart C.D. & Fratini S. (2010) Gene flow and demographic history of the mangrove crab *Neosarmatium meinerti*: A case study from the western Indian Ocean. *Estuarine, Coastal and Shelf Science* **86**, 179-88.

Ragionieri L., Fratini S., Vannini M. & Schubart C.D. (2009) Phylogenetic and morphometric differentiation reveal geographic radiation and pseudo-cryptic speciation in a mangrove crab from the Indo-West Pacific. *Molecular Phylogenetics and Evolution* **52**, 825-834.

Rai A.K. & Srinivasan M.S. (1994) Pleistocene Oceanographic Changes indicated by Deep Sea Benthic Foraminifera in the Northern Indian Ocean. *Proceeding Indian of Academy Science* **103**, 499-517.

Ridderinkhof H., Lutjeharms J.R.E. & Ruijter W.P.M. (2001) A Research Cruise to Investigate the Mozambique Current. *South African Journal of Science* **97**, 461-4.

Rivera M.A.J., Andrews K.R., Kobayashi D.R., Wren J.L.K., Kelley C., Roderick G.K. & Toonen R.J. (2010) Genetic Analyses and Simulations of Larval Dispersal Reveal Distinct Populations and Directional Connectivity across the Range of the Hawaiian Grouper (*Epinephelus quernus*). *Journal of Marine Biology* **2011**, 1-11.

Robey J. (2013) An assessment of abundance trends and biology of langoustines (*Metanephrops mozambicus*) and pink prawns (*Haliporoides triarthrus*) from the deep-water trawl fishery off eastern South Africa. MSc Thesis, University of KwaZulu-Natal, South Africa.

Robey J., Groeneveld J.C. & Fennessy S.T. (2011) Trawling for prawns and langoustines in the deep: trends in abundance, biology and catches in the South West Indian Ocean. 7th Western Indian Ocean Marine Science Association Scientific Symposium. Coping with Global Change. 24-29 October, 2011. Mombasa, Kenya. Oral presentation, Abstract, pp. 167.

Rogers A.R. (1995) Genetic Evidence for a Pleistocene Population Explosion. *Evolution* **49**, 608-15.

Rogers A.R. & Harpending H. (1992) Population Growth Makes Waves in the Distribution of Pairwise Genetic Differences *Molecular Biology and Evolution* **9**, 552-69.

Roldán M., Heras S., Patellani R. & Maltagliati F. (2009) Analysis of genetic structure of the red shrimp *Aristeus antennatus* from the Western Mediterranean employing two mitochondrial regions. *Genetica* **136**, 1-4.

Ronquist F. & Huelsenbeck J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-4.

Sacco F. (2011) Population structure of aristeid shrimps (Decapoda, *Aristeidae*) in the Western Mediterranean Sea inferred by microsatellite loci. PhD Thesis, Università di Bologna. In: *Biodiversità ed Evoluzione*.

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

Sardà F., Roldán M.I., Heras S. & Maltagliati F. (2010) Influence of the genetic structure of the red and blue shrimp, *Aristeus antennatus* (Risso, 1816), on the sustainability of a deep-sea population along a depth gradient in the western Mediterranean. *Scientia Marina* **74**, 569-75.

Saul S. (2004) A Review of the Literature and Life History Study of the Caribbean Spiny Lobster, *Panulirus Argus,* pp. 14.

Schenekar T. & Weiss S. (2011) High rate of calculation errors in mismatch distribution analysis results in numerous false inferences of biological importance. *Heredity (Edinb)* **107**, 511-2.

Schleyer M.H., Tomalin B.J., Robertson W.D., Bailey S. & Nellmapius S.J. (1997) KwaZulu-Natal deepwater crustacean trawling. SANCOR/FRD Final Report of Research Project: 23-28.

Schubart C.D., Diesel R. & Hedges B. (1998) Rapid evolution to terrestrial life in Jamaican crabs. *Nature* **393**, 363–5.

Sekiguchi H. & Inoue N. (2002) Recent Advances in Larval Recruitment Processes of Scyllarid and Palinurid Lobsters in Japanese Waters. *Journal of Oceanography* **58**, 747-57.

Sete C., Ruby J. & Dove V. (2002) Seasonal Variation of tides, currents, salinity and temperature along the Coast of Mozambique, pp. 73.

Shen K.N., Jamandre B.W., Hsu C.C., Tzeng W.N. & Durand J.D. (2011) Plio-Pleistocene sea level and temperature fluctuations in the northwestern Pacific promoted speciation in the globally-distributed flathead mullet Mugil cephalus. *Bio Med Central Evolutionary Biology* **11**, 83.

Shih C.-H., Haung H.-L., Chu T.-J., Lee Y.-C., Wang C.-M. & Tzeng T.-D. (2011) Genetic diversity and historical demography of kuruma shrimp ( *Penaeus japonicus* ) species complex off China based on mitochondrial DNA analysis. *African Journal of Biotechnology* **10**, 1065-72.

Shih H., Kamrani E., Davie P.J.F. & Liu M. (2009) Genetic evidence for the recognition of two fiddler crabs, *Uca iranica* and *U. albimana* (Crustacea: Brachyura: Ocypodidae), from the northwestern Indian Ocean, with notes on the *U. lactea* species-complex. *Hydrobiologia* **635**, 373-82.

Silva I.C., Mesquita N. & Paula J. (2010a) 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.

Silva I.C., Mesquita N. & Paula J. (2010b) Lack of population structure in the fiddler crab *Uca annulipes* along an East African latitudinal gradient: genetic and morphometric evidence. *Marine Biology* **157**, 1113-26.

Smith I.P. & Jensen A.C. (2008) Dynamics of closed areas in Norway lobster fisheries. *Journal of Marine Science* **65**, 1600-9.

Sobrino I., Dias N., Balguerias E., Ramil F., Santana A.P., Burgos C., Palha de Sousa B. & Palha de Sousa L. (2007) Relatório do cruzeiro de investigação de recursos de profundidade realizado a bordo do b/o Vizconde de Eza. In: *Instituto Nacional de Investigação Pesqueira,* pp. 62.

Sobrino I., Dias N., Munoz I., Salmeron F. & Varela D. (2009) Distribution Pattern and Biological Characteristics of *Aristeus antennatus* (Risso, 1816) and *Aristeus virilis* (Bate, 1881) in Mozambique Waters of the Western Indian Ocean. *Journal of Marine Science* **8**, 49-59.

Sobrino I. & García T. (2007) Reproductive aspects of the rose shrimp *Parapenaeus longirostris* (Lucas, 1846) in the Gulf of Cadiz (southwestern Iberian Peninsula). *Boletín Instituto Español de Oceanografía* **23**, 57-71.

Sotelo G., Posada D. & Morán P. (2009) Low-mitochondrial diversity and lack of structure in the velvet swimming crab *Necora puber* along the Galician coast. *Marine Biology* **156**, 1039-48.

Sponaugle S., Cowen R.K., Shanks A., Morgan S.G., Leis J.M., Pineda J., Boehlert G.W., Kingsford M.J., Lindeman K.C., Grimes C. & Munro J.L. (2002) Predicting self-recruitment in marine populations: Biophysical correlates and mechanisms. *Bulletin of Marine Science* **70**, 341-75.

Stentiford G.D. & Neil D.M. (2011) Diseases of *Nephrops* and *Metanephrops*: A review. *Journal of Invertebrate Pathology* **106**, 92-109.

Swart N.C., Lutjeharms J.R.E., Ridderinkhof H. & de Ruijter W.P.M. (2010) Observed characteristics of Mozambique Channel eddies. *Jornal of Geophysical Research* **115**, 14.

Swofford D.L. (2002) PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, pp. 142.

Tajima F. (1989) Statistical Method for Testing the Neutral Mutation Hypothesis by DNA Polymorphism. *Genetics Society of America* **123**, 585-95.

Tavaré S. (1986) Some probabilistic and statistical problems in the analysis of DNA sequences. Pp. 57-86 in R. M. Miura, ed. Some mathematical questions in biology - DNA sequence analysis. *American Methodical Society*, Providence, RI.

Teske P.R., McLay C.L., Sandoval-Castillo J., Papadopoulos I., Newman B.K., Griffiths C.L., McQuaid C.D., Barker N.P., Borgonie G. & Beheregaray L.B. (2009a) Tri-locus sequence data reject a "Gondwanan origin hypothesis" for the African/South Pacific crab genus Hymenosoma. *Molecular Phylogenetic and Evolution* **53**, 23-33.

Teske P.R., Papadopoulos I., Newman B.K., Dworschak P.C., McQuaid C.D. & Barker N.P. (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. *Bio Med Central Evolutionary Biology* **8**, 341.

Teske P.R., Papadopoulos I., Zardi G.I., McQuaid D., GriYths C.L., Edkins M.T. & Barker N.P. (2007) Implications of life history for genetic structure and migration rates of five southern African coastal invertebrates: planktonic, abbreviated and direct development. *Marine Biology* **152**, 697–711.

Teske P.R., Winker H., McQuaid C.D. & Barker N.P. (2009b) A tropical/subtropical biogeographic disjunction in southeastern Africa separates two Evolutionarily Signficant Units of an estuarine prawn. *Marine Biology* **156**, 1265-75.

Tilburg C.E., McCartney M.A. & Yund P.O. (2012) Across-Shelf Transport of Bivalve Larvae: Can the Interface between a Coastal Current and Inshore Waters Act as an Ecological Barrier to Larval Dispersal? *Plos One* **7**, 16.

Tolley K.A., Groeneveld J.C., Gopal K. & Matthee C.A. (2005) Mitochondrial DNA panmixia in spiny lobster *Palinurus gilchristi* suggests a population expansion. *Marine Ecology Progress Series* **297**, 225-31.

Torres P., Penha-Lopes G., Macia A. & Paula J. (2007) Population structure and egg production of the seagrass shrimp *Hippolyte kraussiana* Stimpson, 1860 (Decapoda: Hippolytidae) at Inhaca Island, Mozambique. *Invertebrate Reproduction and Development* **50**, 145-53.

Torstensen E. & Pacule H. (1992) Stock assessment of *Haliporoides triarthrus* (Fam. Solenoceridae) off Mozambique: A preliminary analysis. In: *Revista de Investigação Pesqueira*, pp. 14-28.

Torstensen E.P. (1989) Avaliação preliminar dos recursos de camarão de profundidade em Moçambique. In: *Revista de Investigação Pesqueira*, pp. 153-83.

Tsang L.M., Achituv Y., Chu K.H. & Chan B.K.K. (2012) Zoogeography of Intertidal Communities in the West Indian Ocean as Determined by Ocean Circulation Systems: Patterns from the *Tetraclita* Barnacles. *Plos One* **7**, 1-11.

Tsao W.C. & Yeh W.B. (2008) DNA-Based discrimination of subspecies of swallowtail butterflies (Lepidotera: Papilioninae) from Taiwan. *Zoological Studies*, **47**: 633-634.

Tshudy D., Chan T.-Y. & Sorhannus U. (2007) Morphology Based Cladistic Analysis of *Metanephrops*: The Most Diverse Extant Genus of Clawed Lobster (*Nephropidae*). *Journal of Crustacean Biology* **27**, 463–76.

Tsoi K.H., Chan T.-Y. & Chu K.H. (2011) Phylogenetic and biogeographic analysis of the spear lobsters *Linuparus* (Decapoda: Palinuridae), with the description of a new species. *Zoologischer Anzeiger - A Journal of Comparative Zoology* **250**, 302-15.

Van de Putte A.P., Janko K., Kasparova E., Maes G.E., Rock J., Koubbi P., Volckaert F.A.M., Choleva L., Fraser K.P.P., Smykla J., Van Houdt J.K.J. & Marshall C. (2012) Comparative phylogeography of three trematomid fishes reveals contrasting genetic structure patterns in benthic and pelagic species. *Marine Genomics* **8**, 23-34.

Van der Plas G.W., De Boer E.J., Hooghiemstra H., Florens F.B.V., Baider C. & Van der Plicht J. (2012) Mauritius since the last glacial: environmental and climatic reconstruction of the last

38 000 years from Kanaka Crater. *Journal of Quaternary Science* **27,** 159–168. *Biological Journal of the Linnean Society*.

Vogler C., Benzie J., Barber P.H., Erdmann M.V., Ambariyanto, Sheppard C., Tenggardjaja K., Gerard K. & Worheide G. (2012) Phylogeography of the crown-of-thorns starfish in the Indian Ocean. *Plos One* **7**, e43499.

von der Heyden S., Groeneveld J.C. & Matthee C.A. (2007) Long current to nowhere? — Genetic connectivity of *Jasus tristani* populations in the southern Atlantic Ocean. *African Journal of Marine Science* **29**, 491-7.

von der Heyden S., Prochazka K.I.M. & Bowie R.C.K. (2008) Significant population structure and asymmetric gene flow patterns amidst expanding populations of *Clinus cottoides* (Perciformes, Clinidae): application of molecular data to marine conservation planning in South Africa. *Molecular Ecology* **17,** 4812-4826.

Wang T.Y., Wang L., Zhang J.H. & Dong W.H. (2011) A simplified universal genomic DNA extraction protocol suitable for PCR. *Genetics and Molecular Research* **10**, 519-25.

Wang X., Teng D., Tian F., Guan Q. & Wang J. (2012) Comparison of three DNA extraction methods for feed products and four amplification methods for the 5'-junction fragment of Roundup Ready soybean. *Journal of Agricultural and Food Chemistry* **60**, 4586-95.

Waples R.S., Punt A.E. & Cope J.M. (2008) Integrating genetic data into management of marine resources: how can we do it better? *Fish and Fisheries* **9**, 423-49.

Waqairatu S.S., Dierens L., Cowley J.A., Dixon T.J., Johnson K.N., Barnes A.C. & Li Y. (2012) Genetic analysis of Black Tiger shrimp (*Penaeus monodon*) across its natural distribution range reveals more recent colonization of Fiji and other South Pacific islands. *Ecology and Evolution* 2, 2057-71.

Wolstenholme D.R. (1992) Animal mitochondrial DNA: structure and evolution. *International Review of Cytology* **141**, 173-216.

Wright S. (1978) *Evolution and the genetics of populations. A treatise in four volumes. Volume 4. Variability within and among natural populations*. University of Chicago Press., Chicago, London.

Xiao Y.S., Zhang Y., Gao T.X., Yanagimoto T., Yabe M. & Sakurai Y. (2009) Genetic diversity in the mitochondrial DNA control region and population structure in the small yellow croaker *Larimichthys polyactis*. *Environment Biology and Fisheries* **85**, 303–14.

Xu D., Lou B., Shi H., Geng Z., Li S. & Zhang Y. (2012) Genetic diversity and population structure of *Nibea albiflora* in the China Sea revealed by mitochondrial COI sequences. *Biochemical Systematics and Ecology* **45**, 158-65.

Xu J., Perez-Losada M., Jara C.G. & Crandall K.A. (2009a) Pleistocene glaciation leaves deep signature on the freshwater crab *Aegla alacalufi* in Chilean Patagonia. *Molecular Ecology* **18**, 904-18.

Xu S., Hebert P.D., Kotov A.A. & Cristescu M.E. (2009b) The noncosmopolitanism paradigm of freshwater zooplankton: insights from the global phylogeography of the predatory cladoceran *Polyphemus pediculus* (Linnaeus, 1761) (Crustacea, Onychopoda). *Molecular Ecology* **18**, 5161-79.

Zardi G.I., McQuaid C.D., Teske P.R. & Barker N.P. (2007) Unexpected genetic structure of mussel populations in South Africa: indigenous *Perna perna* and invasive *Mytilus galloprovincialis*. *Marine Ecology Progress Series* **337**, 135-44.

Zwickl D.J. (2006) Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets Under the Maximum Likelihood Criterion. PhD Thesis, School of The University of Texas at Austin, Austin, USA, pp. 125. In: *Biological Journal of the Linnean Society*.

## 7. Appendix

Appendix I. *Haliporoides triarthrus.* Haplotype frequencies detected in Bazaruto A, Boa Paz, Inhaca, Durban, Morombe and Tulear sampling sites using 16S gene.

| Haplotype: | BA | BP | DB | IA | MM | TR | Total |
|---|---|---|---|---|---|---|---|
| Hap_1 | 5 | 0 | 0 | 0 | 0 | 0 | 5 |
| Hap_2 | 17 | 0 | 18 | 0 | 0 | 0 | 35 |
| Hap_3 | 3 | 0 | 0 | 0 | 0 | 0 | 3 |
| Hap_4 | 8 | 0 | 7 | 0 | 0 | 0 | 15 |
| Hap_5 | 4 | 0 | 0 | 0 | 0 | 0 | 4 |
| Hap_6 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_7 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_8 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_9 | 0 | 17 | 0 | 0 | 0 | 0 | 17 |
| Hap_10 | 0 | 16 | 0 | 0 | 0 | 0 | 16 |
| Hap_11 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| Hap_12 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_13 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| Hap_14 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_15 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_16 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_17 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_18 | 0 | 0 | 4 | 0 | 0 | 0 | 4 |
| Hap_19 | 0 | 0 | 5 | 0 | 0 | 0 | 5 |
| Hap_20 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_21 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| Hap_22 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_23 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_24 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hap_25 | 0 | 0 | 0 | 6 | 0 | 0 | 6 |
| Hap_26 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_27 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_28 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_29 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_30 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_31 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_32 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_33 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_34 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_35 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_36 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_37 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_38 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_39 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_40 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_41 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_42 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_43 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_44 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_45 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_46 | 0 | 0 | 0 | 0 | 2 | 2 | 4 |
| Hap_47 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_48 | 0 | 0 | 0 | 0 | 8 | 0 | 8 |
| Hap_49 | 0 | 0 | 0 | 0 | 9 | 0 | 9 |
| Hap_50 | 0 | 0 | 0 | 0 | 3 | 0 | 3 |
| Hap_51 | 0 | 0 | 0 | 0 | 1 | 11 | 12 |
| Hap_52 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_53 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_54 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hap_55 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_56 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_57 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| Hap_58 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_59 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_60 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_61 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_62 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_63 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_64 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_65 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_66 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_67 | 0 | 0 | 0 | 0 | 0 | 3 | 1 |
| Hap_68 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_69 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| **Total** | **40** | **40** | **40** | **27** | **30** | **30** | **207** |

Appendix II. *Haliporoides triarthrus.* Haplotype frequencies detected in Bazaruto A, Boa Paz, Inhaca, Durban, Morombe and Tulear sampling sites using COI gene.

| Haplotype: | BA | BP | DB | IA | MM | TR | Total |
|---|---|---|---|---|---|---|---|
| Hap_1 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| Hap_2 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_3 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_4 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_5 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_6 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_7 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_8 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_9 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_10 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_11 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_12 | 8 | 0 | 0 | 0 | 0 | 0 | 8 |
| Hap_13 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_14 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_15 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_16 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_17 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_18 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_19 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_20 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| Hap_21 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_22 | 0 | 14 | 0 | 0 | 0 | 0 | 14 |
| Hap_23 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_24 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_25 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_26 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_27 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hap_28 | 0 | 0 | 8 | 0 | 0 | 0 | 8 |
| Hap_29 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_30 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_31 | 0 | 0 | 5 | 0 | 0 | 0 | 5 |
| Hap_32 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_33 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_34 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_35 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_36 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_37 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_38 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_39 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_40 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_41 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_42 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_43 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_44 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_45 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_46 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_47 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_48 | 0 | 0 | 0 | 4 | 0 | 0 | 4 |
| Hap_49 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_50 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_51 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_52 | 0 | 0 | 0 | 2 | 0 | 0 | 2 |
| Hap_53 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_54 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_55 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_56 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_57 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hap_58 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_59 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_60 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_61 | 0 | 0 | 0 | 2 | 0 | 0 | 2 |
| Hap_62 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_63 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_64 | 0 | 0 | 0 | 0 | 16 | 0 | 16 |
| Hap_65 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_66 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_67 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_68 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_69 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_70 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_71 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_72 | 0 | 0 | 0 | 0 | 0 | 21 | 21 |
| Hap_73 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_74 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_75 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_76 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_77 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_78 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| **Total** | **27** | **22** | **27** | **25** | **23** | **27** | **151** |

Appendix III. *Metanephrops mozambicus.* Haplotype frequencies detected in Bazaruto A, Boa Paz, Inhaca, Durban, Morombe and Tulear sampling sites using 16S gene.

| Haplotype: | BA | BP | DB | IA | MM | TR | Total |
|---|---|---|---|---|---|---|---|
| Hap_1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_2 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_3 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_4 | 2 | 2 | 0 | 4 | 0 | 0 | 8 |
| Hap_5 | 2 | 2 | 0 | 3 | 0 | 0 | 7 |
| Hap_6 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_7 | 7 | 0 | 0 | 0 | 0 | 0 | 7 |
| Hap_8 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_9 | 7 | 0 | 0 | 0 | 0 | 0 | 7 |
| Hap_10 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_11 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_12 | 1 | 0 | 0 | 5 | 0 | 0 | 6 |
| Hap_13 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_14 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_15 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_16 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_17 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_18 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_19 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_20 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_21 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_22 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_23 | 3 | 1 | 0 | 1 | 0 | 0 | 5 |
| Hap_24 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_25 | 0 | 1 | 0 | 1 | 0 | 0 | 2 |
| Hap_26 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_27 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hap_28 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| Hap_29 | 0 | 1 | 0 | 13 | 0 | 0 | 14 |
| Hap_30 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_31 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_32 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| Hap_33 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_34 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_35 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_36 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_37 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| Hap_38 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_39 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_40 | 0 | 3 | 0 | 0 | 0 | 0 | 3 |
| Hap_41 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_42 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_43 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| Hap_44 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_45 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_46 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_47 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_48 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_49 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_50 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_51 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_52 | 0 | 0 | 6 | 0 | 0 | 2 | 8 |
| Hap_53 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_54 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_55 | 0 | 0 | 3 | 0 | 0 | 1 | 4 |
| Hap_56 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_57 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hap_58 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_59 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_60 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_61 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_62 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| Hap_63 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_64 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_65 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| Hap_66 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_67 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| Hap_68 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_69 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_70 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_71 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_72 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_73 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_74 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_75 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_76 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_77 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_78 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_79 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_80 | 0 | 0 | 0 | 3 | 0 | 2 | 5 |
| Hap_81 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_82 | 0 | 0 | 0 | 1 | 3 | 1 | 5 |
| Hap_83 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_84 | 0 | 0 | 0 | 2 | 0 | 0 | 2 |
| Hap_85 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_86 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_87 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hap_88 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_89 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_90 | 0 | 0 | 0 | 0 | 5 | 0 | 5 |
| Hap_91 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_92 | 0 | 0 | 0 | 0 | 7 | 7 | 14 |
| Hap_93 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_94 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_95 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_96 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_97 | 0 | 0 | 0 | 0 | 2 | 8 | 10 |
| Hap_98 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_99 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_100 | 0 | 0 | 0 | 0 | 2 | 0 | 2 |
| Hap_101 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_102 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_103 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_104 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_105 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_106 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| Hap_107 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_108 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_109 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_110 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_111 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_112 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| **Total** | **40** | **40** | **38** | **40** | **30** | **30** | **198** |

Appendix IV. *Metanephrops mozambicus.* Haplotype frequencies detected in Bazaruto A, Boa Paz, Inhaca, Durban, Morombe and Tulear sampling sites using COI gene.

| Haplotype: | BA | BP | DB | IA | MM | TR | Total |
|---|---|---|---|---|---|---|---|
| Hap_1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_2 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_3 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_4 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| Hap_5 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_6 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_7 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_8 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_9 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_10 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_11 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_12 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_13 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_14 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_15 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_16 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_17 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| Hap_18 | 0 | 9 | 0 | 0 | 0 | 0 | 9 |
| Hap_19 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_20 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_21 | 0 | 2 | 0 | 0 | 0 | 0 | 2 |
| Hap_22 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_23 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_24 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_25 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_26 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_27 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hap_28 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_29 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_30 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_31 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_32 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_33 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_34 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_35 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_36 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_37 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_38 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_39 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_40 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_41 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_42 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_43 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_44 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_45 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| Hap_46 | 0 | 0 | 3 | 0 | 0 | 0 | 3 |
| Hap_47 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_48 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_49 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_50 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_51 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_52 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_53 | 0 | 0 | 3 | 0 | 0 | 0 | 3 |
| Hap_54 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_55 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_56 | 0 | 0 | 2 | 0 | 0 | 0 | 2 |
| Hap_57 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hap_58 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_59 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_60 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_61 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Hap_62 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_63 | 0 | 0 | 0 | 8 | 0 | 0 | 8 |
| Hap_64 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_65 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_66 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_67 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_68 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_69 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_70 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_71 | 0 | 0 | 0 | 4 | 0 | 0 | 4 |
| Hap_72 | 0 | 0 | 0 | 3 | 0 | 0 | 3 |
| Hap_73 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_74 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_75 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_76 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_77 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_78 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_79 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_80 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_81 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_82 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_83 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_84 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| Hap_85 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_86 | 0 | 0 | 0 | 0 | 2 | 0 | 2 |
| Hap_87 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hap_88 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_89 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_90 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_91 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_92 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_93 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_94 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_95 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_96 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_97 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_98 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_99 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_100 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_101 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_102 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_103 | 0 | 0 | 0 | 0 | 1 | 0 | 1 |
| Hap_104 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| Hap_105 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_106 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_107 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_108 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_109 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| Hap_110 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_111 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_112 | 0 | 0 | 0 | 0 | 0 | 4 | 4 |
| Hap_113 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_114 | 0 | 0 | 0 | 0 | 0 | 2 | 2 |
| Hap_115 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_116 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_117 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Hap_118 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_119 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_120 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_121 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_122 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_123 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_124 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_125 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_126 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| Hap_127 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| **Total** | **18** | **37** | **21** | **35** | **20** | **30** | **161** |