A Phylogeny of South African east coast intertidal rocky-shore Polychaete worms (Annelida) and the genetic structure and demographic history of an example, *Marphysa corallina*

by

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

Signed: _____________ Name: Dr Angus HH Macdonald Date: ________

Abstract

The Annelida is an evolutionarily ancient invertebrate taxon. Recent studies have found that the formerly described sister taxon of the Polychaeta, Clitellata, is a derived Polychaete group thus making Polychaeta a paraphyletic group. Polychaete worms represent one of the most diverse invertebrate groups and are well represented in a variety of environments such as temporary freshwater puddles, rocky intertidal shores, estuaries and the abyssal plain. Polychaetes are fundamentally important in their environments as many are regarded as ecosystem engineers. Phylogenetic relationships within the Polychaeta are poorly understood and some species level classifications are uncertain due to the large number of polychaete worms present. In Chapter two, the phylogenetic relationships within the commonly found polychaete families (Nereididae and Eunicidae) were analysed using the universal mitochondrial cytochrome oxidase subunit 1 (COI). Within Eunicidae, analyses supported a polyphyletic *Marphysa* and *Eunice* which is consistent with previous results as individuals from both genera are nested among one another. Within Nereididae, relationships between genera and species were poorly supported and complex. Genera did not form exclusive clades but instead grouped with one another. A large degree of homoplasy has been recorded for the family which could have attributed to the convoluted groupings. Thus it has been suggested that genera from both Eunicidae and Nereididae be revised. *Marphysa corallina* is a poorly studied Eunicid polychaete which has a tropical indo-west distribution. It was observed to be a common worm among others on the intertidal rocky shores of KwaZulu-Natal and the Eastern Cape. In Chapter three, the population genetic structure and demographic history of *M. corallina* was investigated using two genes: universal mitochondrial cytochrome oxidase subunit 1 (COI) and the nuclear intron spacer region (ITS1). Diagnostic taxonomic characters were used to identify and validate the specimens as *Marphysa corallina.* The COI marker revealed that populations were highly connected to one another and formed a large panmictic population whereas ITS1 showed shallow genetic structuring of populations. Family Eunicidae individuals are known to lack a long lived planktonic larval stage which could

not have contributed to panmixia as demostrated by the COI marker. Demographic results indicated that populations had recently undergone sudden expansions which could have falsely resembled highly connected populations. Estimation of divergence times places the expansions in the mid to late Pleistocene. Populations had not reached migration-drift equilibrium thus contemporary population distributions of *Marphysa corallina* along the east coast of South Africa are largely shaped by past climatic events such as in the Pleistocene.

Key words: Genetic structure, *Marphysa corallina*, demographic history, population expansions, pleistocene, phylogenetics, Eunicidae, Nereididae.

Preface

The work described in this dissertation was carried out in the School of Life Sciences, University of Natal, Durban, and field work carried out along various sites in the Eastern Cape and KwaZulu-Natal coast, from January 2014 to December 2015, under the supervision of Dr Angus HH 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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Declaration 2 - Publications

DETAILS OF CONTRIBUTION TO PUBLICATIONS that form part and/or include research presented in this thesis:

Publication 1 (in preparation):

Kara J and Macdonald AHH. Phylogeny of South African east coast Eunicidae and Nereididae polychaete worms.

Publication 2 (in preparation):

Kara J and Macdonald AHH. Genetic structure and demographic history of *Marphysa corallina* (Annelida: Eunicidae) from the east coast of South Africa.

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Declaration 3 - Other research outputs

Poster presentations:

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Kara J and Macdonald AHH. July 2014. Assessing the population genetic structure of *Marphysa corallina* (Polychaeta: Eunicidae) along the East Coast of South Africa. South African Marine Science Symposium, Stellenbosch, Cape Town.

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Chapter one: General background

1.1. Introduction

One of the most diverse, successful and evolutionarily ancient taxon among the invertebrates is the Annelida (Fauchald 1974; 1977; Bleidorn et al. 2003b; Glasby 2005; Halanych 2006). Previously, the Annelida was considered to be a monophyletic taxon consisting of three major Classes: Polychaeta (bristle worms), Hirudinea (true leeches) and the Oligochaeta (earthworms) (Rouse & Fauchald 1998; Bleidorn et al. 2003a; Bartolomaeus et al. 2005; Colgan et al. 2006; Rousset et al. 2007). Recently however, molecular analyses have shown that the hirudineans appear to be nested within the oligochaetes; and because they were assigned a Class rank, Oligochaeta is now a paraphyletic clade (McHugh 2000; Rousset et al. 2007). The hirudeneans and oligochaetes share a common ancestor for which several autapomorphies have been described. For this reason, they have been assigned to the class Clitellata (Bartolomaeus et al. 2005). The monophyly of Clitellata has been well supported by both morphological and molecular analyses (Bartolomaeus et al. 2005).

The Echiura, or spoon worms, consists of a small group of approximately 150 unsegmented, coelomate marine worms found in a number of different habitats from intertidal mudflats to depths of 10 000 m (McHugh 1997; 2000; Hessling & Westheide 2002). The Sipuncula, or peanut worms, consists of a small clade of approximately 147 species distributed among 17 genera. The majority of the unsegmented peanut worms are larger than 5 mm and found worldwide in marine sediments from the intertidal zone to the deep sea (Dordel et al. 2010). Previously, the Echiura and Sipuncula formed a clade within Annelida (McHugh 1997). However, due to the absence of segmentation which was considered to be a primary loss they were removed from the Phylum Annelida (one of the apomorphies being segmentation) and placed as separate phyla (McHugh 1997; 2000; Rousset et al. 2006). Recently, many studies focusing on the

development of the nervous system in larvae and adults, cleavage patterns, chaetal formation, and sperm ultrastructure of echiuran worms have shown that they all closely resemble polychaetous annelids (McHugh 1997; Hessling & Westheide 2002). For this reason authors concluded that echiurans are derived from a segmented ancestor (McHugh 1997; Hessling & Westheide 2002; Bartolomaues et al. 2005; Colgan et al. 2006). The nervous system was shown to have metameric organization and the trunk a modified segmented structure and not a single large segment as previously considered (Hessling & Westheide 2002). Studies relating to the morphology of the neural and muscle formation of sipunuclans have shown close resemblance to those of annelids therefore adding to the body of information that suggests them to be a derived clade of polychaetous annelids (Halanych et al. 2006).

The majority of the diversity of the annelids lies within the Class Polychaeta (McHugh 2000; Bartolomaeus et al. 2005). Polychaete worms can be identified by their multi-segmented bodies, the presence of parapodia, chaetae (bristles) and elaborate head appendages (Fauchald 1977). Polychaete worms are well represented and form an integral part of the benthos of aquatic habitats such as estuaries, rocky shores, continental shelves, deep sea hydrothermal vents as well as the water column (Day 1967; McHugh 2000; Hall et al. 2004; Brett 2006; Zhou et al. 2010). The ability of polychaetes to easily adapt to a whole range of habitats and variable environments are displayed by their numerous feeding modes (McHugh 2000; Eklof 2010). Polychaetes include free-living predators, filter feeders, parasites and scavengers (McHugh 2000; Olsgard et al. 2003; Brett 2006; Eklof 2010).

Polychaete worms are essential to the marine environment for many reasons. They have a high reproductive output allowing them to reach high densities contributing to over half of the total biomass of the benthos (Dean 2004; Brett 2006). Polychaetes contribute to the functioning of benthic communities in terms of nutrient cycling through digestion (Olsgard et al. 2003; Brett 2006). Anoxic sediments are aerated by burrowing and tube building polychaetes through the regulation of water flow in the

sediment (Brett 2006). Polychaete worms also serve as an important food source for larger predators such as commercially important demersal fish and constitute the main diet preference of cuttlefish (Brett 2006; Rivera & Romero 2008). The polychaete group contains sensitive and tolerant species and is distributed throughout pristine and heavily disturbed areas (Dean 2004). This broad distribution together with their rapid generation times allow polychaete worms to respond quickly to environmental changes making them good indicator species of anthropogenic disturbances such as pollution (Dean 2004).

1.2. Taxonomy of Polychaeta

Traditionally polychaetes were divided into two equally large groups which were treated by many authors as orders: “Errantia” and “Sedentaria” (Fauchald 1974; 1977; Fauchald & Rouse 1997; Rouse & Fauchald 1998; Bartolomaues et al. 2005; Eklof 2010). These groupings were assigned by de Quatrefages (1866) and are based on the life habits of the worm as well as the development of the anterior end (head region) (Fauchald 1977; Fauchald & Rouse 1997). Errant polychaetes comprised of those rapacious, free- living worms with an equal number of body segments and a few anterior appendages generally differing in the number of palps, antennae, tentacular cirri and occipital antennae (Fauchald 1977; Bartolomaeus et al. 2005). The sedentary polychaetes on the other hand consisted of those filter feeding, sessile, and mostly tubicolous worms that have a limited number of body segments, a separation of the body into distinct regions, and that lack or have a few anterior head appendages (Fauchald 1977; Bartolomaeus et al. 2005). This grouping was considered to be very practical, convenient and arbitrary and did not reflect any type of taxonomic distinction between orders and families nor did it reflect evolutionary history (Fauchald 1974; Rouse & Fauchald 1998; Bartolomaeus et al. 2005; Eklof 2010).

Fauchald (1974) observed that many authors had attempted to assign families to higher clades but failed and thus reverted to using the arbitrary grouping “Errantia” and

“Sedentaria”. With the lack of knowledge on the variability of structure within families and the origin of the group, many taxonomists were unable to distinguish between primitive and advanced traits making it difficult to erect higher clades within Polychaeta (Fauchald 1974). Fauchald (1974) went on to state that the phylogeny of the Class Polychaeta is dependent on the knowledge of the origin of the secondary body cavities and the origin of segmentation. The origin of segmentation and secondary body cavities would ultimately depend on the living conditions of polychaete worms (Fauchald 1977). Dales (1962) looked at the structural relations of the buccal organs and nephridia of polychaetes in an attempt to create a new classification system. Dales successfully divided the polychaetes into 14 more or less inclusive taxa; however, they were unresolved (Fauchald & Rouse 1997; Rouse & Fauchald 1998).

Fauchald (1977) then presented a phylogenetic hypothesis of the ancestral annelid and proposed that it was more like an earthworm which had a simple body of organization, it lacked head appendages, parapodial lobes and pygidial cirri and as time went by, the simple body form evolved into a more complex body form. He based this phylogenetic hypothesis on Clark’s (1964) Hydrostatic theory presented for the origin of segmentation. This theory stated that the primary function of the segmented coelom was due to the selection for the hydrostatic skeleton to aid the peristaltic movement of a burrowing organism in sediments. This occurred by means of strong muscular septa which divided the body into contractible compartments allowing for peristaltic movement. Therefore, the segmental organization for other organ systems was regarded as a secondary subdivision. Due to this suggestion, the oligochaete – like polychaetes such as Capitellidae, Arenicolidae or Ophellidae, that ingest sediment, were proposed to be the closest relatives of the ancestral annelid (Fauchald 1977). This resulted in polychaetes displaying elaborate body plans such as the filter feeding Sabellidae and the rapacious, errant Eunicidae to be more derived families (Fauchald 1977).

Considering the morpho-functional state of the ancestral annelid proposed by Fauchald

(1977) which was based on the descriptions of Clarke (1964; 1977), Fauchald then presented a new systematic organization of polychaetes dividing them into 17 orders of equal rank. This new classification was similar to that presented by Dales (1962). Fauchald (1977) listed the families in a sequence which reflected their morphological distance from an ancestral polychaete. The families listed last (eg: Terebellidae, Sabellidae) were more morphologically distinct from the ancestral polychaete compared to the families listed first. However, many problems were encountered with this classification system. In a paper discussing the progress of polychaete systematics, Fauchald & Rouse (1997) stated that the majority of the arguments presented in Fauchald's (1977) classification were inaccurate due to many new intensive studies describing new taxa. The classification produced in Fauchald (1977) was based on the differences observed between taxa, whereas phylogeny should be based on the similarities (Cladistics) between and within taxa (Fauchald & Rouse 1997).

Rouse & Fauchald (1995) conducted a study in which they examined a series of homology assessments in terms of character congruence of all taxa within the Annelida. This eliminated all assumptions relating to the evolutionary development of the annelids, the proposed theory of the ancestral annelid and the evolution of any morphological features (Rouse & Fauchald, 1998). This analysis by Rouse & Fauchald (1995) supported the monophyly of Polychaeta; however no synapomorphy was described for this class. Rouse & Fauchald (1998) revised their initial classification to include morphological similarities between the taxa. Since then, this new classification has been accepted and is used as a working hypothesis for the interrelationships of major polychaete taxa (Rouse & Fauchald 1998; McHugh 2000; Bartolomaeus et al. 2005; Halanych 2006). The analysis was performed using traditional multistate characters and the presence / absence data with weighted or unweighted characters (Brown et al. 1999; McHugh 2000). The Annelida were found to be a monophyletic group consisting of the well supported monophyletic Clitellata and Polychaeta (Rouse & Fauchald 1998; McHugh 2000). Polychaeta was weakly supported by the presence of mixonephridia and by nuchal organs as pits or grooves and parapodia (Rouse &

Fauchald 1998). It had been proposed that many polychaetes had lost some of these key features (Rouse & Fauchald 1998). The groupings in this analysis were seen as problematic because the authors did not identify the difference between features that were lost secondarily and features that were absent primarily (Rouse & Fauchald 1998; Rouse & Pleijel 2001; Halanych 2006). Instead they simply scored these in the same way which is misleading (Rouse & Fauchald 1998; McHugh 2000; Rouse & Pleijel 2001; Halanych 2006). For example, key morphological features such as nuchal organs, segmentation, parapodia and state of the coelom were subjected to this unequal scoring (Halanych 2006).

Knowledge on the evolutionary history of the group has to be considered in order to differentiate between primary absence and secondary loss (Halanych 2006), which Rouse & Fauchald (1998) omitted in their classification. The most interesting result from this analysis was that Pogonophora which was previously considered to be a separate phylum, was actually found within Annelida and represented a derived polychaete taxon (Rouse & Fauchald 1998; Bartolomaeus et al. 2005). The Vestimentifera was positioned in the polychaete clade and found to be a derived pogonophoran (Rouse & Fauchald 1998). This finding resulted in the resurrection of Siboglinidae which traditionally included both the Vestimentifera and Pognophora (Rouse & Fauchald 1998; Bartolomaeus et al. 2005).

In a study analysing the larval and adult development of siboglinids, it was found that developmental features closely resemble those of polychaetes, further justifying their placement as a derived polychaete taxon (McHugh 2000). The new classification recognised two major clades in the weakly supported monophlyetic Polychaeta: Palpata and Scolecida. Scolecida is weakly supported by the presence of the following two apomorphies: presence of parapodia with similar rami and presence of two or more pairs of pygidial cirri (Rouse & Fauchlad 1998). Polychaetes that form part of Scolecida are the simple bodied forms that lack head appendages but possess nuchal organs (Rouse & Pleijel 2001). The presence of palps (from which the name arose) and

a limited peristomium are the two synapomorphies for the Palpata clade (Rouse & Fauchald 1998). Palps can either be grooved, used in feeding or as ventral sensory palps (Rouse & Pleijel 2001).

Palpata comprises two clades: Aciculata and Canalipalpata (Rouse & Fauchald 1998). Aciculata was the most strongly supported clade in their analysis which consists of active polychaetes bearing parapodia (Rouse & Pleijel 2001). The name of this clade was derived from one of the apomorphies, presence of stout chaetae called "aciculae" (Rouse & Pleijel 2001). The monophyly of this clade was strongly supported by the presence of several synapomorphies: aciculae found in the parapodia resulting in stability, lateral and medial antenna present on the prostomium, ventral sensory palps, dorsal and ventral cirri, single pair of pygidial cirri and segmented organs (Rouse & Pleijel 2001). Aciculata consists of three major clades: Amphinomida, Eunicida and Phyllodocida (Rouse & Fauchald 1998). Amphinomida commonly known as "fireworms" consists of approximately 200 nominal species divided into two families, Amphinomidae and Euphrosinidae (Rouse & Fauchald 1998; Rouse & Pleijel 2001). The monophyly of this clade was supported by the presence of a caruncle, proboscis shape, chaetal structure and composition (Rouse & Fauchald 1998). The monophyly for the Eunicida clade was suggested to be "very probable" and was supported by the presence of a ventral muscular pharynx, ventral mandibles, dorsal maxillae and a peristome forming a ring or rings (Rouse & Fauchald 1998; Rouse & Pleijel 2001). This clade comprises 7 families (Rouse & Fauchald 1998; Rouse & Pleijel 2001). Phyllodocida consists of Nereidiformia (commonly known as "rag-worms") and Aphroditiformia (commonly known as "scale-worms") (Rouse & Fauchald 1998). The monophyly of Phyllodocida was strongly supported by the presence of ventral sensory palps, anterior enlarged cirri, loss of dorsolateral folds and compound chaetae with a single filament (Rouse & Fauchald 1998). However, according to Rouse & Pleijel (2001) this clade is paraphyletic.

Canalipalpata was a weakly supported group by one apomorphy, the presence of

grooved palps (Fauchald & Rouse 1997; Rouse & Fauchald 1998; Rouse & Pleijel 2001). This clade encompasses almost half of the polychaetes and is divided into three major clades: Sabellida (fanworms and christmas tree worms), Spionida and Terebellida (spaghetti worms). The relationships within this clade are unresolved (Fauchald & Rouse 1997; Rouse & Fauchald 1998; Rouse & Pleijel 2001). Rouse & Fauchald (1998) placed the Siboglinidae among other families (contains Vestimentifera and Pogonophora) within this clade. The monophyly of Sabellida was weakly supported by the presence of one apomorphy, the fusion of the prostomium to the persitomium (Rouse & Fauchald 1998; Rouse & Pleijel 2001). Spionida was a strongly supported clade by the presence of a pair peristomial grooved palps, posterior projecting nuchal organs, anterior excreting nephridia, and segmental organs in the posterior end for gamete release (Fauchald & Rouse 1997; Rouse & Fauchald 1998). Terebellida was supported by the presence of an achaetous segment (segment without chaetae), and gular membrane and heart body (Fauchald & Rouse 1997; Rouse & Fauchald 1998).

Westheide (1997) on the other hand proposed a different approach for the evolution of the ancestral annelid and stated that it occurred from a more complex worm such as the vagile, predatory Amphinomidae and Nereididae to the more simple bodied form. The segmentation of the body was thought to have evolved from annelids bearing protective dorsal and lateral calcareous chaetae present along the axis of the body (Westheide 1997). The parapodia were suggested to have evolved as outfolds from the sides of the body lacking chaetae and the dorsal chaetae were lost (Westheide 1997). The remaining chaetae were divided into notochaetae and used as protective and defensive structures and the neurochaetae were used for locomotion (Westheide 1997). Breathing structures such as branchiae may have developed to sustain the oxygen supply or alternatively it was suggested that the parapodial lobes may have functioned as the branchiae (Westheide 1997). In this case, segmental organisation arose for the need to transport blood to external appendages therefore it is considered to be secondary to the segmental organisation of the body’s extremities (Westheide 1997).

Looking at the fossil record, earlier annelids from the Sirius Passet exhibited biramous parapodia with capillary chaetae (Vinther et al. 2011; Struck 2011). Unfortunately, nothing can be deduced about the anterior appendages as the anterior part of the fossils were either missing or were not well preserved (Struck 2011). Fossils from the Burgess Shales, do however show the presence of one pair of anterior appendages which were considered to be homologous to palps (Struck 2011; Eibye-Jacobsen, 2012). These appendages are considered by Eibye-Jacobsen (2012) and Vinther et al. (2011) to be solid, possible sensory palps due to their distinct morphology. The parapodia from these fossils seem to have a range of different orientations, (sub biramous, biramous and uniramous), which all have capillary chaetae (Struck, 2011). Therefore, it has been concluded that the ancestral annelid seems to be more similar to that of the scenario presented by Westheide (1997) and could possibly belong to a clade within the Phyllodocida (Eibye-Jacobsen & Vinther 2012). Based on this assumption polychaetes were suggested to be ancestral and clitellates more derived. In a molecular analysis conducted by McHugh (1997) using the Elongation Factor – 1α gene, it was found that epifaunal polychaetes such as *Nereis* (Nereidiformia) and *Harmothoe* (Polynoidae) formed the two most basal taxa within the polychaete clade, thus providing molecular evidence that the ancestral annelid was most likely a rapacious worm with elaborate head appendages.

1.3. Phylogeny of Polychaeta

Unravelling deep-level annelid relationships using morphological analysis have proven difficult for many authors (McHugh 2000). This has been attributed to the difficulty in assessing the homology of morphological characters of some extant polychaete families as well as character state scoring. As mentioned above in the classification presented by Rouse & Fauchald (1998) the primary absence of characters and the secondary loss of characters were scored in the same way. This presented a problem as it is misleading and results in conflicting interpretations (Rouse & Fauchald 1995; McHugh 2000; Halanych 2006). To solve this problem of deep-level annelid

relationships and to avoid misinterpretation of character states, molecular data has proven to be a very productive approach (McHugh 2000; Halanych 2006; Colgan et al. 2006; Struck et al. 2011). Molecular-data provides an abundance of characters across a large number of taxa to assess homology of characters making it a more feasible approach (Brown et al. 1999; McHugh 2000; Halanych 2006). With regard to secondary character losses and character coding, they are not problematic and uncontroversial because molecular data presents a limited range of possible character states and patterns (Brown et al. 1999; McHugh 2000). Regardless, it should be stressed that morphological analysis should not be ruled out completely because it is essential in identifying the basic body plans of many organisms. Thus morphology together with molecular data can produce a better understanding of polychaete systematics (Brown et al. 1999; McHugh 2000; Colgan et al. 2006). Over the past 10 years, a large number of studies have been conducted using molecular data to try and resolve the confusion surrounding the interrelationships and placement of polychaete families. The most influential studies will be discussed below.

Winnepenninckx et al. (1998) conducted a study to determine the relationships among metazoan families. In the study, 57 metazoan taxa, among them 15 polychaete families, were analysed using the 18S rDNA gene sequence. The resulting tree did not support the monophyly of Annelida and the 7 polychaete orders analysed did not appear to have a common ancestor. This result could have been due to a limited sampling of taxa. Kojima (1998) conducted a study using the Elongation factor 1-α gene (EF-1α) to determine whether Polychaeta is a paraphyletic group. The study included 13 polychaetes, 4 clitellates, 2 vetimentiferan and 2 molluscs. The results from the study did not clearly indicate any detailed relationships among polychaetes but it did strongly suggest that Polychaeta is a paraphyletic group from which clitellates and pogonophorans (now known as Siboglinidae) have derived independently (Kojima 1998).

Many other molecular studies have been conducted using the 18S rDNA gene but have

failed to produce well supported polychaete relationships (Halanych 2006). During this period, it was suggested that the reason for such poor resolution of polychaete relationships could be due to a limited taxon sampling and the usage of a small range of molecular genes. As a result Brown et al. (1999) used a multigene dataset and a broader sampling range of polychaetes in an attempt to assess the major clades erected by Rouse & Fauchald (1998). In the study they used the nuclear histone H3, U2 snRNA and two fragments of the 28S rDNA (D1 and D9-D10 expansion groups). The results showed that two of the major clades, Canalipalpata and Phyllodocida, were actually paraphyletic and the strongly supported monophyletic Aciculata was not recovered and was suggested to be either paraphyletic or polyphyletic. By this time McHugh (2000) summarized all the molecular work that had been done on the phylogeny of polychaetes and annelids and concluded that the monophyly of polychaetes and annelids were not supported by molecular evidence. Bleidorn et al. (2003a; 2003b) assessed the monophyly of the clade Scolecida using the 18S rDNA gene together with an increase in taxon sampling. They did not recover a monophyletic Polychaeta, Annelida or any of the major clades proposed by Fauchald & Rouse (1997).

In a broad scale analysis conducted by Rousset et al. (2007) another attempt was made to assess the clades proposed by Fauchald & Rouse (1997). In their analysis they included four loci: the 18S rDNA (small nuclear ribosomal subunit) and the D1 region of 28S rDNA (large nuclear ribosomal subunit), histone H3 (nuclear protein-coding gene) and the 16S rDNA (mitochondrial ribosomal gene). The inclusion of a large number of taxa together with numerous nucleotides employed in this study still produced a weak phylogenetic signal which was regarded as a rare phenomenon (Rousset et al. 2007). The monophyly of the more inclusive clades presented by Fauchald & Rouse (1997) were not recovered in their analyses (Rousset et al. 2007). A larger molecular data set was used by Struck et al. (2007) to determine whether the major polychaete clades were monophyletic. They used two datasets: the first was called Nuc and it included ~6.5kb of sequence from three nuclear genes. The second dataset was called NucMt and included ~13.4kb of sequence from 3 nuclear and 8 mitochondrial genes. The

monophyly of Scolecida, Palpata and Canalipalpata was rejected by their analysis.

In recent years the contribution of molecular analyses to determine the relationships within Polychaeta has been enormous. The majority of the families within Annelida are now represented by the small subunit nuclear gene 18S rDNA (McHugh 2005; Colgan et al. 2006; Halanych 2006; Struck et al. 2007; Zrzavy et al. 2009). None of these studies together with others that used multiple genes and increased taxon sampling have been able to recover the monophyletic clades of those presented by Rouse & Fauchald (1998) (McHugh 2005; Colgan et al. 2006; Halanych 2006; Struck et al. 2007; Zrzavy et al. 2009). Many authors have suggested that the possible reason for such a weak phylogenetic signal resulting in a poor resolution of basal nodes could be attributed to a rapid radiation of polychaetous annelids (Fauchald 1974; McHugh 2000; McHugh 2005; Colgan et al. 2006; Halanych 2006; Rousset et al. 2007; Struck et al. 2007; Zrzavy et al. 2009).

The idea of a rapid radiation of the polychaetes was first suggested by Fauchald (1974). Fauchald (1974) defined this radiation as the evolution of different polychaete families from dissimilar ancestors that had adapted to life in the semi-consolidated detrital layer above the sea bottom. The possibility exists that morphologically similar but not identical groups of polychaetes gave rise to different families which adapted to hard bottoms, burrowing or tube-building forms from the detrital layer (Fauchald 1974). This could explain why there are different numbers of anterior appendages, variations in chaetal morphologies, the oddly distributed nephridia and variations in the development of the nervous system in the different families present today (Fauchald 1974).

A number of genes have been utilised in recent molecular studies to determine deep level annelid phylogenies as well as relationships among the annelids and polychaetes. These will be discussed here. The 18S nuclear small ribosomal subunit gene (SSU) is approximately 1800-2000 nucleotides in length. This gene has been used in countless studies to assess the intra-annelid relationships and thus will remain an important tool

in annelid molecular phylogeny (Halanych 2006). Unfortunately, this gene has failed to uncover the deeper level relationships between annelids but has been helpful in placing recognised families within other families as well as determining sister clades. The 18S nuclear gene has also been very useful in the analysis of the Clitellate relationships (Halanych 2006).

The 28S large nuclear ribosomal subunit gene ranges between ~2800-3000 nucleotides in length. In terms of annelid phylogeny the usefulness of this gene is still unknown (Halanych 2006). Studies using this gene showed that it is either too variable or too short to convey any important information regarding annelid phylogeny (Halanych 2006). The fragment of this gene that bears the most information can be amplified from a ~2.1kb excluding the 3` end. Some studies have been able to sequence the entire length of this gene for polychaetes (Struck et al. 2002). When comparing the 18S with the 28S gene, it has been found that the full length of the 28S gene conveys slightly more information for uncovering annelid monophyly (Halanych 2006).

Mitochondrial genomes found in most animals have a length of ~15000bp and contains phylogenetic information regarding gene rearrangement data, amino acid data and nucleotide data. A total of 25 complete annelid genomes are available on NCBI GenBank as off January 2015 (Benson et al. 2005). Fifteen of those complete genomes have been sequenced for the Polychaeta group and 10 for the Clitellate group (Benson et al. 2005). All annelid mtDNA genomes are reported to have an outstanding degree of conservation in gene order; an indication that concatenated coding and ribosomal genes may deliver more promising results in the future (Halanych 2006). Elongation Factor - 1α is associated with the cell's protein synthesis machinery. This nuclear gene has been used to determine the origin and the inclusiveness of annelids. To some extent it can be useful within annelids and can be used at both the amino acid and nucleotide levels (Halanych 2006).

Table 1.1: Summary of molecular markers used in various annelid phylogenetic studies.

Molecular marker	Description	Reference
18 S nuclear small ribosomal subunit	Interfamilial relationships Clitellate relationships	Erseus et al. 2002, Nygren & Sundberg 2003 Erseus et al. 2000
28S nuclear large ribosomal subunit	Overall phylogenetic relationships Complete gene sequencing	Brown et al. 1999 Struck et al. 2006
16S mitochondrial large ribosomal subunit	Intraspecific and intrageneric relationships Higher level relationships	Dahlgren et al. 2001 Struck et al. 2006
Elongation factor -1α	Deep level annelid relationships and origin	Kojima et al. 1993, Kojima 1998 and McHugh 1997
12S small ribosomal subunit (mitochondrial)	Deep level annelid relationships	Borda & Sidall 2004
Cyt B	Deep level annelid relationships	Burnette et al. 2005
Histone H3	Deep level annelid relationships	Brown et al. 1999
U2 small nuclear RNA	Deep level annelid relationships	Brown et al. 1999

The mitochondrial 12S, cytB, histone H3 and U2 snRNA have been used in other studies to reveal deep level annelid relationships. There are still a range of other genes that have not been assessed but could potentially help us solve a lot of the problems we are facing with the current genes in use. These include conserved genes such as the largest subunit of the RNA polymerase chain II, Elongation factor 2, and a potential source for

deeper level phylogenetic information include nuclear genes such as Enolase, Na^+, K^+-ATPase (McHugh 2000).

A summary of the molecular markers discussed above can be found in Table 1.1. It should be noted that this is just a summarized version and presents authors that were the first to use these markers for those particular phylogenetic problems. A more detailed version can be found in Halanych (2006).

1.4. DNA Barcoding of Polychaeta

The most essential first step in any type of biological study is the succcessful identification of species (Hebert et al. 2003; DeSalle et al. 2005; Ekrem et al. 2007; Hajibabaei et al. 2007; Radulovici et al. 2010; Canales-Aguire et al. 2011; Carr et al. 2011). Unfortunately there has been a decrease in practising taxonomists who can accurately identify polychaetes. Traditional taxonomy requires the gathering of morphological and ecological data, thus requiring expert knowledge. When specimens are collected in their larval stages or have been damaged during the collection process it becomes difficult to identify them using traditional taxonomic tools. As a result DNA barcoding can be used as an easy identification tool for non-experts, fulfilling the initial step of identification for many ecological, biodiversity or bio-monitoring studies (Hajibabaei et al. 2007; Radulovici et al. 2010). DNA barcoding should not be mistaken as a tool that replaces traditional taxonomy altogether (Hajibabaei et al. 2007; Ratnasingham & Hebert 2007). It should rather be used in conjunction with morphological identification to produce a more robust identification system (Hajibabaei et al. 2007; Ratnasingham & Hebert 2007). In the case of poorly studied taxonomic groups, DNA barcoding can be used to rapidly sort specimens into genetically divergent populations (Hajibabaei et al. 2007).

DNA barcoding is useful in phylogenetic studies in that it forms the initial point for selection of ideal taxa which can then be barcoded and contribute to the library of

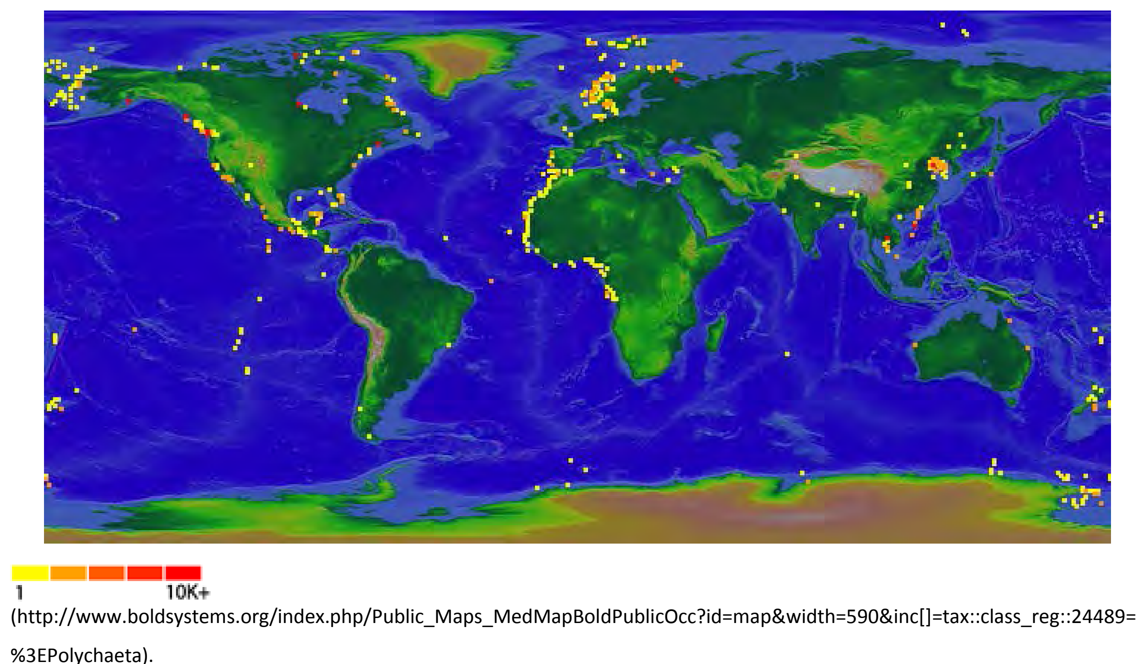
sequences (Hajibabaei et al. 2007). The extent and nature of population divergences can be detected first by DNA barcoding, which may then facilitate comparative studies of population diversity (Hajibabaei et al. 2007).

In order for a specimen to be barcoded, the sequence diversity of a short fragment of ~650 bp of the mitochondrial cytochrome c oxidase subunit 1 (CO1) is detected and for identification purposes compared with other reference barcodes of known individuals (Hebert et al. 2003; Hajibabaei et al. 2007; Ratnasingham & Hebert 2007). As a result, DNA barcoding produces the expert taxonomic data in a converted format which is easily accessible by a wide range of non-expert scientists to identify specimens (Maturana et al. 2011). Many different studies investigating vertebrate and invertebrate taxa have confirmed the effectiveness of the CO1 gene for DNA barcoding and with their studies have significantly contributed to the identification of new and cryptic species (Canales-Aguire et al. 2011).

The Barcode of Life Data System (BOLD - www.barcodinglife.org) integrates bioinformatics tools which facilitates the entire pathway of analytical processes starting from the collection of specimens to the thoroughly validated barcode library (Ratnasingham & Hebert 2007). The BOLD platform serves as an archive for specimen records and sequence records which are the building blocks of all barcoding studies. It can then be used as a workbench where you can perform an array of different analyses with the barcoded data and ensure quality and proper management of the deposited sequences (Ratnasingham & Hebert 2007). As BOLD is a freely accessible web based platform which hosts the specimen data together with sequences, it can be used as a medium from which research communities dispersed across different geographical regions can come together and collaborate. The main goal of the BOLD initiative is to compile a reference library containing barcoded sequences from a wide range of taxonomic groups (Ratnasingham & Hebert 2007).

Polychaeta consists of a highly diverse and abundant group of ecologically important

marine worms (Canales-Aguire et al. 2011). Due to their complex taxonomic history and high levels of homoplasy, the application of DNA barcoding may be essential to the assessment of polychaete phylogenetic processes (Canales-Aguire et al. 2011; Carr et al. 2011). These processes have resulted in their diversification as well as the identification and description of currently undescribed species (Maturana et al. 2011; Canales-Aguire et al. 2011; Carr et al. 2011). As per BOLD's records, there are currently 12055 polychaete specimen records distributed over 14 Orders. Only 7981 specimens of this total have been barcoded. The majority of these specimens have been collected by researchers in Northern Europe, North West Africa, Asia, Canada and the United States (Figure 1.1). There exist no barcoded polychaete specimens for South Africa (Figure 1.1). As a result, all the polychaete worms collected along the Southern African coastline will be barcoded in the hope of creating a complete library containing valid DNA sequences.



(http://www.boldsystems.org/index.php/Public_Maps_MedMapBoldPublicOcc?id=map&width=590&inc[]=tax::class_reg::24489=%3EPolychaeta).

Figure 1.1: Map of the world taken from BOLD systems reflecting the collection sites of polychaete worms thus far. Number legend found at the bottom left-hand corner.

1.5. Population genetics of Polychaeta

The marine environment is influenced by anthropogenic effects and climate change, therefore understanding the degree of connectivity, structure and demography of marine assemblages is important for the management of species (Lowe & Allendorf 2010; Kelly & Palumbi 2010; Ayata et al. 2010). Connectivity of populations and their subsequent structuring relies primarily on larval dispersal estimates (Palumbi 1994; Bohonak 1999; Weersig & Toonen 2009). Additionally, when populations are not in mutation-drift equilibrium, the structuring and connectivity of populations should be interpreted in terms of demographic history rather than gene flow (Bohonak 1999; Marko & Hart 2011). Dispersal on the other hand is influenced by factors such as oceanic currents, occurrence of reproductive barriers (physical and biological) and overall larval biology of the species in question (Grosberg & Cunningham 2001; Skillings et al. 2011; Villamor et al. 2014). The individual tracking of larvae across ocean basins is a daunting task, as a result indirect methods such as population genetic tools have been developed using molecular markers to identify the genetic trail left behind by larvae (Grosberg & Cunningham 2001; Ayata et al. 2010).

Since the 1960's a large number of population genetic studies were conducted on a variety of marine taxa due to the availability of cheap molecular techniques (Bohonak 1999). From these studies a general trend was observed for marine taxa where those species exhibiting high dispersal larvae (long lived planktotrophic larvae) were found to be genetically homogeneous across large geographic distances (Palumbi 1994; Bohonak 1999; Kamel et al. 2014; Villamor et al. 2014). On the other hand, species characteristic of short-lived larvae were found to have genetically structured populations (Palumbi 1994; Bohonak 1999; Kamel et al. 2014; Villamor et al. 2014). With the increase in the availability of population genetic studies on a large number of marine taxa displaying varieties of reproductive strategies, significant genetic structuring of populations have been found, contrary to the idea that marine populations are highly connected (Uthicke & Benzie 2003; Marko & Hart 2011; Kamel et al. 2014). In some cases where

larval dispersal patterns are not congruent with gene flow estimates, coalescent times need to be estimated and past demographic history need to be investigated to obtain a plausible explanation for the structuring patterns of communities (Marko & Hart 2011). Over the past years, a few studies analysing the population structure of polychaete worms have been carried out, however, there seems to be a lack of studies on Southern African polychaetes.

Population genetic studies in the past have used allozyme electrophoresis to identify patterns of genetic diversity and connectivity of polychaete worms (Knowlton 2000). A lot of attention was given to the genetic structuring of brackish water nereidid species namely: *Alitta succinea, Hediste diversicolor* and *Hediste japonica.* These populations were found to be well-structured due to the presence of geographic barriers and reproductive cycles that lacked a pelagic stage (Abbiati & Maltagliati 1992; Abbiati & Maltagliati 1996; Rohner et al. 1997). Beckwitt (1980) analysed the genetic structure of a sedentary tube dwelling worm *Pileolaria pseudomilitaris* using allozyme electrophoresis, populations analysed in this study were highly differentiated from one another because of isolation and founder effects. Upon analysis of the larval biology of these spirorbid species, it was found that they produce brooding larvae with the absence of a planktonic larva (Beckwitt 1980). As a result, one would infer that the absence of planktonic larvae could have contributed to the isolation and differentiation of *Pileolaria pseudomilitaris* populations.

In an analysis of interstitial polychaetes using RAPD-PCR techniques, Von Soosten et al. (1998) found that populations of *Petitia amphophthalma* were genetically differentiated and geographically structured. Schmidt & Westheide (1999) analysed another interstitial polychaete *Hesionides gohari* from three different continents and in contrast to the above findings, all populations were genetically similar to one another regardless of the large geographic distances between them. Patti & Gambi (2001) analysed an invasive species from the Mediterranean, French Atlantic and Southern Australia using the ITS2 nuclear ribosomal DNA. This sedentary *Sabella*

spallanzanii were reported to have long-lived pelagic larvae but were found to be genetically and geographically structured (Patti & Gambi 2001). In the Mediterranean, it was found that this species formed 3 groups while the Atlantic populations were strikingly different to Mediterranean and Australian populations (Patti & Gambi 2001).

Jolly et al. (2004) investigated the processes governing the subdivision of two genetically distinct populations of *Pectinaria koreni* from the Brittany and English channels. Using enzymatic and mitochondrial data, they uncovered deep phylogenetic breaks between these two populations. The Brittany population displayed unimodal mismatch curves indicative of recent population expansion and the English Channel population displayed a bimodal mismatch curve which resembled stable populations (Jolly et al. 2004). Jolly et al. (2004) was the first polychaete study to include demographic analyses to explain the distinct lineages. Similarly Schulze (2006) investigated the genetic structure and phylogeography of *Palolo* worms from the Caribbean and tropical north Pacific using COI and 16S rDNA. Schulze (2006) reported widespread *Palolo* haplotypes across large geographical areas regardless of the fact that eunicid larvae are short lived and lecithotrophic. The idea of incomplete lineage sorting was explored due to a lack of population structuring thus indicating that the genus is relatively young and had undergone recent expansions and colonisations, meaning that not enough time had elapsed to enable large genetic differences between populations to result in distinct lineages (Schulze 2006).

Iannotta et al. (2007) analysed two species of *Lysidice* and found that the two species exhibited different demographic histories. *Lysidice collaris* populations were not genetically structured and it was hypothesised that these populations might have undergone founder effects and recent colonisations in the Mediterranean basin (Iannotta et al. 2007). As a result, populations were not separated long enough to evolve differences resembling one another. On the other hand *Lysidice ninetta* was found to be composed of at least two cryptic species (Iannotta et al. 2007). This would lead one to believe that *L. ninetta* is the older species of the two and these two

lineages would have accumulated differences and thus represent cryptic species (Iannotta et al. 2007).

Recently, two studies investigating the genetic diversity and population structuring of the commonly known "fireworm", *Hermodice carunculata,* have revealed contrasting results. One study was conducted in northern Crete (Aegan Sea) and the other sampling a wider distributional range from the Mediterranean, Gulf of Guinea, Gulf of Mexico and Caribbean Sea (Ahrens et al. 2013; Chatzigeorgiou et al. 2014). Two populations sampled from the Aegan Sea were highly divergent from one another regardless of the short geographical distances between them and the teleplanic larvae recorded for family Amphinomidae (Chatzigeorgiou et al. 2014). Populations analysed in the Mediterranean, Caribbean Sea, Gulf of Mexico and Guinea had geographically distant populations with very low divergences (Ahrens et al. 2013). These contrasting results in my opinion could most likely indicate that the Aegan Sea populations were evolutionarily older and had diverged due to the presence of a geographical barrier. Populations analysed in Ahrens et al. (2013) could be representative of recent expansions and colonisations.

1.6. Oceanic currents of the east coast of South Africa

The presumed predominant driver of pelagic larval dispersal along the the east coast of South Africa is a major western boundary current known as the Agulhas current (Lutjeharms & van Ballegooyen 1988; Lutjeharms & Roberts 1988; Hutchings et al. 2002). It forms part of the anticyclonic Indian Ocean gyre with the topmost layer constituting a mix of tropical and subtropical surface water (Lutjeharms & van Ballegooyen 1988; Beckley & van Ballegooyen 1992; Hutchings et al. 2002). The high velocities documented for the Agulhas current permits the close movements of the current along the edge of the shelf break southward to a broader shelf forming the Agulhas Bank (Lutjeharms & Roberts 1988; Beckley & van Ballegooyen 1992; Lutjeharms et al. 2000). Retroflection of the Agulhas current then occurs between 16

and 20° E back into the South-west Indian Ocean as the Agulhas Return Current (Lutjeharms & Roberts 1988; Beckley & van Ballegooyen 1992). Around the Cape of Good Hope the Agulhas current has been observed to shed eddies and rings into the South-east Atlantic Ocean (Lutjeharms & Roberts 1988; Beckley & van Ballegooyen 1992).

The northern part of the KwaZulu-Natal (KZN) continental shelf is classified as a linear clastic coastline comprising a narrow shelf and a steep slope (Green 2011a; Green 2011b). The unique physiography of this coastline acts to stabilize the strong Agulhas current thus reducing the occurance of sideways meandering (Lujeharms & De Ruijter 1996; Lutjeharms et al. 2000). The Agulhas current in this northern region is characteristic of an inshore boundary with an intense cyclonic shear; the core of the current is 3 km in width and has a mean peak surface speed of 1.4 $m.s^{-1}$ (Lutjeharms & De Ruijter 1996). The shelf begins to widen off at Richards Bay extending southward toward Durban (Lutjeharms & Roberts 1988; Lutjeharms et al. 2000). This area of the shelf creates an unusual change in the shape of the continental shelf of KwaZulu-Natal and is called the Natal Bight (Lutjeharms et al. 2000; Meyer et al. 2002). The Natal Bight stretches for 160 km long and has a width of 50 km which is at its broadest off the Tugela mouth (Lutjeharms et al. 2000; Meyer et al. 2002). The Agulhas current with a width of 100 km, travelling at high velocities follows the relatively steep shelf break meticulously thus containing the shelf waters to the Natal Bight which are dominated by a system of eddies (Lutjeharms & Roberts 1988; Lutjeharms & De Ruijter 1996; Lutjeharms et al. 2000). A solitary meander known as the Natal Pulse originates from the Natal Bight and is considered to be a major disruption of the Agulhas current that is triggered by the instability of the core of the current (Lutjeharms & de Ruijer 1996; Lutjeharms et al. 2000). The continental shelf south of Durban becomes narrow with a very steep shelf slope similar to that found in the northern shelf of KZN (Beckley & van Ballegooyen 1992). The continental shelf then widens again downstream of Port Elizabeth forming the extensive Agulhas Bank (Lutjeharms & Roberts 1988).

1.7. Polychaeta from South Africa

John Day has earned the title of the father of polychaete taxonomy in South Africa. He has contributed an enormous amount of taxonomic accounts, distributional records and species descriptions of polychaete worms from intertidal rocky shores and estuaries in South Africa (Simon & Van Niekerk 2012). He has written several polychaete monographs dating from 1930 to 1960 for the region and has described several new species (Day 1967). Many of his earlier monographs were based primarily on rocky intertidal species, but later several papers and revisions included pelagic species (Day 1967). The most influential works of Day include: A monograph on the Polychaeta of Southern Africa. The monograph included taxonomic descriptions and range distributions of South African polychaete worms. The monograph was published in two volumes, the first volume was dedicated to the active polychaetes which were known as "Errantia" and the second was on sedentary polychaetes known as "Sedentaria" (Day 1967). These arbitrary groupings were used because the presence of homologous feeding structures of numerous families made it difficult to place families into higher orders (Day 1967). The monograph included approximately 36% of polychaete species that are considered endemic to the South African region (Day 1967). These monographs are the most comprehensive taxonomic guides compiled for South African polychaetes and thus are currently still in use. However, these guides urgently need revision.

Carol Simon was the next most influential polychaete biologist from South Africa. Simon conducted various studies on pest polychaete worms, specifically paying attention to pests of cultured abalone Haliotis midae. Various studies were conducted on the endemic sabellid Terebrasabella heterouncinata which ranged from growth rates, infestation rates on cultured abalone, ultrastructure of oogenesis and spermiogenesis, reproductive outputs and life history stages (Simon et al. 2002, Simon 2004, Simon et al. 2004, Simon et al. 2005a, Simon et al. 2005b, Simon & Rouse 2005). Thereafter she looked at various spionid genera and investigated many topics such as the general infestation of spionid worms on cultured abalone, population structure

and growth of polydorid polychaetes and the general polydorid and diploydorid infestation on mollusc shells (Simon et al. 2006, Simon & Booth 2007, Simon 2009, Simon et al. 2010, Simon 2011). Simon et al. (2014) also described two new species belonging to the genus Syllis from South Africa. David et al. (2014), David and Simon (2014) investigated poecilogony in Polydora hoplura which are pests of commercially important molluscs. He also looked at the effect that temperature has on the development of two non-indigenous spionid species, as well as range expansions of this species and establishment.

1.8. Rationale for this study

Polychaete worms are important constituants of benthic and pelagic habitats. The phylogenetic relationships within this family are unresolved and not many studies focus on South African polychaete phylogenetics or population genetics. In addition, the last comprehensive survey on South African intertidal rocky shore polychaetes was done over 30 years ago. Therefore, in Chapter 2 the aim of the study is to determine the phylogenetic relationships of polychaete families/communities that are commonly found on the intertidal rocky shores of the Eastern Cape and KwaZulu-Natal Coasts.

Marphysa corallina is considered to be an ecologically important member of its habitat because it was found to burrow in mucus-sand tubes. Polychaetes that burrow in the benthos are known to aerate anoxic sediments thus contributing to a healthier environment for other intertidal invertebrates to live in. In Chapter 3 the aims are to determine whether populations of *Marphysa corallina* are genetically structured and subsequently to assess the past demographic events that have shaped contemporary distributions of this ecologically important species. This presents the first study investigating the population genetic structure and demographic history of *Marphysa corallina* from South Africa and will contribute significantly to the knowledge of the processes that have shaped many important invertebrate species' distributional patterns.

Chapter two: Phylogeny of common intertidal Nereididae and Eunicidae rocky-shore polychaetes (Annelida) from South Africa

2.1. Introduction

Polychaete worms are diverse multi-segmented worms that are well represented throughout aquatic habitats from shallow continental shelves to deep abyssal plains, from brackish estuarine environments to temporary puddles in terrestrial environments (Day 1967; Hall et al. 2004; Zhou et al. 2010). Polychaete worms are found in a variety of habitats resulting in the development of a diversity of feeding habits and reproductive strategies, thus making polychaete worms very successful invertebrates. (Wilson 1991; Gambi & Cigliano 2006). Over the years, polychaetes have been classified into approximately 80 families however, the phylogenetic relationships among them are poorly understood and the monophyly of the families are subject to much debate (Fauchald 1974; Bleidorn et al. 2003a; Bleidorn et al. 2003b; Halanych 2006).

Polychaete worms are constantly evolving and therefore require the need to be continuously ranked into higher taxa such as orders (Bartolomaeus et al. 2005). Together with the complex evolutionary histories of polychaetes and diversity of morphological differences, many gaps are found in the classification of the polychaete group (Zhou et al. 2010). As a result further classification of polychaete worms still remain a challenge. The majority of studies describing the relationships within the Polychaeta have been using morphological data (Bleidorn et al. 2003a). Recently,

molecular characters combined with morphological characteristics have gained much popularity in providing a more insightful tool in determining deeper polychaete relationships (Bleidorn et al. 2003a).

The first and last comprehensive account of Polychaete fauna in South Africa was composed by Day (1967) more than 30 years ago. Day (1967) provided a thorough description of all polychaetes distributed from the northern parts of southwest Africa, around the Cape of Good Hope to the port of Beira in Mozambique. Not many studies have focused on defining the phylogenetic relationships of polychaete worms found in South African waters. Specimens belonging to families Nereididae and Eunicdae dominated the intertidal rocky shores that were sampled along the Eastern Cape and KwaZulu-Natal coasts. As a result individuals belonging to these families constituted a major portion of our sample set. Thus phylogenetic relationships within Eunicidae and Nereididae will be investigated in the present study.

The Eunicida comprises a diverse group of annelids found in a range of habitats (Struck et al. 2007; Zanol et al. 2010). Eunicids burrow into hard coral or calcareous algae, live in rock crevices and form an essential part of coral reef and rocky shore communities (Hutchings 1986; Sorokin 1995). The Eunicida clade is currently defined by seven families: Dorvelleidae, Eunicidae, Hartmaniellidae, Histriobdellidae, Lumbrineridae, Oenonidae and Onuphidae (Rouse & Pleijel 2001; Struck et al. 2002; Struck et al. 2007; Zanol et al. 2010). Eunicidae is one of the most speciose taxon within the Annelida comprising 900 nominal species distributed among 100 genera (Rouse & Pleijel 2001; Struck et al. 2002; Struck et al. 2007). The descriptions of species of all families within Eunicida were carried out during the late 18th and early 19th centuries. Individuals belonging to Eunicida are considered to have a long scientific history (Struck et al. 2007).

The largest known polychaetes appear within the family Eunicidae with sizes ranging up to 6 m whereas the smallest interstitial forms are approximately 250 µm in length

(Eibye-Jacobsen & Kristensen 1994; Struck et al. 2007; Zanol et al. 2010). The epitokes (a sexually mature life stage filled with gametes) of the *Palola* worms (Eunicidae) are consumed by the natives inhabiting the South Pacific Islands (Schulze 2006). *Diopatra aculata*, *Marphysa mulawa* together with other eunicid species are used as bait for leisure and commercial fishing in a number of regions such as the United States, Japan, Mediterranean Coast and Australia (Hutchings & Karageorgopolous 2003; Struck et al. 2007). As a result they are considered to be culturally and commercially important polychaetes.

Polychaete worms are soft-bodied animals and the jaw apparatus, called scolecodonts, of the eunicids are the sole representation of polychaete worms in the fossil record (Struck et al. 2007; Paxton 2009). The Furongian period (final series of the Cambrian) was the earliest known period to contain eunicid scolecodont fossils (Paxton 2006; 2009). However, the Ordovician was seen as the main period for the radiation of eunicids, which contained fossils of more than 50 known genera belonging to 15 – 20 families (Paxton 2006; 2009). Based on the arrangement, number of teeth and shape of elements on the jaw apparatus, five different architectural types were described within Eunicida (Paxton 2006; Struck et al. 2007; Paxton 2009). The different jaw types reflect grades of evolution and do not represent clades of any sort (Paxton 2006; 2009). The Labidognatha (pincer-jaw) and the Priognatha (saw-jaw) were the first two grades to be described by Ehlers in 1868 (Paxton 2006; 2009). Thereafter the Ctenognatha (comb-jaw) and the Placognatha (plate-jaw) were described by Kielan-Jaworawska (1966). Lastly, the Xenognatha (strange-jaw) was described by Mierzejewski & Mierzejewski (1975). The Placognatha and Ctenognatha jaw types were agreed upon by many authors to be the most ancestral types (Paxton 2006; Struck et al. 2007; Paxton 2009).

Within the Eunicida, the only relationship to be significantly supported was that of families Onuphidae and Eunicidae, forming sister taxa, which has been constantly recovered by many morphological and molecular studies (Rouse & Fauchald 1998; Struck et al. 2002; Rousset et al. 2007). However, this relationship has been

characterised by plesiomorphies such as the asymmetrical labidognath jaws, one to five prostomial appendages and a double ringed peristomium with bilobed prostomium (Zanol et al. 2010). As a result, molecular analysis using the 18S rDNA gene produced a paraphyletic Eunicidae with Onuphidae nested within it, whereas with the analysis of a broader range of genes such as CO1, 16S rDNA, 18S rDNA and 28S rDNA, a monophyly in the Eunicidae was recovered (Zanol et al. 2010). Due to conflicting results, the phylogenetic status of Eunicidae remains uncertain (Zanol et al. 2010).

Polychaetes belonging to the family Nereididae are commonly found in a wide range of habitats from shallow marine habitats to freshwater puddles in moist terrestrial environments (Sorokin 1995; Glasby 1999; Dean 2002; Bakken & Wilson 2005). Nereidids have characteristic flat bodies and well developed parapodia and are known to be omnivorous worms that have an eversible pharynx armed with chitonous jaws used to catch prey (Fauchald & Jumars 1979; Sorokin 1995). It has also been documented that some species of nereidids are filter feeders that consume algae, bacteria and detritus (Fauchald & Jumars 1979; Sorokin 1995; Dean 2002). On the intertidal rocky shores, nereidids are commonly found living in dead barnacle shells, mussel beds and seagrass beds (Fauchald & Jumars 1979; Sorokin 1995). Some polychaetes dig burrows and are lined with mucus secreted by the animal in soft bottom bethic habitats (Sorokin 1995; Dean 2002). Nereidids over time have developed special adaptations that enable them to inhabit areas of low salinity and semi-terrestrial habitats where other polychaete families cannot survive (Glasby 1986; Glasby 1999). These adaptations contribute to making the Nereididae a very successful family (Glasby 1986; Glasby 1999).

Nereidid worms are important food sources for many crustacean and fish farms around the world (Hamdy et al. 2014). Nereidids are sold as bait to fisherman and sea anglers and used as indicatiors for pollution (Bakken & Wilson 2005). They have also been the subject of various physiological and endochrinological research (Hamdy et al. 2014). There exists a lot of research on the reproductive strategies of nereidid polychaetes

(Glasby 1986) as compared to the lack of reproductive studies available for eunicids. The release of epitokes in the water column is the dominant reproductive strategy of marine nereidid worms. However, it has been found that nereidids found in low salinity habitats have developed brooding larvae to ensure the protection of their developing eggs and larvae from osmotic stresses (Glasby 1986).

Nereididae, like Eunicidae, is considered to be another very diverse polychaete family of approximately 677 described species split among 44 genera to date (Bakken & Wilson 2005; de León-González & Goethel 2013). Several phylogenetic studies have recovered a monophyletic Nereididae family with the presence of flattened notopodial lobes and notochaetae with compound falicgers and/or spinigers as the synapomorphy for the family (Bakken & Wilson 2005). The large number of species described for the family has resulted in the formation of heterogeneous genera which have been errected primarily based on morphological characters (Bakken & Wilson 2005). Fitzhugh (1987) conducted the first phylogenetic study on nereidid polychaetes and recovered three valid subfamilies: Namanereidinae, Gymnonereidinae and Nereidinae (Bakken & Wilson 2005). Monophyly was found for subfamilies Namanereidinae and Nereidinae while Gymnonereidina was paraphyletic after using parsimony methods (Bakken & Wilson 2005). Within sub-family Nereidinae, 18 speciouse genera were described based primarily on presence, formation, morphology and number of paragnaths (Glasby 1999; Bakken & Wilson 2005; Bakken et al. 2009). Paragnaths are hardened scleroprotein structures that are found in different patterns on the eversible pharynx (Glasby 1999; Bakken & Wilson 2005; Bakken et al. 2009). The pharynx of nereidids has been divided into areas and assigned Roman numerals I – VII (Figure 2.1) from the basal ring to the maxillary ring (Glasby 1999; Bakken & Wilson 2005; Bakken et al. 2009).

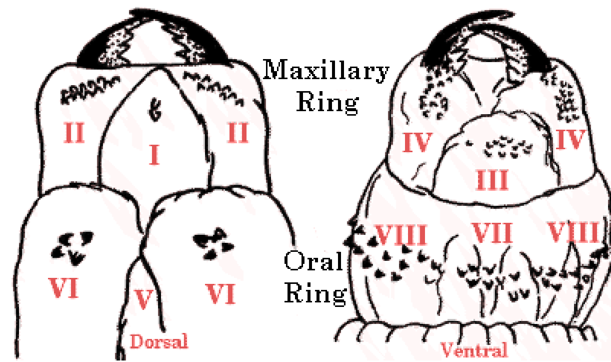


Figure 2.1: Illustration of an everted pharynx of a typical nereidid worm. Each maxillary ring is divided into sections that have been assigned Roman numerals. Figure reproduced from Glasby (1999).

There have been a few taxonomic revisions of the many genera present in the family Nereididae but there seems to be a lack of phylogenetic studies on the interrelationships between the genera and their associated species. The few phylogenetic studies that have been conducted were inconclusive and thus no valid relationships could be drawn from these studies (Bakken et al. 2009).

2.1.1. Aim of the study

There is a lack of phylogenetic studies of the intertidal Nereididae and Eunicidae polychaete worms from South Africa.

The aim of this study is to assess the phylogeny and taxonomy of common polychaete families found, in this case Nereididae and Eunicidae, on the intertidal rocky shores of the Eastern Cape and KwaZulu-Natal Coast in South Africa and to subsequently determine the phylogenetic relationships between and within these families.

2.2. Materials and Methods

2.2.1. Study sites and sample collection

In order to assess the phylogenetic structure and relationships of common intertidal polychaete worms found along the East Coast of South Africa, samples of polychaete worms of different families were collected over a year (June 2013 – April 2014, Table 2.1). Six sites were sampled along the KwaZulu-Natal coast namely: Clansthal, Green Point, Reunion Rocks, Adlams, Ballito and Mabibi and two sites from the Eastern Cape coast namely: Mgazana 1 and Mgazana 2 (Figure 2.2, Table 2.1). Adlams and Mabibi beaches are located near Sodwana Bay, which is the northern-most part of KwaZulu-Natal (Figure 2.2). Mabibi and Adlams are separated from one another by an extensive sandy flat and patches of rocky shores.

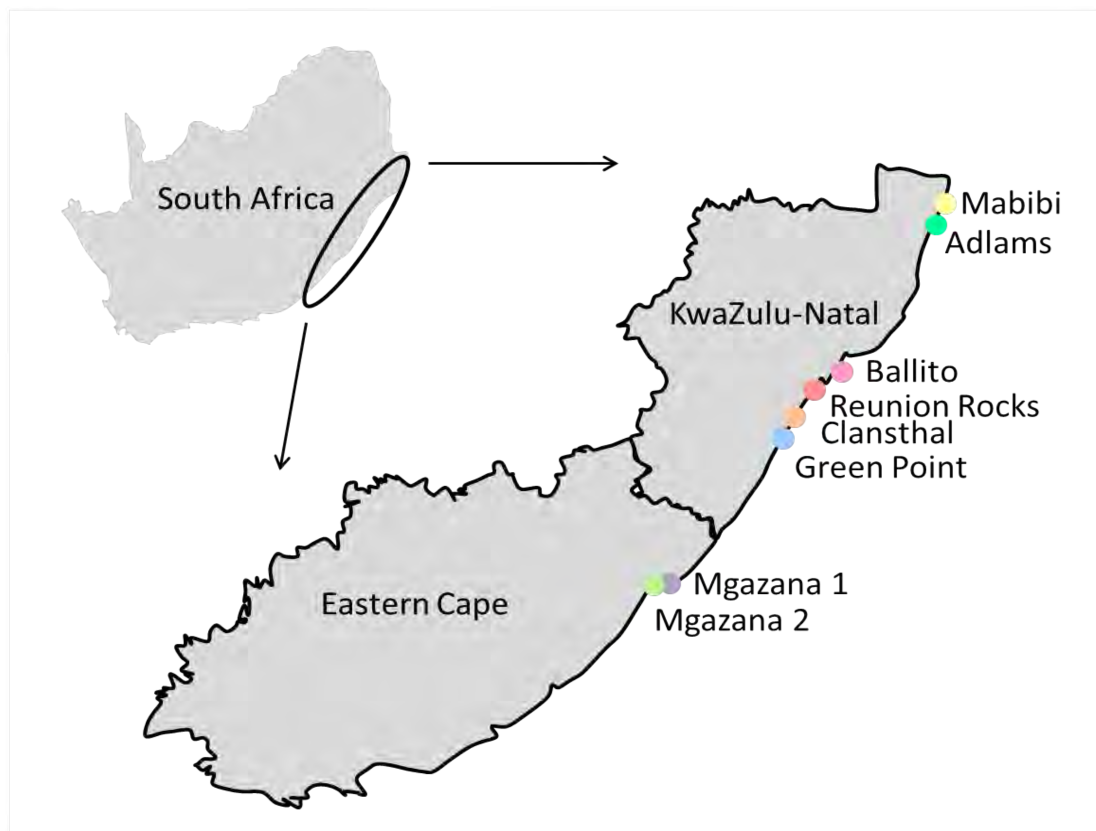


Figure 2.2: Map showing the eight sampling localities from the Eastern Cape and KwaZulu-Natal coasts.

Adlams and Ballito are separated by patches of rocky shore and sandy flat beaches, Ballito and Reunion Rocks separated by sandy and rocky shores. A total of 44 specimens belonging to two different families, Nereididae and Eunicidae were collected from the 8 different sites (Figure 2.2, Table 2.1).

Individuals were found in mucus-sand tubes in algal mats inundated with sand, under worm rock and bait rock on the intertidal rocky shores and under barnacle shells. A crow bar was used to lift up rocks and an oyster knife was used to break off worm rock, barnacles and mussels from hard substrates. Polychaete worms have delicate soft bodies, therefore, to prevent losing body parts and breaking specimens tweezers were used to carefully pick out the encrusting worms.

Worms were put into individual bags of sea water and taken to the lab for processing. At the laboratory, polychaete worms were anesthetized with 10% $MgCl_2$ for approximately 30 minutes after which they were rinsed in distilled water to remove salt and finally preserved in 70% molecular grade ethanol for morphological analysis and DNA extraction.

Table 2.1: Summary of collection data of polychaete worms collected at eight sample sites across two regions, KwaZulu-Natal (KZN) and Eastern Cape (EC) along the South African coast. The COI column indicates the number of individuals in that family sequenced for the COI gene.

Family / Species	Sample Site	Co-ordinates	Date collected	COI
Nereididae *Perinereis cultrifera* *Pseudonereis variegata* *Perinereis sp.* **Eunicidae** *Marphysa corallina*	Clansthal KZN	30°14'10.08"S 30°47'18.21"E	22 June 2013	5

Eunicidae *Marphysa corallina*	Green Point KZN	30°15'0.61"S 30°46'55.91"E	23 June 2013	2
Nereididae *Pseudonereis variegata* **Eunicidae** *Marphysa corallina*	Reunion Rocks KZN	29°59'11.49"S 30°57'51.00"E	13 June 2013	4
Nereididae *Nereis falsa* *Pseudonereis variegata* **Eunicidae** *Marphysa corallina*	Mgazana 1 EC	31°42'19.10"S 29°24'49.19"E	9 July 2013	3
Nereididae *Nereis (Neanthes) indica* *Pseudonereis variegata* **Eunicidae** *Lysidice collaris* *Marphysa corallina*	Mgazana 2 EC	31°41'6.27"S 29°26'20.37"E	10 July 2013	7
Nereididae *Nereis falsa* *Nereis coutierei* **Eunicidae** *Marphysa corallina*	Ballito KZN	29°32'23.16"S 31°13'25.90"E	31 January 2014	8
Nereididae *Pseudonereis variegata* **Eunicidae** *Marphysa corallina*	Adlams KZN	27°37'28.34"S 32°39'22.52"E	30 April 2014	5
Eunicidae *Eunice antennata* *Nicidion cincta* *Marphysa corallina*	Mabibi KZN	27°25'49.06"S 32°42'51.58"E	29 April 2014	10

2.2.2. Morphological identification

Polychaete worms are among the most common and abundant marine organisms that inhabit benthic environments (Fauchald & Jumars 1979). They are characterised by their multi-segmented bodies which are divided into three distinct regions, the prostomium, metastomium and pygidium (Figure 2.3). The prostomium forms the anterior region making up the "head" of the polychaete worm (Day 1967; Fauchald 1977). The prostomium bears a wide variety and arrangement of sense organs which, depending on function, are known as antennae, palps and nuchal organs (Day 1967; Fauchald 1977). The presence, absence, position and arrangement of these sensory organs and the overall shape and size of the prostomium are very important for family and genus level identifications (Day 1967; Fauchald 1977). In some families such as the Nereididae, upon preservation in ethanol, the proboscis is everted and contains numerous chitonous teeth called paragnaths (Day 1967; Fauchald 1977). These paragnaths are different in shape and size and are arranged in various patterns which are useful in the identification to genus level (Day 1967; Fauchald 1977). For other predatory polychaete worms, the arrangement of teeth and the structure of jaws are used for identification to genus level (Day 1967; Fauchald 1977).

The metastomium forms the body which is made up of numerous segments each consisting of a pair of parapodia (Day 1967; Fauchald 1977). Depending on the genus, the parapodia are either biramus including the formation of a dorsal notopodium and ventral neuropodium, or uniramus which consists of a single continuous lobe (Figure 2.3) (Day 1967; Fauchald 1977). These rami consist of a chaetigerous lobe and comprise bundles of chitonous chaetae. These chitonous chaetae are highly important structures as they do not change upon preservation and depending on their position and morphology, are useful for differentiating species. The branchiae are respiratory organs found on various parts of the body and depending on family and genus they could be found concentrated in a bundle on the head region or as individual filaments on the dorso-lateral parts of the body (Day 1967; Fauchald 1977). The pygidium forms the posterior, anal, region of the body (Day 1967; Fauchald 1977). This region is not of

great importance for identification but the morphology of the pygidium differs between families and genera and is occasionally used. Pictures of the above-mentioned characters were viewed using a Nikon Az1000 AS stereo microscope and dissecting microscope and were photographed. Due to the chitonous setae being transparent in nature, methylated blue dye was used to stain the setae in order to identify the different morphological structures. All specimens were identified to species level by myself and none were sent to specialists for identifications. Day's monographs on the polychaeta of Southern Africa (1967) were used to identify all specimens.

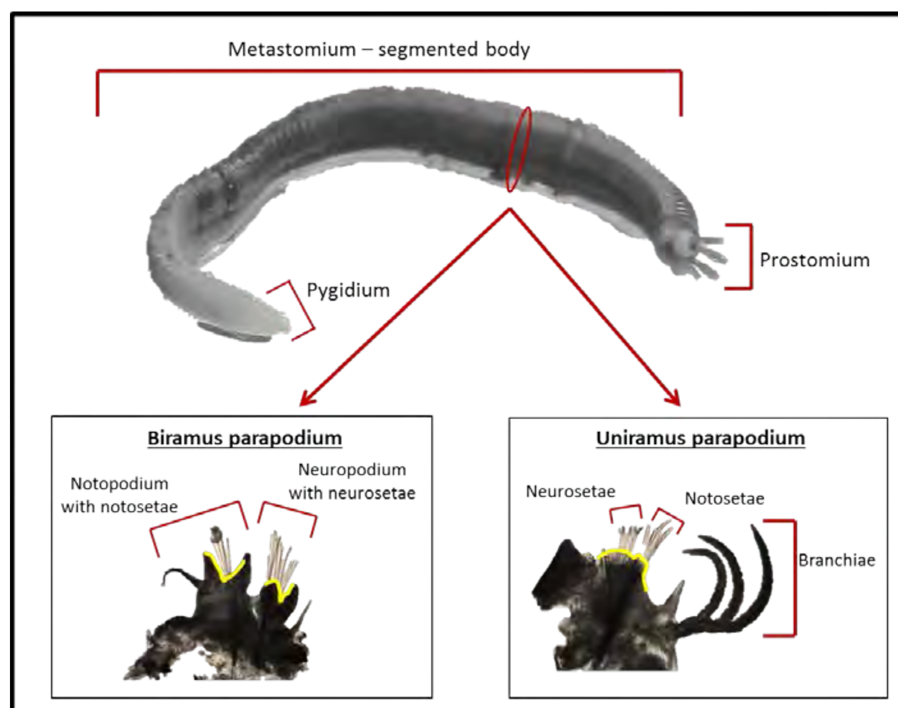


Figure 2.3: Anatomy of a typical polychaete worm. Each segment on the body bears a pair of lateral parapodia which can either be biramus (two rami and setal lobes, outlined in yellow), or uniramus (one rami and one setal lobe, outlined in yellow).

2.2.3. DNA extraction and amplification

Genomic DNA was isolated from polychaete tissue using the ZR Genomic DNA Tissue MiniPrep extraction kit (www.zymoreasearch.com) according to standard

protocol. Approximately 25 mg of tissue was cut from the posterior end of the worm and rinsed with distilled water to remove excess ethanol that could potentially inhibit activity of poteinase-K. Tissue was cut up into tiny pieces to ensure breakdown of cell walls and maximum yield of DNA. Tissue was placed in eppendorf tubes containing 95µl of molecular biology grade water, 95 µl of 2X digestion buffer and 10 µl of proteinase-K. Samples were vortexed and incubated in a dry bath incubator overnight at 55° C on a gyro- rocker.

Samples were removed from the incubator the next day and to each eppendorf tube, 700 µl of Genomic Lysis Buffer was added. Samples were vortexed and spun down for one minute at 10,000x *g* using an eppendorf centrifuge 5418 to remove insoluble debris. The supernatant was transferred to a Zymo-Spin IIC Column with a collection tube and centrifuged for one minute at 10,000x *g.*

DNA pre-Wash buffer with a total volume of 200 µl was added to each Spin Column in a new Collection Tube and centrifuged at 10,000x *g* for one minute. Thereafter, 400 µl of g-DNA Wash Buffer was added to the spin column and centrifuged at 10,000x *g* for one minute. Lastly the spin column was put into a clean eppendorf tube and 100 µl of DNA Elusion Buffer was added to each sample and left to incubate for approximately 60 minutes at room temperature after which the tubes were centrifuged at high speed for 30 seconds to allow for elution of DNA. The DNA was then stored in -80° C freezer until PCR.

The isolated genomic DNA was amplified using the Polymerase chain Reaction (PCR) using the universal mitochondrial primers LCO1490 and HCO2198 (Vrijenhoek 1994). PCR amplifications were conducted using 12.5 µl of EconoTaq® PLUS GREEN 2X Mastermix (Lucigen), 7.82 µl of molecular biology grade water, 0.84 µl of forward and reverse primer, 1 µl/ml of Bovine Serum Albumin (BSA) and 2 µl of template DNA to make up a total reaction volume of 25 µl. COI reactions were amplified using a BioRad T100-Thermal Cycler. The PCR thermal cycle conditions for COI was carried out as

follows: 95° C for 3 minutes, followed by 35 cycles of 94° C for 30 seconds, 45° C for 30 seconds 72° C for 1 minute, followed by 72° C for 7 minutes and a final cold storage of 12° C.

PCR products were run on a 1 % agarose gel (1 g agarose powder and 100 ml of 1X TBE buffer) using 3 µl of PCR product and 3 µl of 100bp ladder. Images were taken of gels using a BioRad Molecular Imager, Gel Doc™ XR+. PCR products were sent to Inqaba Biotechnical Industries (Pty) Ltd, Hatfield, South Africa for sequencing using an ABI 3730 Capillary sequencer.

2.2.4. Genetic analysis

Sequences were individually aligned and edited in BioEdit v7.0.9.0 (Hall 1999). Nucleotide ambiguities were found at different loci and corrected by searching for the strongest signal on the chromatograms of the respective sequences using the IUPAC ambiguity codes. The edited sequences were aligned using the ClustalW multiple alignment method, after which the mtDNA sequences were trimmed to a length of 560bp. A search for highly similar sequences was conducted using BLAST algorithm in NCBI GenBank and these sequences were downloaded and used for further phylogenetic analyses (species names and accession numbers in Table 2.2). Sequences that could not be found in NCBI GenBank were downloaded from the Barcode of Life Database; BOLD (Sample ID's and species names are found in Table 2.2).

DnaSp v5 (Librado & Rozas 2009) was used to generate a haplotype data file for both COI and ITS1 sequences for phylogenetic analyses and polymorphism data. The generated haplotype data file was first used in MrModelTest 2.3 (Nylander 2008) to calculate a best-fit model of evolution. The Akaike Information Criterion (AIC) GTR+G model was chosen to construct a Bayesian tree using Mr Bayes 3.1.2 (Huelsenbeck & Ronquist 2001) and a Maximum Likelihood tree using Garli's (Zwickl 2006) online web service. Garli parameters were set to default and were run for 1000 bootstrap

replicates. The Bayesian and Maximum Likelihood trees were rooted with *Micrura dellechiajei* which belong to the Phylum Nemertea and represent a distant ancestor of the polychaete group (accession numbers in Table 2.2). The Bayesian tree was calculated using 4 Markov chains of 1000 000 generations each, with every 100th tree sampled. The first 25 % trees were discarded as burn in, and the remaining was used to construct a 50 % majority-rule consensus tree with Bayesian Posterior Probability support for each clade. To estimate convergence between runs (stationarity of parameters), a graph of the log likelihood of sampled trees was plotted in Tracer v1.5 (Rambaut & Drummond 2007). The mixing quality of all parameters was verified by analyzing the plot of the log likelihood versus sampled trees and the effective sample sizes (ESS) for all parameters calculated in Tracer v1.5. An ESS of greater than 200 for all parameters when the two runs combined was considered as good mixing. An ESS of >200 was obtained thus the results were accepted.

Table 2.2: List of species used for phylogenetic analyses and their respective accession numbers from Genbank and Sample ID's from the Barcode of Life Database (BOLD indicated in parenthesis).

Family	Species Name	Accession Number
Aphroditidae	*Aphrodita negligens*	AY894309.1
	Aphrodita longipalpa	HM473298.1
	Aphroditella hastata	HQ023985.1
Dorvilleidae	*Schistomeringos longicornis*	HM473664 (BOLD)
Eunicidae	*Eunice amoureuxi*	GQ497538.1
	Eunice antarctica	GQ497532.1
	Eunice cf. antilensis	GQ497533.1
	Eunice cf. insularis	GQ497537.1
	Eunice mutilata	GQ497540.1
	Eunice notata	GQ497544.1
	Eunice roussaei	GQ497543.1
	Eunice torquata	GQ497539.1

Family	Species	Accession number	
	Eunice cariboea	DQ317859.1	
	Eunice mikeli	GQ497558.1	
	Eunice valens	GQ497534.1	
	Lysidice collaris	GQ497557.1	
	Lysidice ninetta	GQ497564.1	
	Marphysa angeli	GQ497550.1	
	Marphysa mossambica	JX559751.1	
	Marphysa sp.	KF931024.1	
	Marphysa brevitentaculata	GQ497548.1	
	Marphysa californica	GQ497552.1	
	Marphysa cf. hentscheli	GQ497551.1	
	Maprhysa disjuncta	GQ497549.1	
	Marphysa regalis	GQ497562.1	
	Marphysa sanguinea	GQ497547.1	
	Marphysa viridis	GQ497553.1	
Lumbrineridae	*Lumbrinereis erecta*	HM473450	(BOLD)
	Lumbrinereis fragilis	GU672261	(BOLD)
	Lumbrinereis japonica	HM473451	(BOLD)
Nereididae	*Alitta sp.*	HM473289.1	
	Alitta virens	GU672562.1	
	Hediste japonica	AB603758.1	
	Hediste atoka	AB603887.1	
	Neanthes acuminata	KJ539130.1	
	Nereis aibuhitensis	JX661455.1	
	Nereis denhamensis	JX392068.1	
	Nereis pelagica	HQ023592.1	
	Nereis heterocirrata	KC800626.1	
	Perinereis falklandica	HQ705184.1	
	Perinereis longidonta	HQ705191.1	
	Perinereis gualpensis	HQ705188.1	
	Perenereis sp.	EU352319.1	
	Perinereis vallata	HQ705196.1	
	Platynereis sp.	HM473612.1	

	Platynereis dumerilii	KF815726.1
	Pseudonereis anomala	JX420271.1
	Pseudonereis variegata	HQ705197.1
	Simplisetia cf. erythraensis	EU835670.1
Onuphidae	*Diopatra dentata*	GQ497522.1
	Diopatra macroensis	FJ646632.1
	Diopatra micrura	GQ456161.1
	Diopatra neopolitana	GQ456164.1
	Diopatra sp.	JQ769509.1
	Onuphis elegans	GQ497525.1
	Nothria conchylega	HM473514
	Paradiopatra quadricuspis	GQ497523.1
OUTGROUP		
Lineidae	*Micrura dellechiajei*	KF935514.1

2.3. Results

2.3.1. Morphology

A total of 44 individuals were collected from eight different sites across two regions: KwaZulu-Natal and the Eastern Cape in South Africa (Table 2.1). Using the diagnostic features mentioned in the Material and Methods (section 2.2, p.30) together with Day's polychaete guides for Southern Africa, the 44 specimens identified belonged to two families: Nereididae and Eunicidae. The various species names, collection sites, dates and geographic co-ordinates can be found in Table 2.1 and a map of sample sites in Figure 2.2. For family Nereididae, 6 species were identified across 3 genera (*Perinereis, Pseudonereis* and *Nereis*) (Table 2.1). Identifications for family Eunicidae included a total of 4 genera (*Marphysa, Lysidice, Eunice* and *Nicidion*) and 4 species (Table 2.1). *Marphysa corallina* was commonly found throughout all sample sites and found in large numbers.

2.3.2. Genetic analyses

A fragment of 560bp was sequenced for 44 individuals for the universal mitochondrial cytochrome oxidase subunit 1 marker. A total of 548 sites were analysed with 12 of them counted as missing data or gaps. There were 103 monomorphic or invariable sites and 445 variable (polymorphic) sites. There were 763 mutations, with 358 parsimony informative sites and 87 singleton haplotypes. The GC content for the 548 analysed sites was 0.428.

The haplotype diversity for 44 sets of sequences was 0.963 and the nucleotide diversity was 0.246 with the average number of nucleotide differences of 135. A total of 29 haplotypes were obtained from the 44 sequences spread across 8 samples sites. Haplotype frequency was the highest for Ballito and Mabibi (24%) indicative of a very diverse population with a large number of singleton haplotypes. Clansthal, Mgazana 2

and Adlams had frequencies of 17%. Reunion Rocks and Mgazana both had a haplotype frequency of 10% and the lowest frequency was recorded for Green Point (3%).

Individuals sampled from both families, Eunicidae and Nereididae formed two distinct clades in their respective families as in the consensus tree (Figure 2.4). Nereididae formed a monophyletic clade and had strong support for both bayesian and maximum likelihood analysis (1/100). However, Eunicidae formed a polyphyletic clade with Lubrineridae and Onuphidae nested within it. This polyphyletic Eunicidae clade was strongly supported by bayesian analysis only (0.99) (Figure 2.4).

In the Eunicidae clade, *Nicidion cincta* (sampled for this study) grouped closely with *Nicidion notata* in a clade strongly supported by Bayesian and ML analyses (1/100) (Figure 2.4). *Nicidion mikeli* grouped as a sister taxon to the *N. notata* and *N. cincta* grouping with moderate to weak support (B: 0.92, ML: 70 respectively). A second *Nicidion* clade was observed with high bayesian support (1) and weak ML support (60) (Figure 2.4) and consisted of species previously belonging to *Marphysa* and *Eunice.* The majority of species belonging to *Marphysa* grouped into a strongly supported clade for bayesian analysis (1) and was weakly supported by ML analysis (70). *Marphysa corallina* (sampled in this study) formed an exclusive strongly supported clade (1/100) and is positioned as sister taxa to other *Marphysa* species (Figure 2.4).

Lysidice collaris sampled in this study did not group in with other *Lysidice* species and instead grouped as a sister taxon to the bigger *Marphysa* clade with strong Bayesian support (0.97). The grouping of *Leodice antennata* as a sister taxon to *L. antarctica, L. valens, L. cf. antillensis* and *L. torquata* had strong bayesian support (1) and weak ML support (60). Onuphidae in my analysis was found to be polyphyletic with *Onuphis elegans* grouping as an outgroup and two other species (*Paradiopatra quadricuspis* and *Nothria conchylega*) nesting within Eunicidae (Figure 2.4). The larger Onuphidae clade is a sister clade to Eunicidae and occupies a more basal position with weak support

(Figure 2.4).

Perinereis cultrifera and most *Pseudonereis variegata*, from the strongly supported monophyletic Nereididae clade, grouped into a well-supported clade (1/100) and forms a sister clade to *Perinereis sp.* This *Perinereis-Pseudonereis* clade forms a sister clade to the *Alitta* clade with little support (Figure 2.4). *Nereis falsa, Nereis coutierei* and *Nereis indica* forms a sister grouping to *Neanthes acuminata* which has no bayesian support and is weakly supported by ML analysis (70) (Figure 2.4). The remaining *Nereis coutierei* individuals group together with *Platynereis* sp. which are well-supported by Bayesian analysis (1) and weakly supported by ML analysis (70) (Figure 2.4). The last *Pseudonereis variegata* species groups together with *Platynereis dumerilii* and has weak Bayesian support (0.7) (Figure 2.4). The overall well-supported monophyletic Nereididae clade is observed to be a sister clade to the polyphyletic Aphroditoididae clade which assumes a more basal grouping that has strong Bayesian support (1) (Figure 2.4). All inner and outer nodes for Nereididae and Eunicidae have overall moderate to strong support for bayesian and maximum likelihood analyses (Figure 2.4).

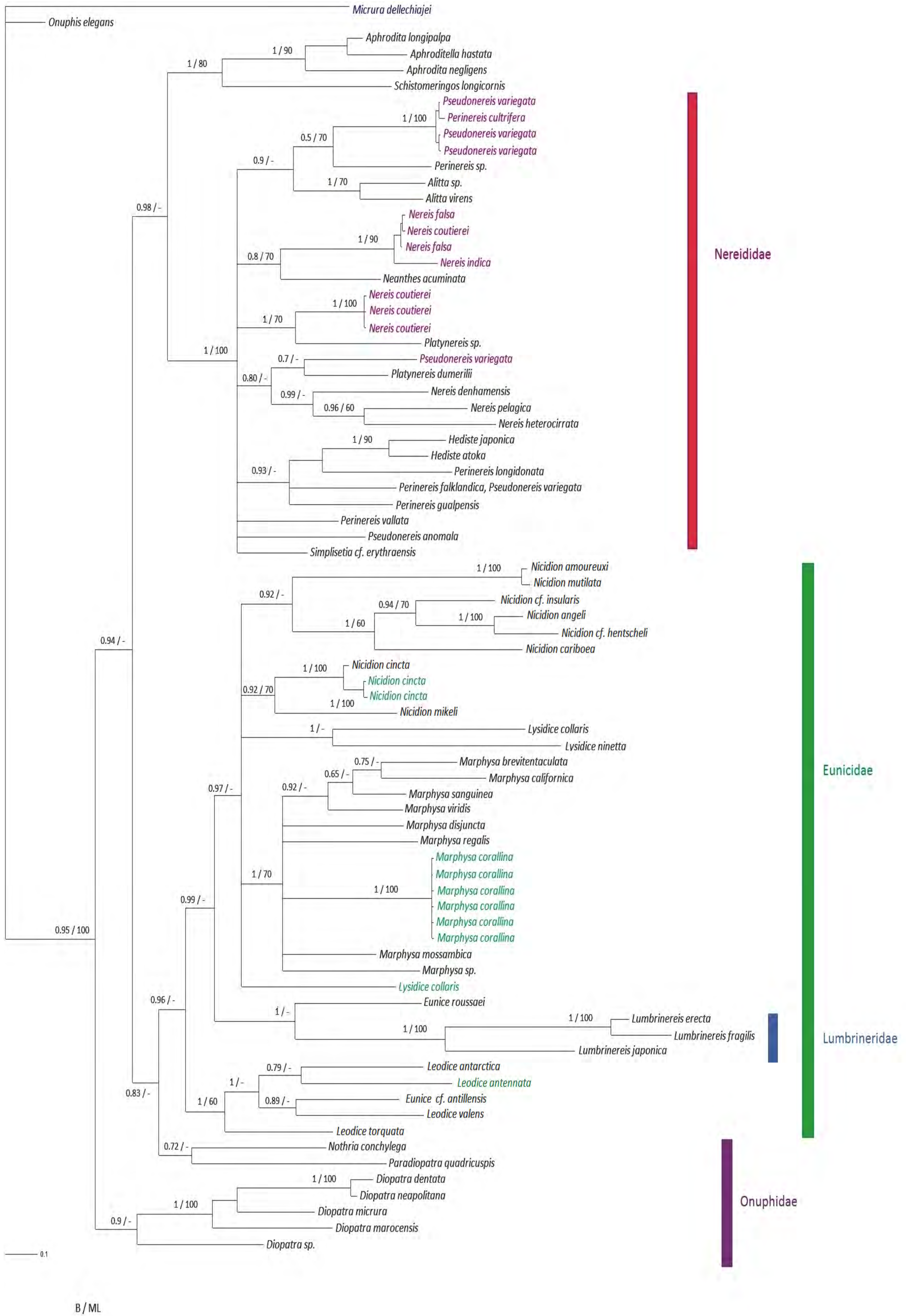


Figure 2.4: Bayesian 50% majority-rule consensus tree of Polychaete worms. Families Eunicidae (Green) and Nereididae (pink) were collected from South Africa. Support for clades: Bayesian and Maximum Likelihood (%).

2.4. Discussion

The results from the present phylogenetic study have not recovered a monophyletic Eunicidae family based on the placement of Lumbrineridae and Onuphidae species nesting within Eunicidae. The larger eunicid clade groups as a sister group to the Onuphidae. This result is consistent with several other phylogenetic studies. Zanol et al. (2014) recovered a moderately supported monophyletic Eunicidae clade that formed a sister clade to Onuphidae using four different genes. Struck et al. (2006) obtained similar results when using 16 taxa in his analysis, however, when he included 43 taxa he found that Onuphidae was nested within a monophyletic Eunicidae. The nesting of Onuphidae within Eunicidae was also observed in the present study where *Paradiopatra quadricuspis* and *Nothria conchylega* (Figure 2.4) were placed as a moderately supported sister clade to *Leodice*. Eunicidae species do not share any synapomorphies with Onuphidae. These include distinct frontal lips and well developed ceratophores at the base of the antennae and palp. As a result the nesting of Onuphidae within a monophyletic Eunicidae is considered uncertain (Struck et al. 2006). Nonetheless, the grouping of Onuphidae as a sister clade to Eunicidae observed in this study is a more plausible hypothesis. Nereididae formed a sister clade to the Aphroditoidea and had strong Bayesian support. Aphroditoidea assumed a more basal position on the tree and thus could have diverged from the last common ancestor first. Alternatively, it should be noted that a limited number of polychaete families were included in this analysis and the grouping of Aphroditoidea as a basal clade could be a consequence of incomplete taxa analysis.

The eight currently valid genera belonging to Eunicidae were analysed by Zanol et al. (2014). Their study revealed a monophyletic *Palola* and *Euniphysa,* a polyphyletic *Marphysa* and *Eunice* and a paraphyletic *Lysidice* with *Nematonereis* nested within it. *Palola, Euniphysa* and *Nematonereis* were not analysed in the present study as no specimens were obtained in the field. The remaining two genera *Nicidion* and *Leodice* were previously invalid but were resurrected in Zanol et al (2014). Five of the eight genera in the Eunicidae investigated in Zanol et al. (2014) have been included in the

present analysis; *Eunice, Lysidice, Marphysa, Leodice* and *Nicidion*. *Eunice* and *Marphysa* in the present study were observed to be polyphyletic which is consistent with the results presented by Zanol et al. (2007; 2010; 2014). Fauchald (1970) and Orensanz (1990) divided *Marphysa* into informal groupings based on the morphology of the chaetae present. Individuals placed into the *sanguinea* grouping include *M. sanguinea, M. viridis, M. brevitentaculata, M. californica* and *M. regalis* (Fauchald 1970; Orensanz 1990; Glasby & Hutchings 2010). Results from the present study recovered this *sanguinea* group but *M. regalis* has been placed out and its relationship is unresolved (Figure 2.4). Nonetheless, Zanol et al. (2010; 2014) found that the *sanguinea*-like individuals grouped into a separate clade and assumed a basal position to the *aenae* group, thus, the observed groupings in the present study are congruent with their results.

The *aenae* group described by Glasby & Hutchings (2010) includes *Marphysa corallina* and *M. disjuncta* also sampled in this study, among other individuals, and has been found to form an exclusive well supported clade (excluding *M. disjuncta*). *Marphysa disjuncta* has been placed outside the *aenae* grouping and its relationship is also unresolved. In the phylogenetic study conducted by Zanol et al. (2014), *M. disjuncta* grouped into the *sanguinea*-like morpho-group but presented as a polytomy. Other studies investigating the phylogeny of Eunicidae have not included *M. corallina* in their analyses making it difficult to draw comparisons with the present findings. Regardless, Zanol et al. (2010) found the formation of another morpho-group described for the genus, the *belii*-group. These informal morpho-groups described by Glasby & Hutchings (2010) should be taken into consideration. It is proposed that a large scale study be carried out to include all the morpho-groups to determine whether they should each be assigned the status of genus or remain as different lineages.

The recently resurrected *Nicidion* was recovered in the present study and comprises of two clades. *Nicidion amoureuxi, N. mutilata, N. cf insularis, N. angeli, N. cf hentscheli* and *N. cariboea* all comprise of a moderately supported clade (Figure

2.4). However, the type species: *N. cincta* described for this genus grouped into a sister clade to the above-mentioned group comprising of *N. mikeli* and *N. notata.* Previously, *Nicidion* was described based on the absence of branchiae but it was decided that this character was not appropriate in defining a genus thus *Nicidion* was considered obsolete (Zanol et al. 2010). Nonetheless Zanol et al. (2014) resurrected the genus and revised it to include branchiae and abranchiate species such as those previously belonging to *Marphysa* and *Eunice* and species possesing dark subacicular hooks such as *N. mutilata* and *N. cincta*. Thus our results strongly support the resurrection of *Nicidion* as the species described for the emended genus in Zanol et al. (2014) have all been analysed in the present study and group into two moderately supported *Nicidion* clades.

Leodice was recovered and represents a strongly supported clade in the present analysis (Figure 2.4). *Leodice antennata* is the type species for the genus and groups into an exclusive clade with *L. antarctica, L. cf antillensis, L. valens,* and *L. torquata*. Previously, diagnostic characters for *Leodice* were not described properly and many of the characters were indistinguishable from *Eunice.* As a result *Leodice* was synonymized with *Eunice* (Zanol et al. 2007; 2010; 2014). Despite this, many studies have constantly recovered a separate clade containing *E. antennata* together with other *Eunice* species (Zanol et al. 2007). This led to the resurrection and revision of the genus to include those species with regularly articulated prostomial appendages; tridentate true-compound falcigers and subacicular hooks (Zanol et al. 2014). Within *Leodice, L. cf antillensis, L. torquata* and other *Leodice* species have constantly grouped together as a sister clade to the main clade containing the type species for this genus (*L. antennata*), thus it has been considered that it may represent a separate genus upon further analysis (Zanol et al. 2014). The present results revealed a similar pattern where *Leodice cf antillensis* grouped together with *Leodice valens* in a separate clade to the type species clade with moderate to low Bayesian support. The outer node support for the two clades as sister clades had strong Bayesian support. Thus it may well represent a separate genus. Species described for the emended genus *Leodice* in Zanol et al. (2014)

have been analysed in the present study and form a strongly supported clade, thus, my results support the resurrection of *Leodice.* In addition, my results support the hypothesis proposed by Zanol et al. (2014) regarding the splitting of *Leodice* due to the constant recovery of two separate clades within this genus. As a result more studies including a large number of taxa from the genus *Leodice* are warranted to determine whether these clades are actually separate genera or just separate lineages.

Lysidice collaris examined in this study was placed as a sister taxon to the exclusive *Marphysa* clade and not among the *Lysidice* clade itself and was well supported by Bayesian analysis (Figure 2.4, p.44). During the growth stages of eunicid polychaetes, it has been found that there are many irregular stages of development of the prostomial appendages (Fauchald 1992a). The discontinuous development of the appendages is found to correlate with a different genus and thus have been named accordingly (Fauchald 1992a; Parra-Carrera & Salazar-Vallejo 1998). *Marphysa sanguinea* is an example of a eunicid species that has three developmental stages corresponding with a different stage of prostomial appendage development (Prevedelli et al*.* 2007). The first is the nematonereis stage where at 18 chaetigers one antenna develops. The second is the lysidice stage where approximately 20-30 chaetigers and two antennae develop. The last is the amphiro stage where at 60 chaetigers; the last pair of antennae develops (Prevedelli et al*.* 2007). Taking this into consideration, a plausible explanation would be that *L. collaris* was misidentified. It could have represented a juvenile in the lysidice developmental stage of a species belonging to *Marphysa* that would have resulted in its placement as a sister taxon to the strongly Bayesian supported *Marphysa* clade. To support this claim, the sequence was blasted in GenBank and the most similar sequence found with 97% query cover was that of *Marphysa sanguinea.*

The genera belonging to family Nereididae, analysed in the present study, all belong to the subfamily Nereidinae (Baken & Wilson 2005). Subfamily Nereidinae includes all individuals possessing hardened structures on their pharynx termed paragnaths (Glasby 1999; Bakken & Wilson 2005). The monophyly of Nereidinae has been

recovered and the synapomorphy for the group is a conical shaped paragnath on the maxillary ring and oral ring (Bakken & Wilson 2005). In the present study, the Nereidinae have a well-supported monophyletic clade congruent with that previously reported for the group. However, relationships between the genera and their respective species seem to be complex.

Within the Nereidinae, the assignment of species to genera is based on the number, morphology and arrangement of paragnaths present on the pharynx (Glasby 1999; Bakken & Wilson 2005). In a study conducted by Bakken (2007) the *Pseudonereis* genus with the unique character of closely spaced conical paragnaths in pectinate-like rows on the pharynx represents a monophyletic group. In my study *Pseudonereis* is a polyphyletic group with *Perinereis cultrifera* nested within it forming a well-supported clade with *Perinereis sp.* placed as a sister taxon to the clade.

In a revision by Bakken (2007) of *Pseudonereis* the shield shaped bar paragnaths present on area VI (Figure 2.1, p.29) on the pharynx was shared by both *Perinereis* and *Pseudonereis.* As a result, *Perinereis cultrifera* could have been a misidentification that could actually be a species belonging to *Pseudonereis* due to homoplasy. Alternatively, it could be that *Perinereis cultrifera* was identified correctly and that *Pseudonereis* was not a monophyletic genus due to the strong bayesian and ML support of this grouping. In addition, the grouping of *Perinereis* sp. as a sister taxon to the clade supports the idea that *Perinereis cultrifera* was most likely a correct identification. Nonetheless, the former is a more plausible explanation as the support for *Perinereis sp.* as a sister taxon to the *Perinereis-Pseudonereis* clade was weak. Strikingly, an individual of *Pseudonereis variegata,* sampled in the present study, grouped into a clade with *Platynereis dumerilii*. This could possibly represent a misidentified species, however, due to weak support, this grouping was not considered.

Two other species of *Pseudonereis* (sequences obtained from Genbank, Table 2.2, p.38)

grouped out of the *Pseudonereis* group, with one *Pseudonereis variegata* sharing a haplotype with *Perinereis falklandica,* thus placing it on the same branch and *Pseudonereis anomala* grouped as a polytomy. Due to the large number of species described for many of the genera within the subfamily Nereidinae, a majority of the genera have been split into informal groups (Bakken & Wilson 2005). The unresolved placement of *Pseudonereis anomala* in the larger Nereididae/Nereidinae clade could be attributed to the fact that it possibly resembles its own informal grouping based on the unique character found for this species: notopodial homogomph falcigers and conical shaped paragnaths on area VI of the pharynx (Bakken & Wilson 2005). Due to the unresolved placement of species, this explanation cannot be validated and a larger number of *Pseudonereis* species need to be included to determine whether the suggested informal grouping is viable. It is assumed that *P. variegata* (obtained from Genbank, Table 2.2 p. 38) could probably be a misidentification as it shares a haplotype with *P. falklandica* (Glasby 1999; Bakken & Wilson 2005).

Nereis is one of the largest and most speciose genera within the Nereidinae (Glasby 1999; Bakken & Wilson 2005). It contains approximately 150 described species split into several informal groups (Bakken & Wilson 2005). From the present study *Nereis* has been split into two separate groups with the first resulting in a moderately supported sister grouping with *Neanthes acuminata* and the second a well-supported sister group to *Platynereis* sp. A large number of species previously described in *Neanthes* have been observed to nest among various *Pseudonereis* species (Bakken 2007; Bakken & Wilson 2005). This led to the revision and consequent placement of some *Neanthes* species in *Pseudonereis* because of initial misidentifications (Bakken 2007; Bakken & Wilson 2005). In the phylogeny conducted by Bakken & Wilson (2005) *Neanthes* was found to be a paraphyletic genus and many species of *Nereis* were found nested within a clade containing species belonging to *Neanthes* and *Perinereis,* which is also evident in the current study. These three genera have been found to possess a pointed bar paragnath on area VI next to the jaw with small conical shaped paragnaths on areas VII and VIII (Figure 2.1, p.29) (Bakken & Wilson 2005). Regardless, it should be

noted that the *Nereis* grouping with *Neanthes acuminata* has moderate and weak support for bayesian and maximum- likelihood analyses and will not be considered.

The well supported *Nereis coutierei* clade formed an unusually strongly supported clade with *Platynereis* sp. This is strange because *Platynereis* is known to be a distantly related genus to *Nereis* which is known for unique pectinate paragnaths (Bakken 2007). The present results are thus inconclusive and more studies need to be conducted to include a large number of taxa from both these genera in order to determine whether they are closely related.

In conclusion, monophyly was not obtained for Eunicidae from the present study due to the nesting of Lumbrineridae and Onuphidae within this clade. Genera within Eunicidae were observed to be polyphyletic. Eunicidae was also found to be a monophyletic family from previous studies. The polyphyletic status of genera *Marphysa* and *Eunice* are consistent with previous results obtained from other studies. *Lysidice collaris* sampled in this study is assumed to be a misidentification due to its placement as a sister taxon to the exclusive *Marphysa* clade. The misidentification could be a result of the discontinuous peristomial appendage development during the growth stages evident for this family. Misidentifications of this sort are fairly common as the appendage growth stages mirror that of the different genera found in the Eunicidae family (Fauchald 1992a). *Lysidice collaris* is therefore assumed to be a juvenile of a *Marphysa* species justified by its position on the tree. *Nicidion* and *Leodice* represent recently resurrected and emended genera. Both have been recovered in the present study thus providing strong support for these revisions. Monophyly was recovered for the Nereididae, subfamily Nereidinae in the present analysis but relationships between the speciose genera within this subfamily are intricate and complicated. This could be attributed to the large number of homoplastic paragnath characters observed which overlap in numerous genera. Also a large number of species belonging to family Nereididae are known to be cryptic species (Glasby 1999; Bakken & Wilson 2005). As a result the numerous genera need to be revised and re-described

using characters other than paragnath morphology in order to identify genus level and species level relationships in the sub-family Nereidinae. Thereafter species need to be investigated to separate out cryptic species.

Chapter three: Genetic structure and demographic history of *Marphysa corallina* (Annelida: Eunicidae) from South Africa

3.1. Introduction

Many natural systems, marine and terrestrial, are modified to some extent by anthropogenic influences, thus, understanding the patterns of connectivity and demographic history of taxa is essential knowledge that can be used to manage these systems successfully (Lowe & Allendorf 2010; Kelly & Palumbi 2010; Ayata et al. 2010). In the marine environment, the genetic connectivity of populations is quantified by the dispersal of larvae, juveniles, adults and the successful settlement of recruits (Palumbi 1994; Grosberg & Cunningham 2001; Palumbi 2003; Kusumo et al. 2006; Hellberg 2009; Weersig & Toonen 2009; Kelly & Palumbi 2010; Skillings et al. 2010; Villamor et al. 2014; Kamel et al. 2014). However, the individual movement of larvae is difficult to track in such a vast body of water where passive larvae are influenced by oceanic currents and can travel for thousands of kilometres at a time (Palumbi 1994; Grosberg & Cunningham 2001; Hellberg et al. 2002; Palumbi 2003; Lowe et al. 2009; Marko & Hart 2011, Selkoe & Toonen 2011). Therefore, population genetic tools are indirect methods that are commonly used to detect the genetic trail left behind by migrating individuals (Palumbi 1994; Grosberg & Cunningham 2001; Hellberg et al. 2002; Palumbi 2003; Lowe et al. 2009; Marko & Hart 2011, Selkoe & Toonen 2011).

Populations that have a constant influx of genetic material tend to be more genetically diverse and as a result these populations are at a lower risk of becoming extinct as opposed to populations that are genetically isolated (Lowe et al. 2009; Lowe &

Allendorf 2010; Bijma 2011; Sexton et al. 2011; De Jong et al. 2011; Peery et al. 2012; Hobbs et al. 2013). This is because a low genetic diversity indicates that populations are not equipped with a diverse gene pool to allow for adaptation to changing environments, whereas those populations that exhibit high genetic diversities are most likely to adapt and thus survive (Lowe et al. 2009; Bijma 2011; Sexton et al. 2011; De Jong et al. 2011; Peery et al. 2012; Hobbs et al. 2013).

The majority of the marine taxa are characteristic of having a pelagic larval stage during their life cycle (Palumbi 1994). This pelagic stage and the duration that the larvae stay in the water column allows for greater or lesser dispersal in the ocean (Palumbi 1994; Grosberg & Cunningham 2001; Palumbi 2003; Weersig & Toonen 2009; Larsson 2009; Reece et al. 2010; Kelly & Palumbi 2010; Guzman et al. 2011). It has been concluded that populations which have long lived planktonic stages are likely to be highly connected to one another and have wider distributional ranges, functioning as panmictic populations that have shallow genetic structuring (Palumbi 1994; Grosberg & Cunningham 2001; Palumbi 2003). On the other hand, organisms exhibiting short pelagic larval stages have larvae that do not travel such distances (Palumbi 1994; 2003; Kelly & Palumbi 2010). These populations accumulate genetic differences enabling them to diverge from one another causing them to function as independent self-breeding/ self-recruiting populations resulting in genetically well-structured populations across the ocean (Palumbi 1994; Grosberg & Cunningham 2001; Palumbi 2003; Kelly & Palumbi 2010).

With the advent of cheap molecular techniques a large number of population genetic studies have been conducted on different marine taxa. These studies had found that contrary to the theory that high dispersal potential leads to genetic homogeneity, numerous marine species form genetically well-structured populations regardless of whether they have high dispersal potentials (Uthicke & Benzie 2003; Marko & Hart 2011; Kamel et al. 2014). For example, McGovern et al. (2010) conducted a study to assess the genetic structure of two invertebrate species, the bat star *Patiria miniata*

and the frilled dogwhelk snail *Nucella lamellose;* occupying the same region with contrasting larval biology. The bat star has a long lived planktonic larval stage whereas the dogwhelk snail lacks a planktonic larval stage (McGovern et al. 2010). The gene flow analysis revealed that populations of the bat star were differentiated from one another displaying structured populations whereas the dogwhelk snail displayed high genetic connectivity and no genetic structure. Due to these contradicting results, a theory of recent colonisation was then analysed using nuclear loci and revealed that the bat star populations represented evolutionarily old populations which over time had led to well-structured populations (McGovern et al. 2010). The dogwhelk snail represented populations that were relatively young and thus had not accumulated genetic differences over a short evolutionary time (McGovern et al. 2010; Marko & Hart 2011). In contrast to this study, many recent investigations on shallow water marine invertebrates have revealed that populations with high dispersal abilities are genetically structured such as those displayed by the broadcast spawner species of the genus *Palola* (Schulze 2006). As a result the use of population genetic techniques together with phylogenetic trees in a geographical context; known as phylogeography; provide a very powerful tool to uncovering the contemporary genetic structure of taxa and historical demographic events that have occurred (Bohonak 1999; Silva & Russo 2000; Mila et al. 2000; Grosberg & Cunningham 2001; Zink 2002; Plouviez et al. 2009; Kelly & Palumbi 2010; Marko et al. 2010; Marko & Hart 2011; Kamel et al. 2014).

Phylogeographic studies draw knowledge from a variety of disciplines such as population genetics, ecology, phylogenetic systematics, geology, and palaeontology in order to make plausible conclusions for the spatial distribution and structuring of populations (Avise 2000; Zink 2002; Duran et al. 2004). Dispersal mechanisms and vicariant events are two fundamental theories used for the explanation of differentiated and connected populations over spatial and geological scales (Palumbi 1994). When lineages occupy present day distributional ranges, the theory of the dispersing passive or active larvae are used to interpret these distributions whereas if populations have undergone genetic differentiation, the vicariant event theory is used

to determine how and why these populations have accumulated mutational differences (Avise 2000).

Avise et al. (1987) characterised four phylogeographic structures that natural populations could represent: Type 1 are those populations that have deep haplotype trees and are geographically well structured, Type 2 are those populations that are not structured but have deep haplotype trees, Type 3 are those populations that exhibit a shallow haplotype tree but are genetically structured and lastly Type 4 are those populations that have a shallow haplotype tree and no genetic structure. Types 1 – 3 are characterised by vicariant events where populations become separated and undergo genetic drift due to a geological or ecological barrier and begin to accumulate mutations as a result (Avise et al. 1987). Type 4 populations produce star-like haplotypes and represent populations with either continuous gene flow (dispersal), relatively recent expansions from bottlenecked populations or range-wide selective sweeps (Avise et al. 1987; Nielson 2005; Lowe et al. 2009). In a range-wide selective sweep a favoured haplotype is selected for and consequently spread across the distributional range of the species (Avise et al. 1987; Rogers 1995; Mila et al. 2000; Nielsen 2005; Lowe et al. 2009; Mirol et al. 2008).

Polychaete worms are a characteristically unique class among the metazoans for exhibiting a wide range of reproductive mechanisms (Fauchald & Jumars 1979; Levin 1984; Wilson 1991; Giangrande 1997; McHugh & Rouse 1998; Gambi & Cigliano 2006; Prevedelli et al. 2007; Malathi et al. 2011). Amongst the families and orders of the Polychaete clade, 18 reproductive modes have been identified (Wilson 1991; Giangrande 1997). According to Wilson (1991), closely related species generally do not exhibit such a variety of reproductive mechanisms. The reproductive plasticity of these marine worms enables them to successfully colonise new habitats, ultimately resulting in their tremendous success in the marine environment (Wilson 1991; Prevedelli et al. 2007).

Eunicids are a diverse group of polychaete worms that are found in a variety of habitats (Sorokin 1995; Struck et al. 2006; Zanol et al. 2014; Kurt Sahin 2014). They are also known to be detrimental to reefs by burrowing in to them (Fauchald & Jumars 1979). The epitokes (a sexually mature life stage filled with gametes) of the *Palola* worms (Eunicidae) are consumed by the natives inhabiting the South Pacific Islands (Schulze 2006). *Diopatra aculata*, *Marphysa mulawa* and *Marphysa sanguinea* are used as bait for recreational fishing in a large number of regions such as the United States, Japan, Mediterranean Coast and Australia (Hutchings & Karageorgopolous 2003; Lewis & Karageorgopolous 2008). Polychaete biomass is used in the feed of many finfish and crustacean aquaculture industries (Struck et al. 2007), making them a culturally and commercially important polychaete group.

Marphysa is one of eight valid genera within the family Eunicidae and is considered to be speciose with approximately 79 described species (Glasby & Hutchings 2010; Zanol et al. 2014; Katsiaras et al. 2014; Kurt Sahin 2014). The species are known to have a worldwide distribution inhabiting temperate and tropical seas, estuaries, rocky intertidal areas and ocean depths of 200 m and have a variety of feeding habits ranging from herbivory to omnivory (Fauchald & Jumars 1979; Sorokin 1995; Huthcings & Karageorgopoulos 2003; Lewis & Karageorgopoulos 2008; Glasby & Hutchings 2010; Hutchings 2012). Diagnostic characters such as the presence of 5 antennae and the absence of peristomial cirri, define *Marphysa* (Glasby & Hutchings 2010; Katsiaras et al. 2014; Kurt Sahin 2014). Fauchald (1970) divided *Marphysa* into 4 morpho-groups based on the morphological variation of the chaetae found in the neuropodia (lower bundle). These groups are named according to the oldest species described for that particular variation in chaetae. Group A is the Mossambica group consisting of limbate chaetae only, Group B is named the Sanguinea group and is characterised by compound spinigerous chaetae, Aeana group is Group C and has compound falcigerous chaetae only and lastly group D which is known as the Belli group is represented by both compound falcigerous and spinigerous chaetae (Fauchald 1970). Glasby & Hutchings (2010) described a 5th grouping called the Teretiuscula group which are characterized

by compound spinigerous chaetae and limbate capillaries. Unfortunately, many taxonomists have based some descriptions of species belonging to *Marphysa* on the juvenile stages, thus they have incorrectly identified and named species due to a lack of prostomial appendages at that developmental stage (Kurt Sahin 2014).

Marphysa coralline, a burrowing eunicid, (species of interest in the present study) has been classified by Day (1967) to have a tropical indo-west distribution. Taxonomically, *M. corallina* is characterized by having a bilobed prostomium, 5 antennae, a pair of reniform eyes, branchiae present throughout the body, absence of peristomial cirri, rounded prostomium with a flattened body and tapering pygidium (Day 1967). The type locality of this species is the Senegalese and Hawaiian exclusive economic zones (WoRMS 2015). There have been records of *M. corallina* from Mozambique, Madagascar, New Zealand, the Red Sea, Kahului, Australia, Marshall Islands, Lakshadweep Island and the Jaluit Atoll (Day 1967). *Marphysa corallina* is a common burrowing eunicid found along the KwaZulu-Natal and Eastern Cape coast of South Africa. Burrowing polychaetes are known to aerate anoxic sediments thus contributing to a healthier environment for other invertebrate species (Dean 2002). As a result, *Marphysa corallina* is considered to be an ecologically important species.

There are no known studies that have investigated the life history traits of *Marphysa corallina*. However, the reproductive cycles of closely related species, such as *Marphysa sanguinea, Marphysa gravelyi, Marphysa borradailei* and *Marphysa fauchaldi* have been studied. It has been found that *M. sanguinea* is an iteroparous, gonochoric species that spawns in synchrony at the population level (Prevedelli et al. 2007; El Barhoumi et al. 2013). *Marphysa sanguinea* produces lecithotrophic larvae that spend approximately 60 hours in the water column before hatching (Prevedelli et al. 2007; El Barhoumi et al. 2013). Once the juveniles have hatched they settle to the bottom where they create tubes made of mucus and sediment and live out the rest of the summer (Prevedelli et al. 2007; El Barhoumi et al. 2013). The anterior region of the worm begins to develop musculature at the end of summer allowing the worm to

burrow into the sediment where it will live permanently (Prevedelli et al. 2007; El Barhoumi et al. 2013). *Marphysa gravelyi*, *M. fauchaldi* and *M. borradailei* are characterized by producing embryos encapsulated in jelly sacs on the benthos (Malathi et al. 2010). Various developmental stages occur in this jelly mass for approximately 84 hours, after which lecithotrophic larvae are produced and consequently settle on the sediment (Malathi et al. 2010). As a result species belonging to *Marphysa* that have been studied thus far are known to exhibit short lived and lecithotrophic larvae (Schulze 2006), leading to the assumption that *M. corallina* also exhibit the same larval biology.

3.1.1. Aim of the study

To date, there have been no population genetic studies done on *Marphysa corallina.* It has been reported that polychaete worms in particular, regardless of larval biology, have genetically well-structured populations. *Marphysa corallina* was observed to be a common burrowing worm on the rocky shore communities of KwaZulu-Natal and Eastern Cape.

As a result, the aim of this study is to determine whether populations of *Marphysa corallina* found on the intertidal rocky shores of KwaZulu-Natal and Eastern Cape are (a) genetically differentiated from one another and (b) to assess the past demographic events that have shaped the contemporary patterns of this species' distribution in South Africa.

3.2. Materials and Methods

3.2.1. Study sites and sample collection

To determine the population structure and connectivity of *Marphysa corallina* found along the East Coast of South Africa, samples of *M. corallina* were collected over a one year period (June 2013 – April 2014, Table 3.1). A total of 220 specimens of *M.corallina* were collected from 8 sites across two regions. From KwaZulu-Natal, 6 sites were sampled, namely: Clansthal, Green Point, Reunion Rocks, Ballito, Adlams and Mabibi and from the Eastern Cape region 2 sites were sampled: Mgazana 1 and Mgazana 2 (see Table 3.1, Figure 2.2 p.31). Adlams and Mabibi beach are located near Sodwana Bay. Mabibi and Adlams are separated from one another by an extensive sandy flat and patches of rocky shore. Adlams and Ballito are separated by patches of rocky shore and sandy flats and Ballito and Reunion Rocks are separated by sandy and rocky shores.

Specimens of *M. corallina* were found in mucus-sand tubes in algal mats inundated with sand, under worm rock and bait rock on the intertidal rocky shores at the respective sampling sites. A crow bar was used to lift up rocks and an oyster knife was used to break off worm rock from hard substrates. Polychaete worms have delicate soft bodies and therefore to prevent losing body parts and breaking specimens a tweezer was used to carefully pick out the encrusting worms.

Individuals of *M. corallina* were identified in the field by the white tapering ends on their antenna with the rest of the antenna a light brown colour and bilobed palps that form the prostomium. Worms were put into individual bags of sea water and taken to the laboratory for processing. In the laboratory, *M. corallina* individuals were anesthetized with 10 % $MgCl_2$ for approximately 30 minutes. Thereafter they were rinsed with distilled water to remove salt and preserved in 70 % molecular grade ethanol for morphological analysis and DNA extraction.

Table 3.1: Summary of collection data of *Marphysa corallina* collected at 8 different sites, the geographic co-ordinates of sample sites, the dates of collection, the number of morphological ID's, COI and ITS1 individuals sequenced.

Species	Area Collected	Co-ordinates	Date collected	ID's	CO1	ITS
Marphysa corallina	Clansthal, KZN	30°14'10.08"S 30°47'18.21"E	22 June 2013	47	25	24
	Green Point, KZN	30°15'0.61"S 30°46'55.91"E	23 June 2013	52	23	22
	Reunion Rocks, KZN	29°59'11.49"S 30°57'51.00"E	13 June 2013	22	14	14
	Mgazana 1, EC	31°42'19.10"S 29°24'49.19"E	9 July 2013	20	20	9
	Mgazana 2, EC	31°41'6.27"S 29°26'20.37"E	10 July 2013	13	9	-
	Ballito, KZN	29°32'23.16"S 31°13'25.90"E	31 January 2014	28	25	11
	Adlams, KZN	27°37'28.34"S 32°39'22.52"E	30 April 2014	21	22	-
	Mabibl, KZN	27°25'49.06"S 32°42'51.58"E	29 April 2014	17	16	-
				220	154	80

3.2.2. Morphological analysis

Polychaete worms are among the most common and abundant marine organisms that inhabit benthic environments (Fauchald & Jumars 1979). They are characterised by their multi-segmented bodies, which are divided into three distinct regions, the prostomium, metastomium and pygidium. The prostomium forms the anterior region which makes up the "head" of the polychaete worm (Day 1967; Fauchald 1977). The prostomium bears a wide variety and arrangement of sense organs which depending on function are known as antenna, palps and nuchal organs (Day 1967; Fauchald 1977). The presence, absence, position and arrangement of these sensory organs and the

overall shape and size of the prostomium are very important for family and genus level identifications (Day 1967; Fauchald 1977). For predatory polychaete worms such as those belonging to the Eunicidae, the arrangement of teeth and the structure of jaws are used for generic identification (Day 1967; Fauchald 1977).

The metastomium forms the body which is made up of numerous segments each consisting of a pair of parapodia (Day 1967; Fauchald 1977). Depending on the genus, the parapodia are either biramus, which is the formation of a dorsal nopodium and ventral neuropodium, or uniramus which consists of a single continuous lobe (Day 1967; Fauchald 1977). These rami consist of a chaetigerous lobe and comprise bundles of chitonous chaetae. These chitonous chaetae are highly important structures as they do not change upon preservation and depending on their position and morphology, are used for the identification of different species (Day 1967; Fauchald 1977). The branchiae are respiratory organs found on various parts of the body and depending on family and genus; they could be found concentrated in a bundle on the head region or as individual filaments on the dorso-lateral parts of the body (Day 1967; Fauchald 1977). The pygidium forms the posterior region of the body and forms the anal region (Day 1967; Fauchald 1977). According to Day (1967) this region is not of great importance for identification; however, the morphology of the pygidium differs between families and genera and is occasionally used.

The above mentioned characters were viewed using a Nikon Az1000 AS stereo microscope and a Zeiss dissecting microscope and were photographed. Due to the chitonous chaetae being transparent in nature, methylated blue dye was used to stain the chaetae in order to identify the different morphological structures. All specimens were identified by myself and were not sent to any specialist. Day's 1967 monographs were used as a guide to identify all specimens.

3.2.3. DNA extraction and amplification

Genomic DNA was isolated from *M. corallina* tissue using the ZR Genomic DNA Tissue MiniPrep extraction kit (www.zymoreasearch.com) according to standard protocol. Approximately 25 mg of tissue was cut from the posterior end of the worm and rinsed with distilled water to remove excess ethanol that could potentially inhibit activity of proteinase-K. Tissue was cut up into tiny pieces to ensure the breakdown of cell walls and maximum yield of DNA. Tissue was placed in eppendorf tubes containing 95 µl of molecular biology grade water, 95 µl of 2X digestion buffer and 10 µl of proteinase-K. Samples were vortexed and incubated in a dry bath incubator overnight at 55° C on a gyro- rocker.

Samples were removed from the incubator the next day and to each eppendorf tube, 700 µl of Genomic Lysis Buffer was added. Samples were vortexed and spun down for one minute at 10,000x *g* to remove insoluble debris. The supernatant was transferred to a Zymo-Spin IIC Column with a collection tube and centrifuged for one minute at 10,000x *g.* DNA pre-Wash buffer with a total volume of 200 µl was added to each Spin Column in a new Collection Tube and centrifuged at 10,000x *g* for one minute. Thereafter, 400 µl of g-DNA Wash Buffer was added to the spin column and centrifuged at 10,000x *g* for one minute. Lastly the spin column was put into a clean eppendorf tube and 100 µl of DNA Elution Buffer was added to each sample and left to incubate for approximately 60 minutes at room temperature after which the tubes were centrifuged at high speed for 30 seconds to allow for elution of DNA. The DNA was then stored in -80° C freezer until PCR was conducted.

The isolated genomic DNA was amplified by the Polymerase chain Reaction (PCR) using the universal mitochondrial primers LCO1490 and HCO2198 (Vrijenhoek 1994) and nuclear ITS1 (pITS-R and pITS-F) primers that were designed from the conserved regions of the 18S and 28S rRNA genes (Sugita et al. 1999). PCR amplifications were conducted using 12.5 µl of EconoTaq® PLUS GREEN 2X Mastermix (Lucigen), 7.82 µl of molecular

biology grade water, 0.84 µl of forward and reverse primer, 1 µl of 10 % Bovine Serum Albumin (BSA) and 2 µl of template DNA to make up a total reaction volume of 25 µl. COI reactions were amplified using a BioRad T100-Thermal Cycler. The PCR thermal cycle conditions for COI was carried out as follows: 95° C for 3 minutes, followed by 35 cycles of 94° C for 30 seconds, 45° C for 30 seconds 72° C for 1 minute, followed by 72° C for 7 minutes and a final cold storage of 12° C. The thermal cycle for the ITS 1 region was conducted as follows: 95° C for 3 minutes followed by 35 cycles of 94° C for 30 seconds, 55° C for 1 minute, 72° C for 2 minutes, followed by 72° C for 10 minutes and 12° C for infinity.

PCR products were run on a 1 % agarose gel (1 g agarose powder and 100 ml of 1X TBE buffer) using 3 µl of PCR product and 3 µl of 100bp ladder. Images were taken of gels using a BioRad Molecular Imager, Gel Doc™ XR+. PCR products were sent to Inqaba Biotechnical Industries (Pty) Ltd, Hatfield, South Africa for sequencing using an ABI 3730 Capillary sequencer.

3.2.4. Genetic analysis

Sequences were individually aligned and edited in BioEdit (v7.0.9.0) (Hall 1999). Nucleotide ambiguities were found at different loci and corrected by searching for the strongest signal on the chromatograms of the respective sequences using the IUPAC ambiguity codes. The edited sequences were aligned using a ClustalW multiple alignment method. The COI sequences were trimmed to a length of 560bp and ITS1 to a length of 876bp. A search for highly similar sequences was conducted using BLAST algorithm in NCBI GenBank, however, this returned no sequences for *M. corallina* itself but did yield sequences for different species of the *Marphysa* for both COI and ITS regions (Accession numbers and species names in Table 3.2).

DnaSp v5 (Librado & Rozas 2009) was used to generate a haplotype data file for both COI and ITS1 sequences for various population genetic analyses and phylogenetic analyses. Diversity indices such as the number of haplotypes (*h*), nucleotide diversity

(π) and haplotype diversity (Hd) were computed in DnaSp. Nucleotide diversity estimates the divergence of sequences between individuals in populations independent of haplotypes whereas haplotype diversity estimates the frequency and number of variants at a locus irrespective of sequence clusters (Lowe et al. 2009). These indices are used to assess the molecular diversity of a species or populations and are used as an estimator for historical demographic events (Lowe et al. 2009). Briefly, low haplotype and nucleotide diversities indicate that populations may have experienced extended or severe bottlenecks. Stable populations with large effective population sizes that persist or historical splits that resulted in mixed samples are characteristic of high nucleotide and haplotype diversities (Lowe et al. 2009).

Table 3.2: COI and ITS1 sequences of various species of *Marphysa* and outgroups (*Limulus polyphemus* and *Carcinoscorpius rotundicauda*) that were used for the phylogenetic tree. Sequences were downloaded from NCBI GenBank and their accession numbers are given.

Species Name	COI	ITS
Marphysa angeli	GQ497550.1	GQ497506.1
Marphysa brevitentaculata	GQ497548.1	GQ497503.1
Marphysa disjuncta	GQ497549.1	GQ497504.1
Marphysa regalis	GQ497562.1	-
Marphysa cf. hentscheli	GQ497551.1	GQ497509.1
Marphysa californica	GQ497552.1	GQ497507.1
Maprhysa sanguinea	GQ497547.1	GQ497502.1 AY038861.1
Marphysa viridis	GQ497547.1	GQ497508.1
Marphysa cf. bellii	-	GQ497511.1
Limulus polyphemus	HQ588747.1	-
Carcinoscorpius rotundicauda	-	U91491.1

Populations exhibiting high haplotype and low nucleotide diversities are indicative of

rapid expansions from small populations with accumulated mutations and lastly, ancestral populations that have undergone short-term bottlenecks display low haplotype and high nucleotide diversities (Lowe et al. 2009).

The generated haplotype data files were used in MrModelTest 2.3 (Nylander 2008) to calculate a best fit model of evolution. The Akaike Information Criterion (AIC) selected the GTR+G model to construct a Bayesian tree using Mr Bayes 3.1.2 (Huelsenbeck & Ronquist 2001), maximum parsimony and neighbour joining trees were contructed using PAUP. *Limulus polyphemus* and *Carcinoscorpius rotundicauda,* both horseshoe crabs (Table 3.2) belonging to the Arthropoda represent distant ancestors of the polychaete group and therefore were used as outgroups to root both the COI and ITS trees respectively. Different species belonging to *Marphysa* from around the world were used to determine evolutionary relationships to *M. corallina* from South Africa.

GenAlex v6.5.1 (Peakall & Smouse 2012) was used to calculate the mean number of alleles across all loci known as the number of alleles (N_a), number of effective alleles (N_e) which is an estimation of the number of equally frequent alleles occurring in an ideal population. The probability that two individuals in a population will be different which is gene diversity (H_e), Shannon's diversity index (*I*) which is a measure of allelic and genetic diversity in a population and the percentage of polymorphic loci across all loci which is percentage polymorphism (%Poly) (Lowe et al. 2009; Peakall & Smouse 2012) were calculated in GenAlex. Diversity indices are used to assess the genetic variability of a species or populations. Populations exhibiting high diversities make them less vulnerable to extinction thus allowing for various adaptations (Lowe et al. 2009). Natural selection, positive, negative or neutral tends to imprint unique signatures on molecular data. As a result varying levels of diversity can be used to determine the type of selection acting on a particular population or species (Nielsen 2005; Lowe et al. 2009).

The genetic partitioning of molecular variation within and between populations of both

COI and ITS markers were determined by an Analysis of Molecular Variance (AMOVA) that was calculated in GenAlex and the efficacy of this method was tested using a significance level of 0.05 run for 1000 permutations. AMOVAs were used to explain the relative subdivision of genetic diversity between the hierarchical levels (Lowe et al. 2009).

A minimum spanning network (MSN) was estimated for both COI and ITS1 using a squared distance matrix among haplotypes in Arlequin v3.5 (Excoffier et al. 2005). An MSN is also known as a molecular-variance parsimony technique because it is computed using the squared distance matrix used to calculate F-statistics in an AMOVA and determines the relationships between haplotypes (Excoffier & Lischer 2010; De Jong et al. 2011). The MSN together with its alternative connection lengths between haplotypes were imputed in HapStar v0.5 (Teacher & Griffiths 2011) which uses a force directed method for easy visualisation of the network. As mentioned above, different evolutionary processes leave a unique signature in genetic data and these signatures can be observed in the patterns assumed by gene genealogies or haplotype networks (Nielsen 2005; Lowe et al. 2009). For example, according to Lowe et al. (2009) if positive selection was acting on a population, it would produce a genealogy that is star shaped with a central ancestral haplotype that has short terminal branches. A selective sweep would have wiped out all previous polymorphisms and fixed all populations with advantageous mutations (Nielsen 2005; Lowe et al. 2009). Past demographic events such as bottlenecks, rapid population expansions and population subdivision can also be detected by observing the patterns a gene genealogy adopts (Mirol et al. 2008; Lowe et al. 2009; De Jong et al. 2011).

Pairwise F_{ST} was calculated for pairs of populations in order to determine the genetic distances between populations for both COI and ITS1 sequences (Excoffier & Lischner 2010). A significance level of 0.05 was used to test the calculated genetic distances for 16000 permutations using Arlequin v3.5.

Tests of selective neutrality are used to determine whether populations are evolving

under neutral mutational-drift equilibrium or whether they are evolving under non-neutral processes associated with directional or balancing selection and demographic expansion and contraction (Fu 1997; Ramos-Onsins & Rozas 2002). Tajima's test of selective neutrality is based on the frequency distribution of alleles of segregating nucleotide sites and is used to test population expansions and bottleneck events (Tajima 1996; Aris-Brosou & Excoffier 1996). Positive values of this test suggest that populations contain an abundance of intermediate alleles whereas a negative value indicates that populations have an abundance of rare alleles which is characteristic of a sudden expansion (De Jong et al. 2011; Guzman et al. 2011). Fu's F_S test of selective neutrality uses the distribution of alleles per haplotype and is known to be very sensitive to population expansions yielding large negative F_S values (Fu 1997). The above mentioned tests of neutrality were computed for both COI and ITS1 data in Arlequin v3.5 (p values calculated by 1000 bootstrap replicates).

Mismatch distribution analysis was performed in conjunction with the above mentioned neutrality tests to assess past demographic events and effects of selection on populations (Guzman et al. 2011). This approach uses the distribution of pairwise differences between haplotypes in a sample using the least squares methodology to estimate parameters of sudden population growth (used in this study: Raggedness index, Sum of squared differences and expansion time) (Rogers & Harpending 1992; Rogers 1995; Excoffier & Schneider 1999). Multimodal distributions are characteristic of populations that are in demographic equilibrium whereas populations that have experienced a sudden range expansion with high levels of migration between neighbouring populations display distributions that are smooth or unimodal (Rogers & Harpending 1992). The raggedness index is a measure of the smoothness of the observed mismatch distribution, where large values indicate a multimodal pattern and is observed for stationary populations and smaller values reveal unimodal or smooth distributions which are typical for expanding populations (Rogers & Harpending 1992; Rogers 1995; Excoffier & Schneider 1999). The sum of squared differences (SSD) is used as a test statistic where non-significant values point to population expansions. Estimations of population expansion times were calculated using Tau (τ) = $2\mu t$, where

µ is the mutation rate and t the time of expansion. Tau (t) was calculated in Arlequin and expansion time was estimated with an online mismatch calculator (http://www.uni-graz.at/zoowww/mismatchcalc/mmc1.php) developed by Schenekar & Weiss (2011). A mutation rate of 2.2 % per million years was used for COI calculated by Chevaldonne et al. (2002) and Jolly et al. (2006) for polychaete worms. Where $\tau > 0$, whereas $\tau = 0$ indicates stationary populations (Hurtado et al. 2004). The mismatch distribution together with its population expansion parameters were calculated in Arlequin v3.5 for both COI and ITS markers and the validity of the expansion model was tested using a significance of 0.05 and run for 1600 bootstrap replicates.

In addition to the above demographic analyses, a bayesian skyline plot was estimated using BEAST v1.8.0 (Drummond & Rambaut 2007) and subsequently Tracer v1.6 (Rambaut & Drummond 2011) to estimate the change of effective population sizes through time. The date value was set to default (0), the HKY substitution model (Hasegawa et al. 1985) was used with base frequencies estimated, the site heterogeneity model set to gamma with 4 categories used. A lognormal relaxed clock was used and the tree prior set to Bayesian skyline plot. The prior distributions were set to normal with the mean of 0 and standard deviation of 1. The MCMC runs were set to 10 000 000 with parameters logged every 1000 iterations.

Gene flow is the movement of genes from one population to another and is known to counteract the effects of genetic drift and mutation thus preventing differentiation between populations (Lowe et al. 2009). Gene flow was estimated using the program Migrate-n v3.2.1 (Beerli & Palczewski 2010). Migration rates and effective population sizes are calculated using mutation scaled parameters (Beerli & Palczewski 2010). A custom directional island migration model was used to estimate the directionality of gene flow where migration and theta were both estimated using F_{ST}. A DNA sequence model data type was used with a transition and transversion ratio of 2.00. A Bayesian Inference search strategy was performed and 5000 steps in a chain were computed. Static heating was applied using 4 temperatures. Mutation-scaled effective population

sizes were estimated with the following equation (Beerli & Palczewski 2010):

$$\chi Nm = \theta \times M$$ (Beerli & Palczewski 2010)

Where χ is a multiplier and depends on the ploidy and inheritance ($\chi = 4$ for nuclear data and $\chi = 1$ for mitochondrial data), N_e is the effective population size and μ is the mutation rate per site per generation (Beerli & Palczewski 2010). Thus the number of immigrants per generation was calculated using:

$$\chi Nm = \theta \times M$$ (Beerli & Palczewski 2010)

Where θ is the mutation-scaled effective population size and M is the mutation-scaled effective immigration rate calculated as the immigration rate (m) divided by the mutation rate per site per generation (μ) (Beerli & Palczewski 2010).

The persistence of genetic connectivity between populations is associated with Nm values that are greater than 1. Conversely, Nm values less than 1, with an associated F_{ST} value of >=0.2 indicates that populations over time will eventually diverge from one another and become isolated (Lowe et al. 2009). Two migration models were estimated, a north to southward flow of genes and secondly a south to northward flow of genes. The probability of each model was then calculated using the following equation:

$$\text{Prob (model}_i) = \text{mL model}_i / \Sigma_j^n \text{ mL model}_j$$ (Beerli & Palczewski 2010)

Where, mL model_i is the marginal likelihood of model i divided by the sum of all models computed.

Private alleles are genetic alternatives (new mutations) found in a single population and is commonly used as an indirect estimator of gene flow (Nm) (Hellberg et al. 2002; Lowe et al 2009; Lowe & Allendorf 2010). Populations will generally exhibit a large number of private alleles due to mutations if gene flow between these populations is

particularly low (Lowe & Allendorf 2010). The number of private alleles was computed in GenAlex v6.5.1.

3.3. Results

3.3.1. Morphology

A total of 220 individuals from 8 different sites along the South African coast (Table 3.1) were identified using the diagnostic features mentioned above and were classified as *Marphysa corallina*. They are characterised as having a bilobed prostomium (Figure 3.1B), and 5 antennae each approximately 1.5 times the length of the prostomium (Figure 3.1C). The antenna is smooth and has a white tapering end. A pair of reniform eyes was present on the outer side of the lateral antenna. The anterior end of the worm is rounded thereafter becoming dorso-ventrally flattened posteriorly (Figure 3.1A). Branchiae, depending on the size of the individual are present and begin from approximately the 20-30th chaetiger and reached a maximum length of 1.4 mm (Figure 3.1D). The branchiae usually start as single filaments and reach a maximum of 8 filaments toward the middle of the body thereafter decreasing back to a single filament.

Marphysa corallina belongs to the Eunicdae and is characterised by the possession of Labidognatha jaws, commonly known as pincer jaws (Figure 3.1K). *Marphysa corallina* has uniramous parapodium with both dorsal and ventral cirrus and two bundles of chaetae emerging from a single chaetigerous lobe (Figure 3.1E). Chaetae in the superior position, those closest to the dorsal cirrus are known as the notochaetae and consist of simple winged capillaries and comb chaetae (Figure 3.1H-J). Chaetae found closest to the ventral cirrus are known as the neurochaetae and consist of compound falcigerous chaetae with small guards (Figure 3.1G). Also present are the characteristic true acicula and acicula chaetae. The acicula is attached to the muscle in a unique way in which it will not protrude resulting in “internal skeletal rods” for the parapodia

(Rouse & Pleijel 2001). The acicula chaeta on the other hand is thick and spine-like and project beyond the tip of the parapodia (Rouse & Pleijel 2001). This acicula chaeta is not a true acicula and originates with the other chaetae (Rouse & Pleijel 2001). The acicula generally has a blunt tip and is dark in colour (Figure 3.1F) whereas the acicula chaeta is pale in colour and has a bidentate tip and a small guard (Figure 3.1I).

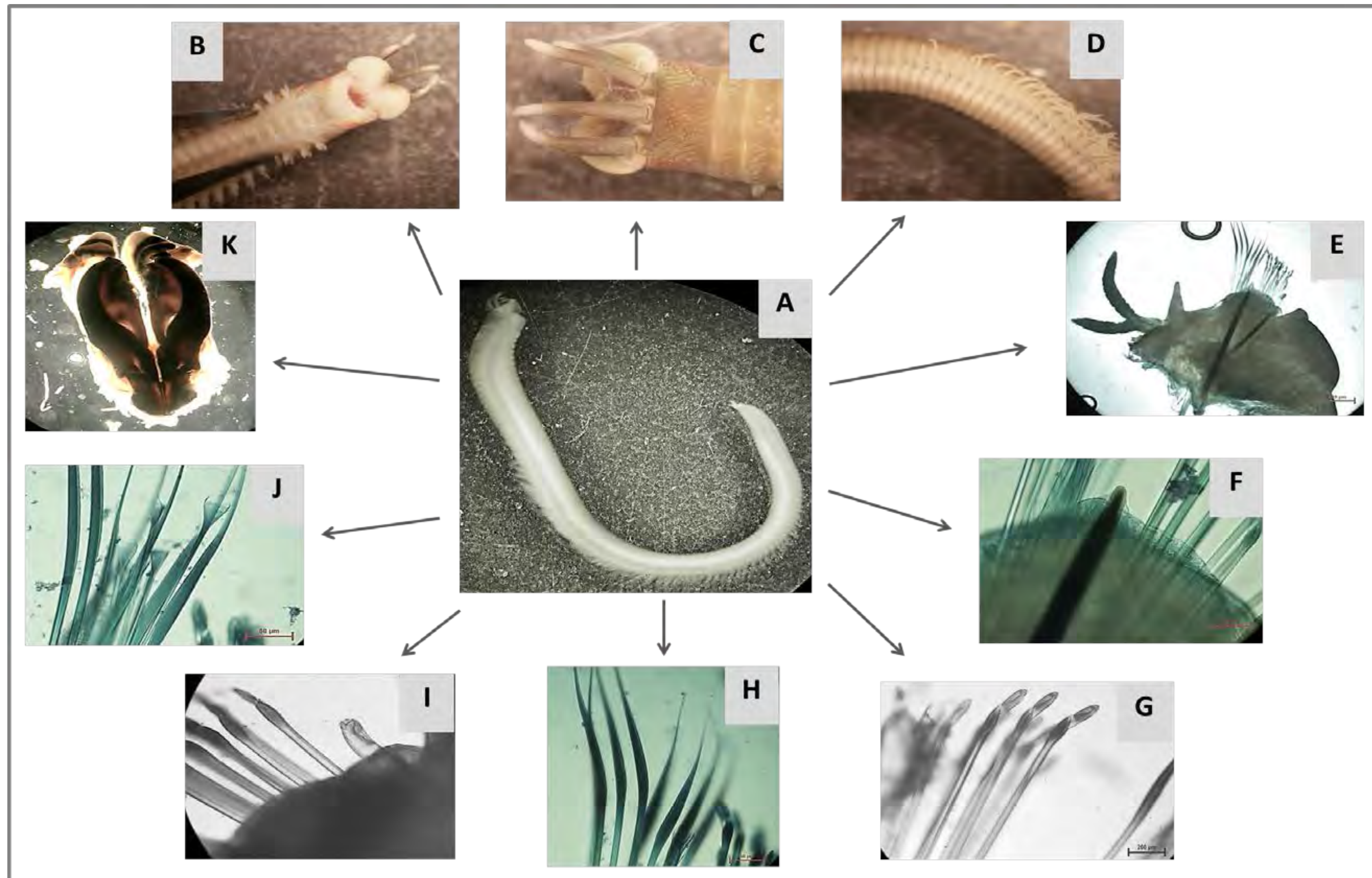


Figure 3.1: Summary of the diagnostic morphological characters used for the identification of *Marphysa corallina*. A – Full body of *M.corallina*, B - ventral view of the bilobed prostomium, C - dorsal view of the prostomium showing the antenna, D – side view of the metastomium showing the branchiae, E – Cross section of the uniramus parapodium, F – Acicula, G – compound falcigerous neurochaetae H – simple winged capilliries (notosetae), I – bidentate acicula chaetae with small guard, J – Comb notoschaetae, K – Labidognath jaw structure.

3.3.2. Genetic analysis

A total of 220 individuals of *Marphysa corallina* inhabiting the rocky shores of South Africa were sampled at 8 different locations from two regions namely: the KwaZulu-Natal coast (6 sites) and the Eastern Cape coast (2 sites) (Table 3.1).

COI:

A fragment of 560bp was sequenced for 154 individuals of the universal mitochondrial cytochrome oxidase subunit 1 marker. An overall number of 560 sites were analysed with 535 monomorphic and 23 sites polymorphic. There were 24 mutations with 7 parsimony informative sites, 15 singleton variable sites and an overall G-C content of 0.438. The overall haplotype diversity was 0.715 and overall nucleotide diversity was 0.001. The average number of nucleotide differences was 1.055. Out of a total of 154 sequences, 21 haplotypes were observed with 8 found in Clansthal and Reunion Rocks, 4 in Green Point, Ballito and Adlams. Five haplotypes were recorded for Mabibi and Mgazana 1 and 7 haplotypes were found in Mgazana 2 (Table 3.1). Six of these 21 haplotypes were shared and 15 were unique to a single population, with them being singleton haplotypes.

ITS1:

A fragment of 876bp was sequenced for 80 individuals of the ITS1 region that comprises of conserved regions of the 18S and 28S rRNA genes. Of the 32 sites that were analysed all were polymorphic. There were 35 mutations with 6 parsimony informative sites, 26 singleton variable sites and an overall G-C content of 0.545. The overall haplotype and nucleotide diversity was 0.609 and 0.002 respectively, with an average number of nucleotide differences of 1.603. Eighty individuals were sequenced and a total of 16 haplotypes were found. These haplotypes consisted of 4 from Ballito, Reunion Rocks and Clansthal, 8 haplotypes from Green Point and 5 haplotypes from Mgazana 1.

3.3.3. Diversity of *Marphysa corallina*

The genetic diversity indices measured in this study for 8 sample sites (COI) and 5 sites (ITS1) are the total number of individuals (N), the number of alleles (N_a), the number of effective alleles (N_e), Shannon's information index (*I*), haplotype number (h), haplotype diversity (Hd), nucleotide diversity (π), gene diversity (He) and percentage polymorphism (%Poly) (Table 3.3). The highest haplotype diversity for COI was observed for Reunion Rocks (Hd = 0.824) and the lowest was recorded for Ballito (Hd = 0.626) (Table 3.3). Haplotype diversity observed for ITS1 sequences were overall much lower (0.609) than those recorded for COI, with the lowest found for Reunion Rocks (0.395) (Table 3.3). Nucleotide diversity for COI was the lowest for Green Point, Ballito, Mabibi and Adlams (π = 0.001) and the highest recorded for Reunion Rocks (π = 0.003) (Table 3.3). Clansthal, Mgazana 1 and 2, displayed the same low nucleotide diversity for COI of π = 0.002 (Table 3.3), whereas ITS1 region showed an overall low nucleotide diversity of 0.001 with Ballito exhibiting the lowest 0.000 (Table 3.3). For all populations of *M. corallina,* a general trend of intermediate to high haplotype diversities (Hd = 0.715) and low nucleotide diversities (π = 0.001) were observed for both COI and ITS1 data (Table 3.3). This indicates that populations may have recently undergone a bottleneck after which a rapid population expansion occurred (Lowe et al. 2009).

The mean number of alleles for any population is a measure of the genetic richness of a population (Lowe et al. 2009). Thus the number of alleles for all populations for COI ranged from N_a = 1.120 (±0.066) to 1.400 (±0.100) with Green Point, Ballito and Adlams displaying the lowest genetic richness, and Reunion Rocks displaying the highest genetic richness (Table 3.3). For ITS1 data, the overall genetic richness (1.586±0.032) was slightly higher than that of COI (1.220±0.290), with the highest genetic richness displayed for Mgazana 1 (2.010±0.067) (Table 3.3). Overall populations show a high genetic richness for both COI and ITS1 data (Table 3.3).

The effective number of alleles is a measure of the diversity of alleles across all loci in a population (Silva & Russo 2000). Reunion Rocks had the highest allelic richness across

all populations of *M. corallina* for COI (Table 3.3). Mgazana 1 displayed the highest allelic richness for ITS1 data (1.313±0.013). Mgazana 1, 2, Clansthal and Mabibi displayed similar N_e values for COI ranging from N_e = 1.059 (±0.033) – 1.063 (± 0.027). Clansthal, Green Point and Ballito on the other hand displayed similar allelic richness for ITS1 data indicating that populations are similar in allelic richness (Table 3.3). Ballito was recorded as having the lowest Shannon's information index for both COI and ITS1 data which is another measure of allelic and genetic diversity (Peakall & Smouse, 2012). Reunion Rocks was found to have the highest Shannon's information index (I = 0.126±0.034) for COI and Mgazana 1 had the highest scores for ITS1 data (0.365±0.025).

The largest number of individuals sampled for a population was Ballito and Clansthal (N = 25), whereas Mgazana 2 had the smallest population (N = 9) (Table 3.3). Clansthal and Reunion Rocks had the highest number of haplotypes for COI (8) whilst Green Point, Ballito and Adlams had the lowest (4) (Table 3.3). The highest number of haplotypes was observed for Green Point for ITS1 data (8) and the lowest was found for Clansthal, Reunion Rocks and Ballito (4). The highest percentage of polymorphic loci was recorded for the geographically intermediate population, Reunion Rocks (40%) indicating high diversity, whereas Ballito, Adlams and Green Point displayed the lowest polymorphic diversity (12%, Table 3.3). The highest percent polymorphism was observed for Mgazana 1 (80%) and the lowest for Ballito (14%, Table 3.3). Overall, for COI, Reunion Rocks was observed to be the most genetically diverse population of *M. corallina* (Table 3.3). In contrast, ITS1 data indicated that Mgazana 1 was the most genetically diverse population due to the highest percent polymorphism, haplotype diversity, Shannon's Information Index, number of alleles and number of effective alleles (Table 3.3).

Table 3.3: Diversity indices for populations of *Marphysa corallina* sampled at eight different sites (Clansthal, Green Point, Reunion Rocks, Ballito, Mabibi, Adlams, Mgazana 1 and Mgazana 2) across two regions (KwaZulu-Natal and Eastern Cape) in South Africa for the COI and ITS1 genes.

	COI									ITS								
Population	N	N_a	N_e	*I*	h	Hd	π	H_e	%Poly	N	N_a	N_e	*I*	h	Hd	π	H_e	%Poly
Clansthal	25	1,360 (±0,098)	1,061 (±0,026)	0,087 (±0,029)	8	0,763	0,002	0,047 (±0,018)	36	24	1,404 (±0,070)	1,096 (±0,030)	0,105 (±0,022)	4	0,510	0,001	0,056 (±0,013)	30
GreenPoint	23	1,120 (±0,066)	1,058 (±0,038)	0,058 (±0,033)	4	0,695	0,001	0,037 (±0,022)	12	22	1,414 (±0,066)	1,068 (±0,013)	0,101 (±0,017)	8	0,688	0,002	0,053 (±0,009)	34
ReunionRocks	14	1,400 (±0,100)	1,089 (±0,027)	0,126 (±0,034)	8	0,824	0,003	0,070 (±0,020)	40	14	1,909 (±0,064)	1,197 (±0,028)	0,258 (±0,023)	4	0,395	0,002	0,136 (±0,013)	76
Ballito	25	1,120 (±0,066)	1,041 (±0,027)	0,046 (±0,028)	4	0,626	0,001	0,028 (±0,018)	12	11	1,192 (±0,055)	1,090 (±0,033)	0,078 (±0,023)	4	0,600	0,000	0,045 (±0,013)	14
Mabibi	16	1,160 (±0,075)	1,059 (±0,033)	0,064 (±0,033)	5	0,750	0,001	0,039 (±0,021)	16	-	-	-	-	-	-	-	-	-
Adlams	22	1,120 (±0,066)	1,051 (±0,034)	0,051 (±0,031)	4	0,688	0,001	0,033 (±0,021)	12	-	-	-	-	-	-	-	-	-
Mgazana 1	20	1,320 (±0,095)	1,063 (±0,027)	0,087 (±0,030)	7	0,784	0,002	0,048 (±0,018)	32	9	2,010 (±0,067)	1,313 (±0,033)	0,365 (±0,025)	5	0,722	0,001	0,206 (±0,014)	80
Mgazana 2	9	1,160 (±0,075)	1,062 (±0,031)	0,070 (±0,034)	5	0,861	0,002	0,043 (±0,021)	16	-	-	-	-	-	-	-	-	-
	154	1,220 (±0,29)	1,060 (±0,011)	0,074 (±0,011)	21	0,715	0,001	0,043 (±0,007)	22	80	1,586 (±0,032)	1,153 (±0,013)	0,181 (±0,011)	16	0,609	0,001	0,099 (±0,006)	42

N = number of individuals; N_a = Number of alleles; N_e = number of effective alleles; *I* = Shannon's Information Index; *h* = number of haplotypes; *Hd* = haplotype diversity, π = nucleotide diversity; *He* = expected heterozygosity; *%Poly* = Percentage of polymorphic loci

Dominant haplotypes 1, 2 and 3 are found in all populations for COI data and are present in high frequencies (Figure 3.2). Haplotypes 3 and 4 generated for ITS1 data are present in high frequencies in all sample sites besides Green Point and Mgazana 1 (Figure 3.2). Haplotype frequencies for populations of *M. corallina* were highest for Clansthal and Reunion Rocks (38%) and Mgazana 1 (33%) for COI. For ITS1 Green Point (50%) had the highest diversity. This indicates that these populations are diverse with regard to a large number of singleton haplotypes present (Figure 3.2). Green Point, Ballito and Adlams had the lowest haplotype frequencies for COI indicating that these populations of *M. corallina* are not as diverse. This is further supported by the low polymorphism present for these populations (Table 3.3).

No private alleles for COI were recorded for Mabibi and Ballito leading to the conclusion that these populations are well mixed, however, Reunion rocks, Mgazana 1 and Clansthal displayed a significant ($p < 0,05$) number of private alleles for COI (0.240 and 0.200, respectively). For ITS1 data, Green Point, Clansthal, Ballito and Mgazana 1 displayed the highest number of private alleles (Figure 3.2, adjacent bar graph), indicating that genetic drift or selection in conjunction, with gene flow is acting on these populations. These frequency patterns further support the diversity indices suggesting that *Marphysa corallina* from Reunion Rocks is the most genetically diverse population for COI data.

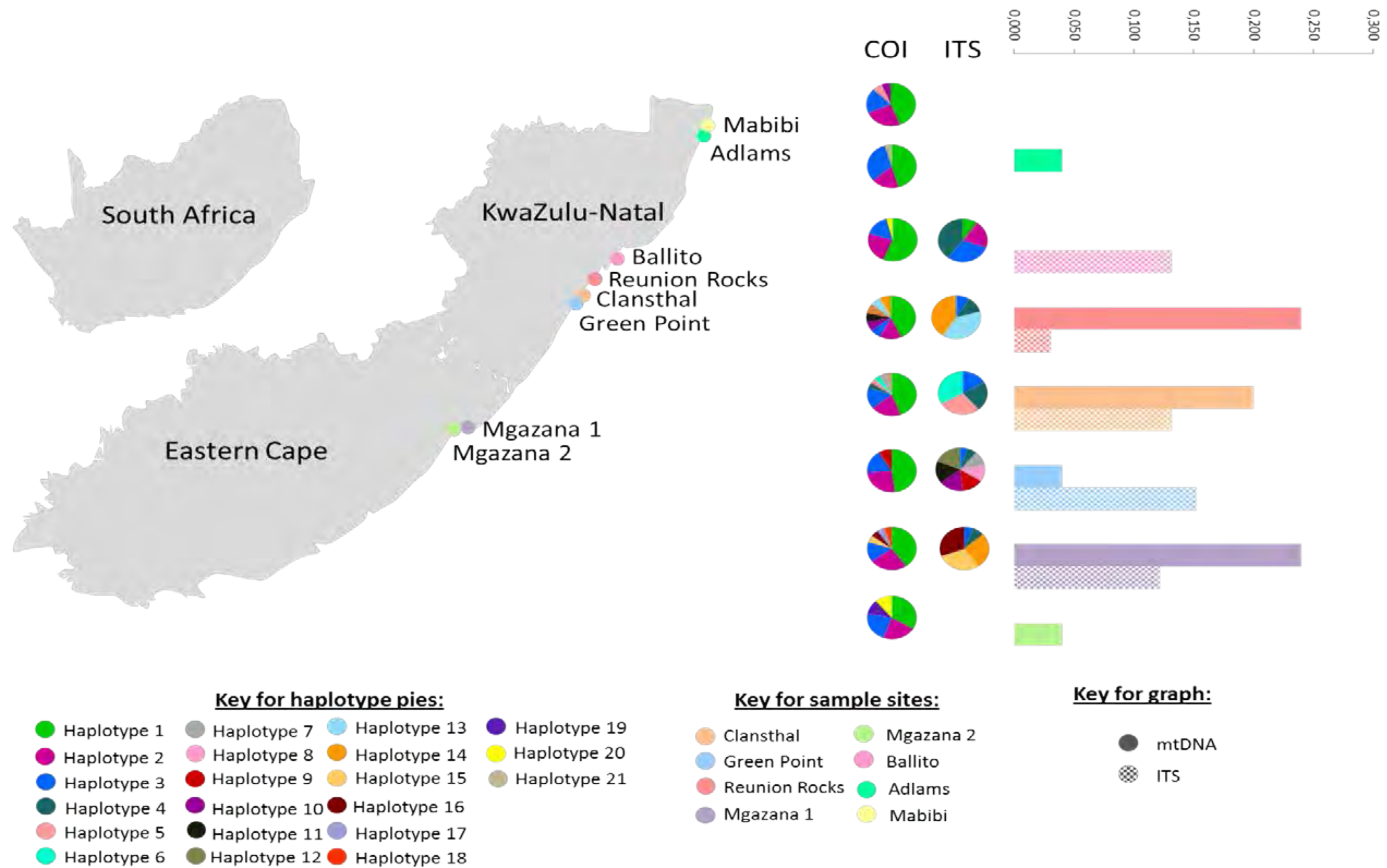


Figure 3.2: Map showing *Marphysa corallina* sample sites with the geographical distribution of haplotypes and haplotype frequencies per population sampled per gene; COI and ITS1. The adjacent bar graph represents the average frequency of private alleles for each sample site for COI and ITS1.

Table 3.4: AMOVA results computed for COI and ITS1 for eight different populations of *Marphysa corallina* sampled along the South African coastline. COI had a ϕ_{ST} value of -0.021 ($p > 0.05$) and ITS1 had a ϕ_{ST} value of 0.071 ($p < 0.05$).

	COI					ITS				
Source of variation	**d.f**	**Sum of squares**	**MS**	**Est.Var.**	**%**	**d.f**	**Sum of squares**	**MS**	**Est.Var.**	**%**
Among pops	7	2,356	0,337	0,000	0	4	38,890	9,722	0,341	7
Within pops	146	80,345	0,550	0,550	100	75	334,698	4,463	4,463	93
Total	153	82,701	-	0,550	100	79	373,588	-	4,803	100

3.3.4. Genetic structure, gene flow and migration patterns

AMOVAs were conducted to determine the sources of genetic variation and to determine whether populations display any genetic structure. The results from the AMOVA showed that 100% of the genetic variation for COI is found within populations and 0% of the variation can be explained among populations (Table 3.4). Intra-population differences are responsible for all of the molecular variation found for eight populations of *M. corallina* for COI, demonstrating that populations are similar to one another and display no genetic structure (Table 3.4). This is further supported by the ϕ_{ST} value, -0.021 ($p > 0.05$).

Consequently, ITS1 data are slightly different. The variation among populations show a 7% variation and within populations exhibit 93 % of variation (Table 3.4) further confirming that majority of the variation is found within populations of *Marphysa corallina.* The ϕ_{ST} statistic reveals fine-scale genetic structuring between populations, 0.071 ($p < 0.05$). Genetic distances were calculated using the population pairwise distance method for populations of *M. corallina* and resulted in substantially low F_{ST} values that were not significant for COI data (Table 3.5). A similar pattern is observed

for ITS1 data where the majority of the F_{ST} values are extremely low, negligible and non-significant (Table 3.5). These low F_{ST} values are characteristic of either extensively mixed populations or a recent colonisation of new habitats. The lowest genetic distance observed was for the southern-most population, Mgazana 1 and northern-most population, Adlams 0.000 ($p < 0.05$), indicating that these populations regularly exchange genes. The genetic distances observed for Mgazana 1 and Clansthal, Mgazana 1 and Green Point display moderately differentiated populations (0.140 – 0.109, Table 3.5). Overall, the genetic distances between pairs of populations were low and not significant indicating that populations share a lot of genetic material and are not genetically structured. This is further supported by the AMOVA results displaying no genetic variation among populations (Table 3.4).

Migration was estimated using the directional island model of migration, in a north to south direction and subsequently from a south to north direction. The probability of each model was calculated and results showed that both models for COI and ITS1 data yielded a probability that was significant ($p < 0.05$). This result suggests that there is a bidirectional flow of immigrants. The highest number of immigrants per generation was observed from Green Point to Clansthal (Nm = 46) and thereafter from Green Point to Mgazana 1 (Nm = 37) (Figure 3.3).

High gene flow observed from Green Point to Clansthal could be due to these populations present in close proximity to one another thus allowing for the successful recruitment and subsequent settlement of larvae. Gene flow was observed to be less than 1 from Mabibi to Adlams (Nm = 0.3), Clansthal to Green Point (Nm = 0.09), Mgazana 1 to Green Point (Nm = 0.1) and Ballito to Adlams (Nm = 0.3, Figure 3.3). The number of immigrants moving per generation to and from these populations is significant enough to prevent genetic drift and subsequent isolation. The low gene flow observed could be due to a geographical barrier present between these populations.

Table 3.5: Pairwise F_{ST} values calculated for eight populations of *Marphysa corallina* sampled along the South African coast from two regions (KwaZulu-Natal and Eastern Cape). COI values are below diagonal and ITS1 values are above diagonal. (F_{ST} p values are presented in parentheses and those that are significant are in bold).

	Clansthal	Green Point	Reunion Rocks	Mgazana 1	Mgazana 2	Ballito	Adlams	Mabibi
Clansthal	0	-0,007 (0,588)	0,010 (0,283)	**0,140 (0,000)**	-	-0,030 (0,695)	-	-
Green Point	-0,018 (0,708)	0	-0,003 (0,474)	**0,109 (0,004)**	-	-0,023 (0,784)	-	-
Reunion Rocks	-0,011 (0,603)	0,003 (0,342)	0	0,088 (0,142)	-	-0,020 (0,748)	-	-
Mgazana 1	-0,021 (0,896)	-0,015 (0,573)	-0,005 (0,490)	0	-	**0,124 (0,007)**	-	-
Mgazana 2	-0,047 (0,940)	-0,045 (0752)	-0,039 (0,894)	-0,046 (0,928)	0	-	-	-
Ballito	-0,024 (0,999)	-0,018 (0,613)	0,005 (0,318)	-0,028 (0,921)	-0,053 (0,832)	0	-	-
Adlams	-0,016 (0,668)	-0,024 (0,684)	-0,002 (0,428)	-0,000 (0,321)	-0,040 (0,692)	-0,002 (0,383)	0	-
Mabibi	-0,036 (0,988)	-0,038 (0,865)	-0,020 (0,672)	-0,029 (0,893)	-0,059 (0,832)	-0,031 (0,820)	-0,034 (0,714)	0

Gene flow was higher from a South to North direction with an average number of 19 immigrants per generation, whereas gene flow from a North to South direction yields an average number of 16 immigrants per generation. The overall high values of immigrants per generation for all populations correspond to the low values of genetic distances between populations. This indicates that populations share a lot of genetic material making them panmictic. No genetic structuring of populations was observed from the AMOVA results (Table 3.4). The same pattern can be observed for ITS1 data. Overall, the number of immigrants per generation for ITS1 data is significantly higher than that for COI data (Figure 3.3). The highest gene flow observed for ITS1 data was from Clansthal to Green Point with 181 immigrants per generation and Reunion Rocks to Ballito with 131 immigrants per generation (Figure 3.3). The results from ITS1 data is in agreement with patterns displayed for COI, thus strongly suggesting a large panmictic population.

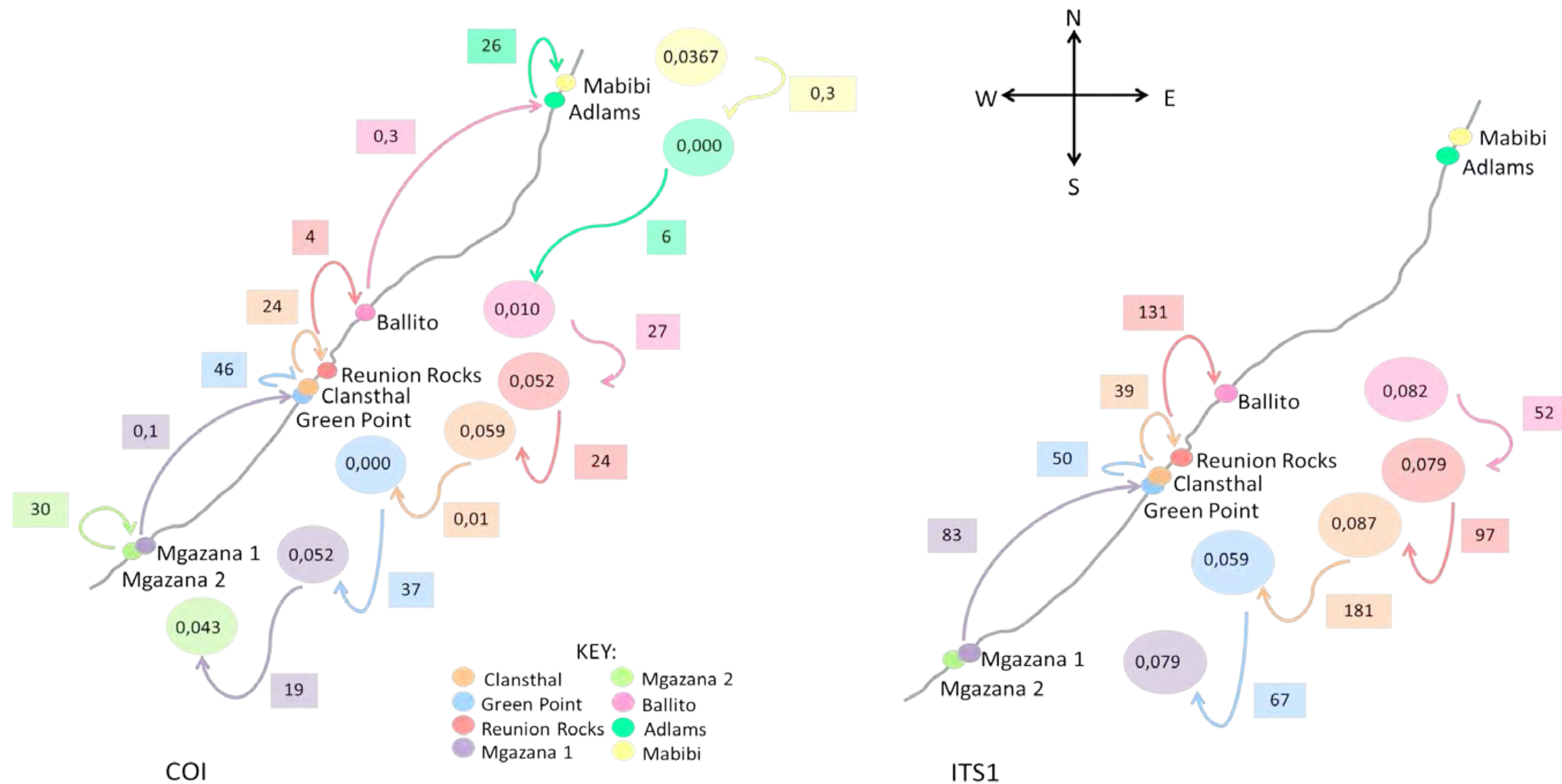


Figure 3.3: Migration patterns among eight (COI) and five (ITS1) populations of *Marphysa corallina* sampled along the South African coast from two regions namely: KwaZulu-Natal and Eastern Cape for both COI and ITS1 genes. Mutation-scaled effective population sizes (Θ) are in coloured ovals and correspond to each population and the estimated number of immigrants per generation (xNm) in coloured rectangles corresponding to populations.

The minimum spanning network assumes a star-like pattern for both COI and ITS1 data, with the ancestral haplotype in the star's centre (Figure 3.4). These ancestral haplotypes are made up of a mixture of all eight COI populations of *M. corallina* sampled with many derived singleton haplotypes around it that are separated by a one nucleotide change. For ITS1 data, only 5 sample sites were presented and the ancestral haplotype (Haplotype 3) consists of all 5 populations (Figure 3.4). These mixed ancestral haplotypes for both COI and ITS1 are indicative of panmixia where gene flow persists resulting in very diverse and highly connected populations (Mirol et al. 2008; Lowe et al. 2009). An individual from Mgazana 1 is separated by two nucleotide differences from the main ancestral haplotypes, whereas individuals from Clansthal are separated by three nucleotide differences from the ancestral haplotype (Figure 3.4). For ITS1 data, haplotypes consisting of individuals from Mgazana 1 and Reunion Rocks seem to have diverged from the main ancestral haplotype by 10 nucleotide differences (Figure 3.4).

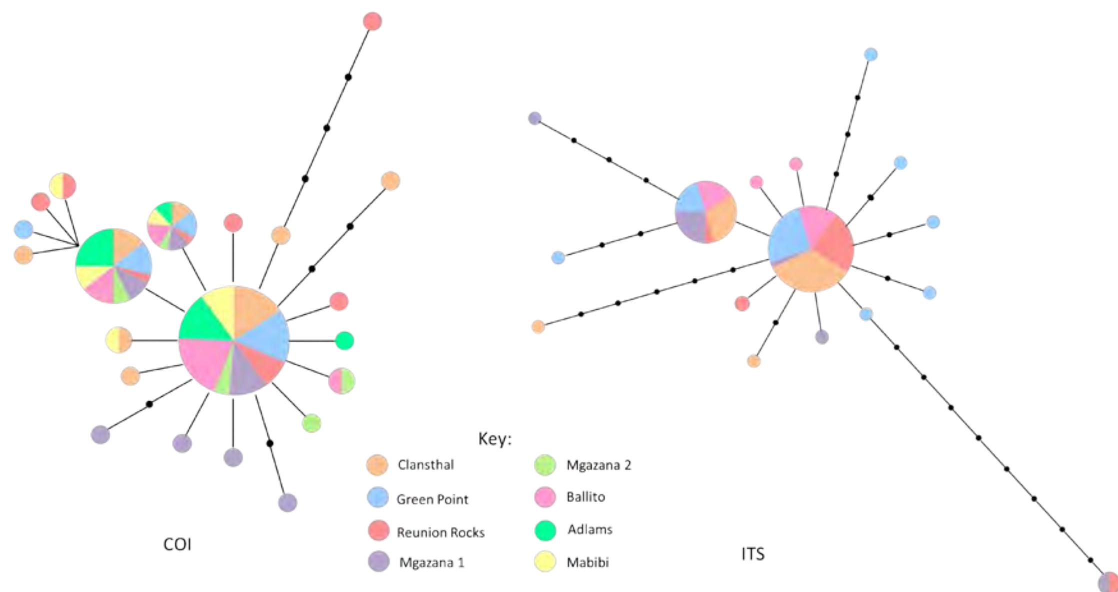


Figure 3.4: Minimum spanning haplotype network for eight populations of *Marphysa corallina* sampled along the South African Coast from two regions. Circle sizes are proportional to the frequency of individuals present in each haplotype; solid lines represent nucleotide differences and dots represent inferred missing haplotypes.

The network illustrates that populations of *M. corallina* are not genetically or geographically structured and exhibit a high level of genetic homogeneity. These results are supported by the high diversity indices (Table 3.3, p.75), low genetic distances indicating no structure (Table 3.5, p.80) and extensive gene flow patterns (F_{ST} and migration) among these populations (Figure 3.3, p.81). The overall high haplotype diversities and low nucleotide diversities (Table 3.3, p.75) observed for all populations reinforces the hypothesis of a rapid population expansion and a large accumulation of mutations as suggested from the observed star-like pattern of the minimum spanning networks presented (Figure 3.4).

3.3.5. Demographic analysis

Tests of neutrality were performed to determine whether populations have undergone a recent and sudden expansion or bottleneck. Six populations of *M. corallina* display negative, non-significant ($p > 0.05$) results for Tajima's D test for COI (Table 3.6). Green Point and Adlams have positive values indicating a collection of intermediate alleles nonetheless these are not significant (Table 3.6).

ITS1 data showed similar results, however, only 5 populations of *Marphysa corallina* were analysed and 4 populations displayed negative significant values ($p < 0.05$) for Tajima's D (Table 3.6). Fu's F_S test showed that Clansthal, Reunion Rocks, Mgazana 1 and 2 all show large negative significant values for COI suggesting these populations had undergone a sudden expansion (Table 3.6). Green Point and Ballito display positive significant values for Fu's F_S test for ITS1 data (Table 3.6). All of these values point to a sudden recent population expansion of the eight different COI populations and five ITS1 resulting in an abundance of rare alleles.

The mismatch distribution for COI presented results that demonstrate support for neutrality tests where the distributions for all populations are considered to be unimodal or "smooth" (Figure 3.5). These "smooth" distributions signify sudden

expansions. The mismatch distribution displayed for ITS1 data showed contrasting results to that of COI. The ITS1 mismatch distributions for Green Point, Mgazana 1

Table 3.6: Demographic expansion statistics (Tajima's D, Fu's F_S) and population expansion indices for mismatch distribution: Sum of squared distances (SSD), Raggedness index and τ–estimated time of expansion, for eight populations of *Marphysa corallina* sampled in South Africa. The associated p-values are in parenthesis.

	COI				ITS1			
Population	**Tajima's D**	**Fu's and F**	**SSD**	**Raggedness Index**	**Tajima's D**	**Fu's and F**	**SSD**	**Raggedness Index**
Clansthal	-1,456 (0,058)	-3,515 (0,005)	0,012 (0,265)	0,130 (0,111)	-1,838 (0,009)	0,318 (0,577)	0,007 (0,385)	0,110 (0,563)
Green Point	0,500 (0,735)	0,072 (0,463)	0,011 (0,267)	0,127 (0,207)	-1,785 (0,025)	-2,737 (0,047)	0,005 (0,826)	0,031 (0,953)
Reunion Rocks	-1,468 (0,069)	-3,553 (0,007)	0,010 (0,506)	0,077 (0,499)	-2,000 (0,009)	0,791 (0,691)	0,025 (0,318)	0,217 (0,647)
Mgazana 1	-1,468 (0,052)	-2,696 (0,025)	0,008 (0,416)	0,102 (0,304)	-1,619 (0,042)	0,491 (0,592)	0,618 (0,000)	0,114 (1,000)
Mgazana 2	-0,689 (0,266)	-1,994 (0,021)	0,045 (0,181)	0,280 (0,116)	-	-	-	-
Ballito	-0,170 (0,434)	-0,477 (0,330)	0,025 (0,098)	0,192 (0,048)	-1,113 (0,209)	-1,524 (0,024)	0,258 (0,164)	0,195 (0,323)
Adlams	0,107 (0,620)	-0,264 (0,374)	0,024 (0,091)	0,194 (0,049)	-	-	-	-
Mabibi	-0,403 (0,391)	-1,302 (0,124)	0,021 (0,236)	0,166 (0,152)	-	-	-	-

and Clansthal represent multimodal distributions, which are indicative of stable populations (Figure 3.5). The raggedness index reported for all populations of *M. corallina* are small (R < 0.5) which is typical for populations undergoing rapid expansions (Rogers & Harpending 1992).

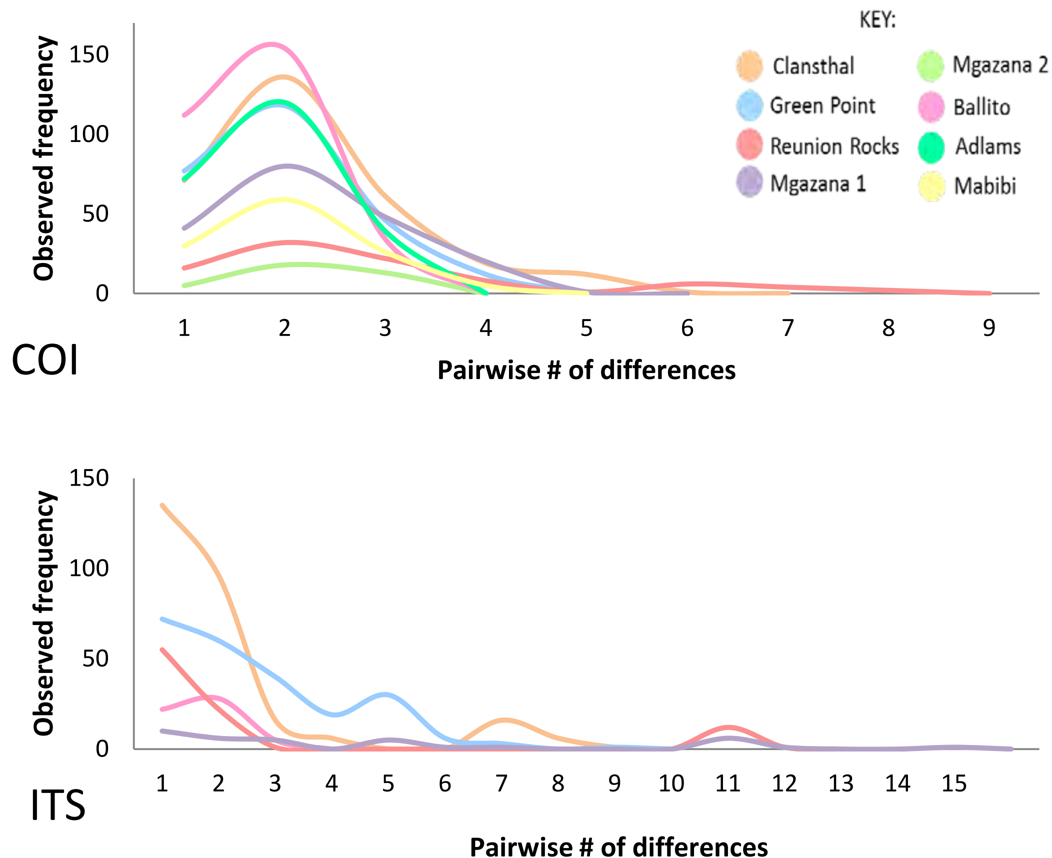


Figure 3.5: Mismatch distribution for populations of *Marphysa corallina* from two regions along the South African coast, sampled for two genes. The x-axis represents the pairwise number of differences. The y-axis represents the observed frequencies. Each population is coded with a different colour.

The Bayesian skyline plot presented in Figure 3.6 shows that *Marphysa corallina* experienced a gradual population decline that started approximately 113 636 years before present. The population then went through a rapid expansion about 22 727 years before present. These results are consistent with those produced for the

mismatch distributions which also indicated that the individual populations had undergone expansions in the past. A Bayesian skyline plot was analysed for ITS1 data but unfortunately did not yield any informative results therefore it was not presented.

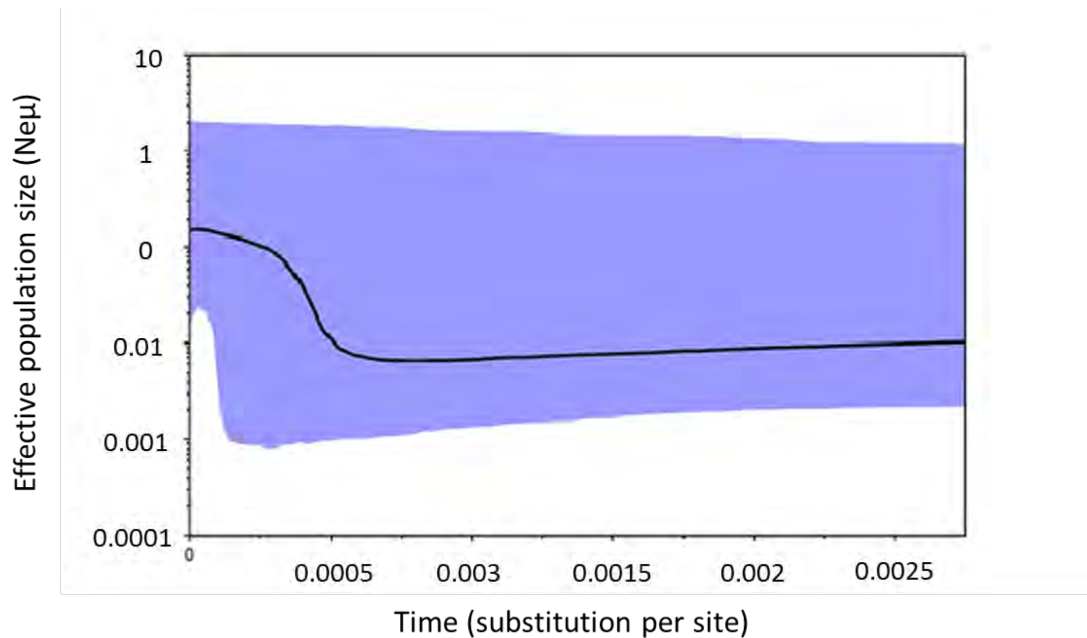


Figure 3.6: Bayesian Skyline Plot based on the COI gene of *Marphysa corallina*. All eight populations were considered as one meta-population. The x-axis represents time in terms of the substitution rate per nucleotide. The y-axis represents the effective population size. The black bold line represents the effective population size and the shaded areas represent the upper limits of the 95 % confidence intervals.

3.3.6. Phylogenetic analysis

The phylogenetic trees were constructed to determine the evolutionary relationships of *M. corallina* as well as to validate that it is an exclusive species. Three distinct clades were observed from the COI phylogenetic tree (Figure 3.7). *Marphysa corallina* (grey) forms an exclusive clade with the remaining species of *Marphysa* grouped into two separated clades (purple and green). The *Marphysa corallina* clade and the purple clade

constitute one clade and are sister to one another with moderate bootstrap support (< 90%, Figure 3.7). The green clade containing *M. angeli* and *M. cf. hentscheli* do not group into the bigger *Marphysa* clade, but groups as the sister clade with very strong support (> 90%, Figure 3.7). This grouping represents a different genus that was recently resurrected by Zanol et al. (2014). As a result, *Marphysa angeli* and *Marphysa cf hentscheli* were placed into the *Nicidion* genus. The grey and green clades have very strong bootstrap support (> 90%, Figure 3.7), whereas the purple clade is moderately supported (< 90%, Figure 3.7).

Results from the minimum spanning network (Figure 3.4), demographic expansion tests (Table 3.6), mismatch distribution analyses (Figure 3.5) and the nucleotide and haplotype diversities (Table 3.3, p.75) all support the hypothesis that populations of *M. corallina* have undergone a recent rapid population expansion from a bottleneck event. This can also be observed from the 15 singleton haplotypes (unique mutations or private alleles) for three of the populations found on the phylogenetic tree (Figure 3.7).

The ITS1 tree shows a similar pattern to that of the COI tree in that the *M. corallina* obtained from South Africa all group into one exclusive clade which has strong bootstrap support (0.99/1/1) (Figure 3.7). *Marphysa sanguinea* groups as a sister taxon to the bigger exclusive *M. corallina* clade with high bayesian support (1, Figure 3.7). Other species of *Marphysa* group as a sister clade to the *M. corallina* clade with low support from bayesian analysis and high support from neighbour joining and parsimony methods (Figure 3.7). *M. angeli* and *M. cf hentscheli* belonging to the newly emended *Nicidion* genus share a haplotype and thus are placed on the same branch. *Nicidion* (green) is placed as a sister taxon to the remaining *Marphysa* species (purple) with strong bootstrap support. A few polytomies were observed for the *M. corallina* clade, and as mentioned above this could be indicative of a rapid recent expansion event due to incomplete lineage sorting (Avise et al. 1987). It is also evident from the COI and ITS1 trees that no genetic or geographic structuring is observed for

populations of *M. corallina* which is further confirmed by the AMOVA results (Table 3.4, p.78) and the gene flow results (Figure 3.3, p.81). Overall, the phylogenetic results support a rapid expansion event.

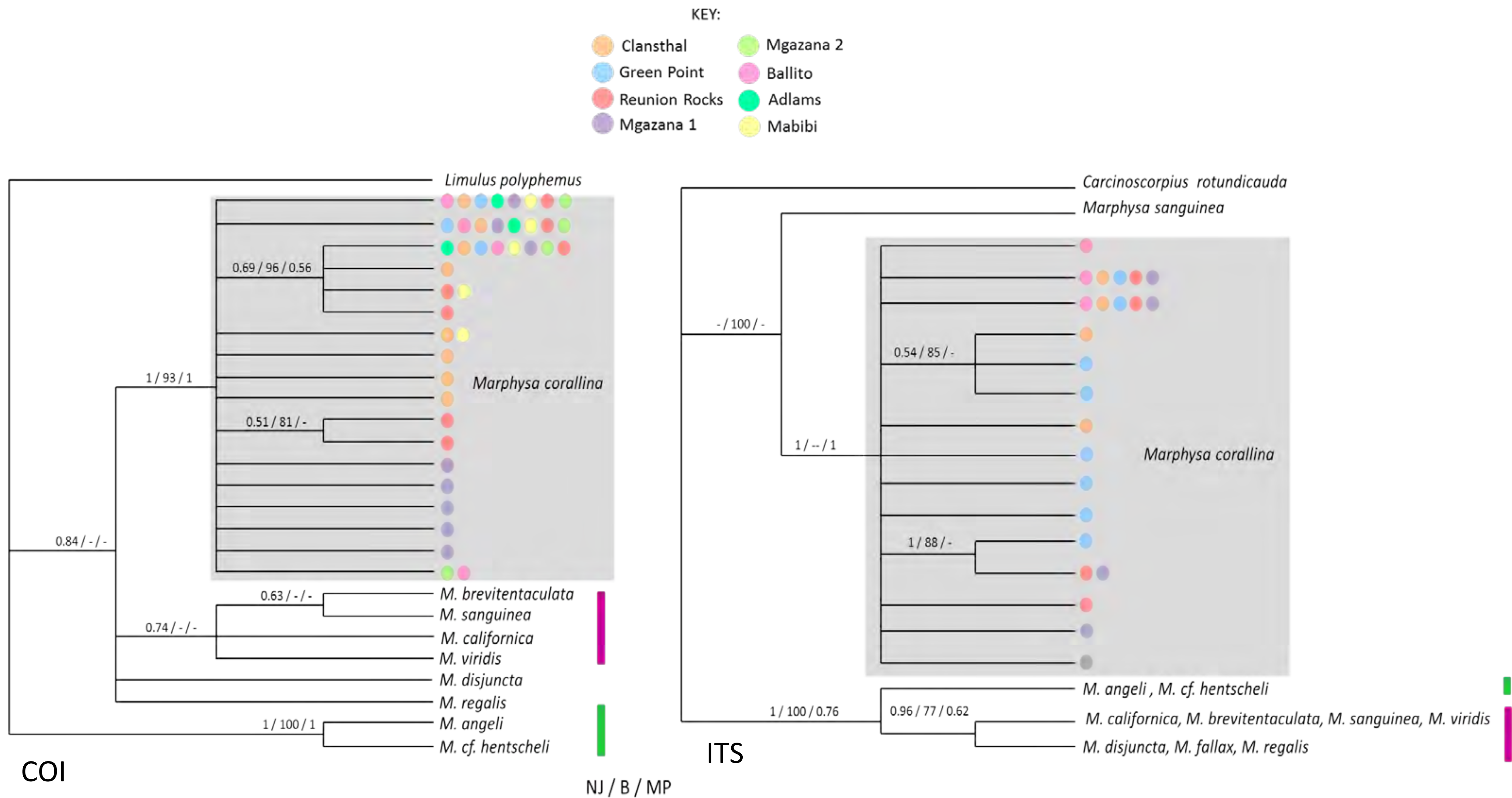


Figure 3.7: Bayesian phylogenetic trees of the genus *Marphysa*, with *M. corallina* sampled for COI and ITS1 markers respectively along the South African coast (Grey Clade). Other species of *Marphysa* are from around the world and the trees are rooted with the horseshoe crab; *Limulus polyphemus* and *Carcinoscorpius rotundicauda* (Accession numbers in Table 3.2, p.64). NJ, B and MP support in parenthes.

3.4. Discussion

The aim of this study was to determine whether populations of *Marphysa corallina* are differentiated from one another and subsequently analyse past demographic events that contributed to shaping their current distributional patterns across the Eastern Cape and KwaZulu-Natal regions of South Africa.

3.4.1. Genetic diversity of *Marphysa corallina*

Genetic diversity varied slightly among populations of *Marphysa corallina* for COI. The highest overall diversity was recorded for Reunion Rocks, which is the population in the centre of the sampling regime. Clansthal and Mgazana 1 displayed diversities that were significantly higher than other populations but slightly lower than Reunion Rocks. The overall genetic diversity indices for ITS1 indicated a similar pattern to COI. The genetic diversity indices varied slightly between populations. Mgazana 1 had the highest genetic diversity as opposed to other populations of *M. corallina.* In contrast to the Reunion Rocks population present in a highly polluted area, the Mgazana populations were sampled from an unpolluted habitat. It should also be noted that the sample size was relatively small (N = 9, Table 3.3, p.75) in comparison to other populations.

Genetic variability is the platform upon which evolution occurs and is influenced by mutation, effective population sizes and gene flow (Vellend & Geber 2005; Nabholz et al. 2009). There are a number of explanations as to why certain sample locations are more genetically diverse than others. High genetic diversities in a population can be indicative of large population sizes maintained over evolutionary time as a result of a stable habitat, an evolutionarily old population which has accumulated mutations over time or it could be a genetic signal of refugial areas during glacial events at ice free regions relative to glaciated regions (Stevens et al. 2007; Hobbs et al. 2013; Reynolds et al. 2014; Zhang et al. 2014). The rocky shore present at Reunion Rocks is highly polluted due to the presence of a petroleum refinery outlet. This petroleum outlet has been

operational for the past 50 years. The possibility exists that *M. corallina* at this sample site could have maintained large population sizes prior to the development of the refinery resulting in such high genetic diversities. The expansion time for populations at Reunion Rocks, Clansthal and Mgazana 1 was estimated to be between 105 000 - 121 753 years ago placing these expansions in the middle to late Pleistocene glacial cycles. Clansthal and Mgazana are relatively unpolluted sample sites. Populations that have survived the series of glacial cycles have a longer demographic history and display high genetic diversities due to a large accumulation of mutations (Provan & Bennett 2008). Alternatively, these sample sites could have been refugial areas during the Pleistocene glacial cycles resulting in high genetic diversities. However, this should be interpreted with caution as the genetic signatures of admixture zones are commonly confused with refugial zones (Petit et al. 2003; Provan & Bennett 2008; Hu et al. 2011). Thus, the entire distributional range of *M. corallina* needs to be sampled for the identification and separation of refugial zones and zones of admixture.

A study investigating the genetic diversity of a marine invertebrate by Aguirre & Marshall (2012) found that populations have more stability, are more productive and resistant to disturbances when they have high genetic diversities (Amos & Harwood 1998). One could infer that the high genetic diversities obtained by the *M. corallina* population at Reunion Rocks as a result of their supposed past demographic history have equipped these individuals with advantageous alleles to enable them to adapt and survive in an extensively polluted area.

Refugial populations can be identified by the presence of a large number of private haplotypes specific to one population (Maggs et al. 2008; Provan & Bennett 2008). For the COI gene, Reunion Rocks, Clansthal and Mgazana 1 populations all have a large number of private haplotypes possibly suggesting that these populations served as refugial zones during the Pleistocene climatic events. For the ITS1 gene, Green Point, Clansthal and Ballito all displayed a high number of private haplotypes. The only population containing private haplotypes for the two genes is Clansthal. This would

imply that Clansthal could have been the refugial population. Contact zones or zones of admixture constitute soley of ancestral haplotypes and are assumed to have recently colonized or recolonised that particular area (Maggs et al. 2008; Provan & Bennett 2008). As a result it is assumed that for COI, Ballito and Mabibi could have been recently colonized as they displayed no private alleles. Since the diversity indices for both markers for all populations of *M. corallina* vary slightly from one another, it is hypothesized that the entire region could be representative of an evolutionarily old population that could have been a refuge. Thus emphasis is placed on more adequate sampling of haplotypes spanning the distributional range of *Marphysa corallina* as the limited sampling within this proposed refugial area resulted in the absence of haplotypes found elsewhere.

Haplotype diversities for all populations of *M. corallina* were intermediate to high for COI (Hd = 0.715, Table 3.3, p.75) and slightly lower for ITS1. In contrast to Reunion Rocks displaying the highest haplotype diversity for COI (Hd = 0.824, Table 3.3, p.75), it has the lowest haplotype diversity for ITS1 data (Hd = 0.395, Table 3.3, p.75). The over-estimation of diversity in the COI gene could be due to mitochondrial DNA having a higher mutation rate as opposed to nuclear DNA (Nabholz et al. 2009). This high mutation rate gives rise to homoplasy when using the COI gene for phylogenetic and population genetics studies (McMillen-Jackson & Bert 2004; Nabholz et al. 2009; Hobbs et al. 2013); as could be the case in the present study. Nonetheless, high haplotype diversities were also recorded for *Palola* worms using COI as documented by Schulze (2006).

The relatively low haplotype and nucleotide diversities recorded for the ITS1 gene for Reunion Rocks and Clansthal signify that these populations might have undergone or are currently going through a severe population bottleneck (Lowe et al. 2009; Winkelmann et al. 2013). These populations could have recently colonized their respective habitats shown by the loss of haplotypes, low nucleotide differences and the low number of private alleles (as mentioned above). The overall high haplotype and

low nucleotide diversities found for all populations of *M. corallina* for the COI gene is the genetic imprint of a recent population expansion (McMillen-Jackson & Bert 2004; Lowe et al. 2009; Hellberg 2009; Marko et al. 2010). Avise et al. (1987) documented that during rapid population expansion events, expanding populations retain haplotypes due to the absence of genetic drift, which significantly reduces haplotypes. Thus a plausible reason for such high haplotype and low nucleotide diversities found for all populations for both COI and ITS1 could be that of a recent population expansion from an ancestral population.

Geographically widespread haplotypes and their abundant presence in a population are indications of an ancestral lineage (Avise 2000; Beheregaray & Sunnucks 2001; Duran et al. 2004; Maggs et al. 2008). Thus in this study, haplotype 1 for COI is the ancestral lineage since it is present in high frequencies in all populations whereas haplotype 3 for ITS1 is the most ancestral lineage since it is also present in all populations but in intermediate frequencies (Figure 3.2, p.77). It is expected that the ancestral haplotype is generally more frequent around its place of origin and the majority of the ancestors' descendants occur near the place of the ancestral lineages' origins (Templeton 1993; Avise 2000; Maggs et al. 2008). As a result, this then acts as the centre of origin and radiation to other areas.

Interestingly COI haplotype 1 and ITS1 haplotype 3 occur in their highest frequencies in Ballito therefore it is assumed that the Ballito population is possibly the ancestral lineage. However, this assumption is not likely because looking at COI data, the frequencies of haplotype 1 differ very slightly between populations which could also mean that the ancestral population could have not been sampled. In addition to the above, no private alleles were recorded for Ballito further implying that a recent colonization had taken place. In contrast to COI, ITS1 data reflect a large accumulation of private alleles for Ballito which could infer that this could have been a refugial population. It should be noted that only 5 populations of *Marphysa corallina* were analysed for ITS1 data thus, there could be missing links and the source population

cannot be identified with certainty. Alternatively, if we take into consideration that evolutionarily old populations are characterised by a high diversity of haplotypes this would lead to the assumption that the Mgazana populations of *M. corallina* for both ITS1 and COI would be the oldest population due to high haplotype and low nucleotide diversities. However, this may not be ascertained as the distribution of haplotypes indicates that the ancestral haplotype is widely distributed, giving no indication as to the direction and origin of the expansion (Hellberg 2009).

3.4.2. Population structure and gene flow

The AMOVA analyses reveal no genetic structuring for populations of *Marphysa corallina* for COI and very shallow structuring for ITS1. The genetic structuring of populations are influenced by a wide range of factors such as genetic drift, local adaptations, gene flow which is dependent on larval biology, mobile adults and oceanic currents (Nabholz et al*.* 2009; Marko & Hart 2011). Extensive gene flow among populations can drastically slow down the process of genetic drift producing highly connected panmictic populations (Lowe et al. 2009; Marko & Hart 2011). Populations that receive little or no gene flow from neighbouring populations are assumed to be separated leading to fragmented population distributions (Grosberg & Cuningham 2001). The gene flow analysis for populations of *Marphysa corallina* for COI reveal highly connected populations. In contrast, ITS1 data suggest shallow genetic structuring.

The majority of the F_{ST} values recorded for COI populations do not differ from 0 and others are negative (Table 3.5, p.80). According to Popa et al. (2013), F_{ST} values that are negative and negligible are an indication of excessive outbreeding. Due to the lack of information available on the larval biology of *Marphysa corallina*, the general larval biology of the *Marphysa* has been applied when considering *M. corallina*. The genus is known to be iteroparous and to produce lecithotrophic larvae that spend approximately 7-10 days in the water column before settling down and creating permanent burrows on the benthos.

The results from this study found no geographic or genetic structure regardless of the assumed short-lived lecithotrophic larvae that would otherwise result in structuring of *Marphysa corallina* populations. The growing literature on the phylogeography of marine invertebrates in South Africa has identified different lineages of species that coincide with the biogeographic provinces described for the region (Teske et al. 2011). These phylogeographic breaks are found on the south-west coast near Cape Point and Cape Agulhas forming a transition zone between the cool temperate and warm temperate regions, the south-east coast between Algoa Bay and the Wild Coast creating the second transition zone between the warm temperate and subtropical regions and lastly a new break has been found at St Lucia which is the third transition zone between the subtropical and topical regions (Teske et al. 2011). The sampling range of *M. corallina* in the present study extends over three of these biogeographic provinces thus covering two of the phylogeographic breaks. These are the St. Lucia break in the north-east and the Wild Coast break in the south-east. Taking this sampling range into consideration and assuming that *M. corallina* is a poor disperser one would expect at least two separate lineages. However, from our results it has been found that populations of *M. corallina* are highly connected resembling a meta-population. As a result *M. corallina* is an example of a species that is present in three biogeographic regions described for South Africa but has no apparent genetic structure and thus does not coincide with any of the described phylogeographic breaks.

The high genetic connectivity observed for the current study was also found for a closely related species in the Eunicidae family. Schulze (2006) found that regardless of whether species had long lived lecithotrophic larvae, a high level of genetic connectivity was still observed. It has been suggested that significant genetic and geographic structuring is characteristic of all polychaete worms. In contrast to this assumption, it was found that the fireworm *Hermodice carunculata* as well as the invasive nereidid species *Platynereis dumerilii* resemble extensively connected populations across ocean basins (Ahrens et al. 2013; Popa et al. 2013). Therefore in terms of the current study, gene flow was not considered an appropriate estimator for

the high connectivity observed between populations. Instead the hypothesis of recent expansions from ancestral populations discussed in the paragraphs below appears to be a more accurate explanation for such high connectivity.

According to Mirol et al. (2008), a lack of population structure found in COI could resemble a shared ancestry of populations whilst the shallow nuclear genetic structuring of populations could indicate that genetic drift is acting on the nuclear gene thus differentiation is currently in progress. One would take into consideration the effect of concerted evolution on the nuclear ITS1 gene. ITS1 is a multicopy gene containing tandem arrays that evolve under concerted evolution (Liao 1999; Fuertes Aguilar et al. 1999; Naidoo et al. 2013). The process of concerted evolution occurs when multigene copies of repetitive sequences are homogenized due to their evolution as a single unit (Liao 1999; Naidoo 2013). The ITS1 gene had a higher polymorphism percentage (42 %, Table 3.3, p.75) compared to COI, and nucleotide diversity was low (0.001). This result implies that concerted evolution was slow acting as the gene was not completely homogenized yet. The incomplete homogenization ITS1 could be a result of the recent admixture of genes in contact zones. These large polymorphic differences could have contributed to the shallow genetic structuring of the ITS1 populations resulting in a 7 % genetic variation between populations of *Marphsya corallina.* Similar results were obtained by Gao et al. (2012) when analyzing intraspecific differences of *Rhodiola alsia.* Gao et al. (2012) found a high number of polymorphic loci and high nucleotide differences for the ITS gene and concluded that concerted evolution was gradual in this case. Alternatively, it could be a case of retention of males in their natal homes and females dispersing to other habitats (Waser & Jones 1983). This phenomenon could have resulted in the sampling of female mitochondrial haplotypes producing a shared maternal ancestry and male haplotypes under nuclear genetic drift. Unfortunately there has been no evidence of the latter recorded for polychaete worms.

Taking into consideration the former hypothesis, this scenario produces an increased

number of singleton haplotypes due to the unstable population losing haplotypes because of genetic drift (Chatzigeorgiou et al. 2014). In addition, Duran et al. (2004) stated that shallow genetic structuring could be attributed to a relatively young species that has recently spread across its geographic range and thus spent very little time independently isolated to accumulate large genetic differences. This, coupled with high haplotype diversities is characteristic of an evolutionarily old species that has been influenced by demographic events (Duran et al. 2004). It can be assumed that populations of *Marphysa corallina* might have recently spread from an ancestral population and colonised new habitats resulting in a large retention of ancestral genes in the present populations. This is further supported by the 26 singleton haplotypes found for ITS1 gene, which represents recently radiated populations that are undergoing genetic drift. This hypothesis is further supported by the presence of the star-like topology of the haplotype network displayed for both mitochondrial and nuclear genes (Figure 3.4, p.83) (Mirol et al. 2008; Lowe et al. 2009).

The COI star-like network observed is an indication of populations undergoing rapid recent expansions (Mirol et al. 2008; Lowe et al. 2009). This is further validated by the high haplotype and low nucleotide diversities found in the COI gene where populations have a retention of haplotypes and have undergone a recent bottleneck resulting in low nucleotide differences. The ITS1 network on the other hand resembles a somewhat star-like topology with a large number of mutational differences between the haplotypes. This is congruent with the idea that the nuclear gene is undergoing genetic drift resulting in populations accumulating mutational differences. Furthermore, F_{ST} values that are equal to 0 need to be interpreted with caution and a lack of statistical significance for F_{ST} values must not be equated to high genetic connectivity between populations (Hellberg 2009). Due to this common error made by many population geneticists, Marko & Hart (2011) suggested that a more robust method would be to estimate coalescent times and demographic history in conjunction with gene flow analyses in order to explain extensive (F_{ST} = negative and zero) connectivity between populations.

3.4.3. Demographic history

The genetic imprint of a shared ancestry uncovered by the COI marker for populations of *Marphysa corallina* as well as the on-going genetic drift acting on the nuclear gene sampled for the recently split populations coupled with the star like topology for both networks are all indicative of a recent sudden expansion from an ancestral population. This range expansion event is further denoted by the presence of the ancestral haplotype in high frequencies throughout all populations along the East coast of South Africa for both COI and ITS1 genes (Figure 3.2, p.77).

Tajima's D and Fu's Fs tests were conducted to confirm whether populations have undergone a recent sudden expansion. All populations sampled for the COI gene displayed negative, non-significant Tajima's D values whereas for Fu's F_S test half of the populations had negative and significant values (Table 3.6, p.85). Tajima's D values analysed for ITS1 were all negative and significant except for Ballito. Fu's F_S test revealed negative and significant values for Green Point and Ballito populations. These results support the initial hypothesis proposed for a recent population expansion as negative values for both tests imply that there is retention of rare mutations in the population indicative of a population that has recently expanded (De Jong et al. 2011).

A similar pattern of population expansions has been observed for various seahorse species (Zhang et al. 2014) and for the diamondback moth from Africa (Wei et al. 2013). This collection of rare mutations was also identified by the high haplotype and low nucleotide diversities displayed for all populations. However, it should be noted that the discrepancies between the significance of Tajima's D and Fu's F_S test is attributed to sensitivity of the test itself. Fu's F_S test is commonly used as a more reliable and powerful source for population expansion analyses (De Jong et al. 2011). The unimodal distributions shown for the COI gene for populations of *Marphysa corallina* signify recently expanding populations. Unimodal distributions can also be influenced by a selective sweep for advantageous alleles, which would reach high frequencies in a population subject to this process (McMillen-Jackson & Bert 2004;

Mirol et al. 2008). A selective sweep in the COI gene could have occurred for all populations of *Marphysa corallina* as can be shown by the high haplotype frequencies and unimodal distributions found. For the ITS1 gene most of the populations display a multimodal distribution as seen in figure 3.5, p.86. Multimodal distributions are indicative of stable populations and large effective population sizes; however, this distribution is not supported by the non-significant high values for the raggedness index and SSD statistic (Table 3.6, p.85).

Populations of *Marphysa corallina* sampled show only shallow genetic structuring for ITS1 and no structuring for COI. It is therefore assumed that together with the genetic data indicating recent population expansions and shallow genetic structure, populations of *M. corallina* along the South African coast have not yet reached migration-drift equilibrium. As a result the patterns of genetic homogeneity across all sampled populations are attributed to past demographic history, which could have been influenced by climate change (Chatzigeorgiou et al. 2014).

The expansion time calculated for *Marphysa corallina* populations all pre-date the Last Glacial Maximum, ranging between 138 344 – 77 818 years ago. The last glacial maximum was estimated to have occurred approximately 21 000 years ago (Stone 2014), thus placing population expansion of *Marphysa corallina* in the mid to late Pleistocene. Results from the bayesian skyline plot analysed for the overall change in effective population size of *Marphysa corallina* indicate that a gradual population decline had begun aproximately 113 600 years ago. This population decline appears to have occurred in the middle of the Pleistocene glacial cycles, congruent with the above findings. This decline was followed by a rapid expansion around 22 700 years ago during the late Pleistocene. This result deviated from the general consensus found for South African marine taxa which normally expand after glacial episodes as documented by Reynolds et al. (2014). However, there exists a considerable amount of research that has found pre LGM expansions such as the South African barnacle *Tetraclita serrata* (Reynolds et al. 2014) and two seahorse species, *Hippocampus trimaculatus and H.*

mohnikei from the southeast coast of China (Zhang et al. 2014). In addition, Marko et al. (2010) conducted an investigation using multiple rocky shore species from the Northern hemisphere and found that the majority of species expansions occurred after the Pleistocene, before the Last Glacial Maximum.

During glacial periods, climatic conditions fluctuate rapidly affecting the normal distribution of species that are intolerant to extreme changes in temperatures and conditions (McMillen-Jackson & Bert 2004; Zhang et al. 2014). The South African coast did not experience any ice-cover during these glacial cycles but sea levels decreased by approximately 130 m exposing extensive beds of intertidal habitats and consequently destroying those (Reynolds et al. 2014). This could have forced many rocky intertidal species to contract into smaller refugial populations thus decreasing their ranges (De Jong et al. 2011; Zhang et al. 2014). Temperatures increased thereafter and sea levels rose which would have facilitated in-range expansions of many intertidal species from these refugial populations. This would have resulted in the newly colonising populations being identical to one another, implying extensive gene flow (Maggs et al. 2008).

The results obtained in this study are consistent with the hypothesis of multiple colonisations of *Marphysa corallina* that have been shaped by Pleistocene glacial cycles. The phylogenetic tree of *M. corallina* has many unresolved branches that could be a result of incomplete sorting of lineages and, thus, a retention of rare haplotypes caused by rapid population radiations (Avise et al. 1987). The topology of this tree supports the hypothesis of recent expansions of *Marphysa corallina.*

In conclusion, patterns of little or no genetic structure observed for *Marphysa corallina* in the present study lead to the assumption that these populations are not in migration-drift equilibrium due to the climatic events that caused recent expansions. These expansions would then have prevented genetic differentiation thus giving the impression of a large homogenous population which one would readily interpret as high

gene flow. Due to the lack of studies available on the larval biology of *M. corallina* it has proved difficult to ascertain whether genetic homogeneity is a result of high dispersal ability. Nonetheless, looking at the larval biology of closely related species it has been assumed that *Marphysa corallina* could potentially be a low dispersing species therefore the idea of highly connected populations was not expected in the present study. Strong evidence has been found that populations have diverged from its recent common ancestor during the middle to late Pleistocene period and have since been going through large population expansions and colonising new available habitats. Thus the Pleistocene climate appears to have influenced the contemporary distributional ranges of *Marphysa corallina* in South Africa*.*

Chapter four: Trends and future research

4.1. Phylogeny of intertidal Nereididae and Eunicidae polychaetes

Polychaete worms are known to be one of the most diverse groups of worms that are well represented in freshwater and marine environments (Day 1967). The incredible ability of polychaetes to inhabit a variety of habitats is due to the diversity of feeding modes and reproductive strategies displayed by the group, which in evolutionary time resulted in new adaptations and potentially new species (Wilson 1991; Bartolomaeus et al. 2005; Gambi & Cigliano 2006). The plasticity of morphology presented by the group together with their intricate evolutionary histories leave many gaps in the classification of polychaete worms. The consequences of such plasticity have resulted in questionable phylogenetic relationships among taxa (Zhou et al. 2010). In terms of South African polychaete worms, not many studies are available on the phylogenetic relationships of these worms. For this reason, chapter two was set out to investigate the phylogenetic relationships of commonly found intertidal eunicid and nereidid worms from the South African coast.

A bayesian phylogenetic tree revealed two distinct clades, one with the monophyletic Nereididae and the other containing one polyphyletic Eunicidae with Lumbrineridae and Onuphidae nesting among the eunicid taxa. Two of the genera (*Marphysa* and *Eunice*) analysed for the Eunicidae were found to be polyphyletic. *Marphysa* species were found nested within a *Eunice* clade that was recovered in previous studies. This clade strongly supports the resurrection of the *Nicidion* as was recently amended. Those *Marphysa* species have been placed in *Nicidion.* Species belonging to the newly resurrected *Leodice* formed an exclusive grouping with its previously described type specimen *Eunice anetennata* and other *Eunice* species. Those species previously belonging to *Eunice* have been synonymized with *Leodice.* Results from the present study strongly agree with the newly resurrected and emended genera.

In the large *Marphysa* clade, there is evidence that the morpho-groups described for the genus form separate clades. However, some relationships were unresolved in this clade due to insufficient taxon sampling. As a result, it is suggested that more species of *Marphysa* be included in subsequent studies in order to determine whether these morpho-groups actually exist which would then potentially result in the classification of these morpho-groups as subgenera. Overall, the morphology and phylogeny of *Marphysa* and *Eunice* need to be revised. The suggested groupings of these genera could possibly result in natural monophyletic groupings.

The monophyly of family Nereididae was recovered, however, relationships between genera and species were complicated. *Pseudonereis* is a monophyletic genus however in the present study it was polyphyletic with *Perinereis* nested in the clade. With a larger number of taxa included in future studies, the monophyly of this genus could be recovered. Due to *Nereis* being a large speciose genus, species were assigned to numerous informal groupings within the genus. Species belonging to *Nereis* grouped up into separate well supported clades which could resemble one of the many informal groups proposed for the genus. It is suggested that future studies explore these informal groupings for *Nereis* by including a large number of taxa in their analyses. In the Nereididae clade, many of the inner nodes representing species and genus level relationships were weak and not supported. Therefore it is suggested that each genus within the family be thoroughly analysed and revised using both morphological and molecular data which could potentially result in more clarity regarding relationships within and between genera and species.

4.2. Genetic Structure and demographic history of *Marphysa corallina*

Understanding patterns of diversity, connectivity and past demographic histories of populations and how they impact the present distributions of species in the marine environment is a fundamental aspect that is explored in population genetics and

phylogeography (Grosberg & Cunningham 2001; Marko & Hart 2011). Natural populations are largely influenced by anthropogenic effects and climate change. Information about population connectivity and demography will aid ecologists and biologists in monitoring natural populations that are most likely to adapt to changing environmental conditions ensuring their survival and populations that die out due to their inability to adapt (Lowe & Allendorf 2010; Kelly & Palumbi 2010; Ayata et al. 2010).

The genetic structure, connectivity and demography of *Marphysa corallina* have never before been studied. *Marphysa corallina* was observed to be the most abundant polychaete among others on South Africa's east coast rocky shores. Polychaete worms form an integral part of their communities and function as ecosystem engineers, thus it is imperative to understand the demography, structure, diversity and connectivity of these important invertebrates. As a result, chapter three was set out to investigate the genetic structure and demographic history of the abundant rocky shore polychaete *M. corallina* from two regions: KwaZulu-Natal and Eastern Cape in South Africa.

The COI data revealed that all 8 populations of *M.corallina* were extensively connected to one another, which was further supported by the large number of migrants estimated moving between populations and forming a large panmictic population. The ITS1 data in contrast showed shallow genetic structuring for this species. Due to a lack of information available regarding reproductive strategies of this species in question, a general pattern for species belonging to the genus was assumed instead. *Marphysa* species are known to produce short lived lecithotrophic larvae as a result this reproductive strategy was not congruent with the high population connectivity obtained.

Due to the lack of population genetic studies available, comparisons could not be drawn with other studies. Nonetheless, demographic analyses were conducted and all indicated a sudden recent population expansion for all populations of *Marphysa*

corallina. Thereafter coalescent times were estimated and it was found that all populations have recently expanded during the mid to late Pleistocene. The ancestral populations could not be identified which is possibly due to incomplete sampling of the species range.

One of the major drawbacks of this study was the lack of information available on *M.corallina.* A lot of difficulty was encountered when estimating generation times, larval behaviour and general ecology of this species which was required to accurately make assumptions on the demographic history. Also incomplete sampling along the east coast of South Africa posed a problem when estimating various population genetics statistics. It has been documented by Helberg (2009) that an overestimation of gene flow will occur due to the presence of ghost populations. These ghost populations are those populations of *M. corallina* that were not sampled but contributed to the gene flow of populations documented in this study. The uneven sample size could have resulted in an over estimation of effective population sizes, diversities and gene flow.

As a result, future studies should focus on identifying the distributional ranges, reproductive biology and other general ecological aspects of *Marphysa corallina*. More sample sites need to be included for the entire distributional range of *M. corallina* to allow for proper estimations of gene flow and to identify ancestral populations and the origin of the multiple radiations that took place. And lastly, a more uniform sample size should be employed to prevent the over estimation of various population genetic indices.

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