Taxonomic and molecular assessment of the apparently alien and cosmopolitan Nereididae Blainville, 1818 polychaetes from South Africa

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Declaration

By submitting this dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

This dissertation includes one original paper published in a peer-reviewed journal and three unpublished publications. The collection, processing and analysis of data and writing of the papers (published and unpublished) were the principal responsibility of myself whereas Dr Carol Simon and Dr Angus Macdonalad were responsible for editing of the manuscripts thereafter.

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Abstract

Polychaete worms provide many ecosystem services and are useful indicator species. Some are invasive and may threaten biodiversity and ocean economies. However, the convoluted taxonomic history of polychaetes has led to incorrect classifications of indigenous and alien species as they are commonly mistaken for cosmopolitan species. Thus, species with a dubious taxonomic status are termed pseudocosmopolitan species. Three such species, *Pseudonereis variegata* Grube, 1857*, Platynereis dumerilii* Audouin & Milne Edwards, 1833 and *Platynereis australis* Schmarda, 1861 were prioritised for investigation owing to their alien or cryptogenic status elsewhere, multiple synonymised names, globally disjunct distribution and a widespread South African distribution. The first overarching aim of this thesis was to determine whether these three species are indigenous or alien to South Africa or harbour cryptic and new species. Thorough morphological revisions and molecular datasets (mtCOI and nDNA) revealed that all three species are indigenous to South Africa. The local *Pseudonereis podocirra* n. comb. (Schmarda, 1861) was incorrectly synonymised as *P. variegata* (type locality: Chile) and *Platynereis* B sp. nov. was misidentified as *P. dumerilii* (type locality: Mediterranean). Individuals identified as *P. australis* (type locality: New Zealand) represent *P. massiliensis* (Moquin-Tandon, 1869) (type locality: Mediterranean), but since individuals from Italy and Portugal nested within the South African clade and had comparatively low genetic diversities, this species is likely indigenous to South Africa and alien in the Mediterranean. However, because *P. massiliensis* is part of a cryptic species complex in the Mediterranean, its name is considered doubtful and hence is referred to as *P. massiliensis s.l*. *Pseudonereis podocirra's* wrongful synonymisation was due to poor species descriptions and conservative taxonomic views whereas misidentifications of the two *Platynereis* species was because they are truly cryptic. These results together with the finding that ~50% of other local nereidids may be pseudocosmopolitan, indicate that diversity of nereidids has been underestimated. Short-term solutions are proposed when dealing with pseudocosmopolitan polychaete species and recommendations are made regarding the clarification of *P. massiliensis s.l.* in the Mediterranean, the molecular identification of the global *Platynereis* species complex and taxonomic revisions of *P. variegata* from Chile. The second overarching aim was to determine factors contributing to the phylogeographic structure of these three

sympatric South African species to determine the underlying factors driving their present-day distributions. Using mtCOI, *P. massiliensis s.l.* displayed three geographically structured lineages separated by the Cape Point and Cape Agulhas phylogeographic breaks whereas *Platynereis* B sp. nov. showed two well-mixed regional lineages separated by the Cape Agulhas break. In contrast, nDNA demonstrated well-mixed populations for both species and the intrinsic properties of each marker was used to explain the differences in patterns. *Pseudonereis podocirra* exhibited a panmictic meta-population using a mitochondrial dataset which persisted even when using a high-throughput SNP dataset. Age, hence resilience, were likely factors contributing to the contrasting patterns of structure and connectivity as *P. podocirra* n. comb. was demonstrated to be an evolutionarily older species. Fluctuating temperatures and paleo-conditions during the Pleistocene probably resulted in the southeastern expansions of all three species while radiations along the west coast were inferred for *Platynereis* B sp. nov. and *P. podocirra. Platynereis* B sp. nov. and *P. massiliensis s.l.* speciated sympatrically due to reproductive isolation and temperature, whereas *P. massiliensis s.l.* and *P. dumerilii* are hypothesised to have speciated allopatrically. All *Platynereis* species display evidence of morphological stasis despite their ancient divergence times. *Pseudonereis podocirra* and *P. variegata* (Chile) speciated allopatrically and have undergone morphological stasis or convergence. Historical climatic oscillations, oceanographic currents, ecoregions and larval development were factors contributing to the phylogeographic structure whilst allopatry and sympatry coupled with morphological stasis were identified as the most likely mode and mechanism of cryptic speciation of these species in South Africa.

Opsomming

Borselwurms verteenwoordig ‘n groot deel van seebodem habitat diversiteit en verskaf vele ekosisteem dienste insluitend voedingstof hersirkulasie en habitat voorsiening. Baie is indringer-spesies en kan die plaaslike diversiteit en mariene-ekonomie bedreig. Bygesê, die ingewikkelde taksonomiese geskiedenis van borselwurms het gelei tot die inkorrekte klassifikasie van inheemse en indringer spesies deur hul as wydverspreide of kosmopolitiese spesies te klassifiseer. Dus, spesies met ‘n twyfelagtige taksonomiese status word pseudo-komopolitaanse spesies genoem. Drie sulke spesies, *Pseudonereis variegata* Grube, 1857*, Platynereis dumerilii* Audouin & Milne Edwards, 1833 en *Platynereis australis* Schmarda, 1861 is as prioriteit beskou vir verdere ondersoek namate hul status as indringer-, twyfelagtige- of kriptogeniese- spesies elders, asook verskeie sinonomiese name, gebroke globale verspreiding en wydverspreide Suid-Afrikaanse verspreiding. Die eerste oorkoepelende doelwit was om vas te stel of hierdie Suid-Afrikaanse spesies wel inheems tot die streek is. Deeglike morfologiese wysigings en molekulêre datastelle (mtCOI en nDNS) het al drie spesies as inheems openbaar. Die plaaslike *Pseudonereis podocirra* n. comb. (Schmarda, 1861) is inkorrek as sinoniem van *P. variegata* (tipe ligging: Chile) aangedui en *Platynereis* B sp. nov. was verkeerdelik identifiseer as *P. dumerilii* (tipe ligging: Mediterreens). Individue identifiseer as *P. australis* (tipe ligging: Nieu-Seeland) verteenwoordig *P. massiliensis* (Moquin-Tandon, 1869) (tipe ligging: Mediterreens), maar sedert individue van Italië en Portugal geneste is binne die Suid-Afrikaanse klade en vergelykend lae genetiese diversiteit gehad het is daar tot die gevolgtrekking gekom dat hierdie spesie inheems tot Suid-Afrika is en ‘n indringer spesie in die Mediterreense see is. Omdat *P. massiliensis* deel vorm van n kriptiese spesie-kompleks in die Mediterreense see word die naam as twyfelagtig beskou en word dus voortaan verwys na “*P. massiliensis*”. Die verkeerdelike sinonomie van *Pseudonereis podocirra* was as gevolg van onvoldoende spesie beskryfwings asook die konservatiewe uitsigte van taksonomiese kenners terwyl die verkeerdelike identifikasie van die twee *Platynereis* spesies plaasgevind het as gevolg van hul kriptiese morfologie. Hierdie resultate tesame met die vinding dat ~50% van ander plaaslike nereidid spesies pseudo-kosmopolitaans kan wees is beduidend daarop dat die diversiteit van nereidid speies onderskat is. Kort-termyn oplossings word voorgestel vir die hantering van pseudo-kosmopolitaanse spesies en aanbevelings

word gemaak met betrekking tot die klassifikasie van *"P. massiliensis"* in die Mediterreense see, die molekulêre identifikasie van die globale *Platynereis* spesie kompleks en taksonomiese wysigings van *P. variegate* van Chile. Die tweede oorkoepelende doelwit was om vas te stel watter faktore bydrae tot die filo-geografiese strukture van die drie simpatriese Suid-Afrikaanse spesies om sodoende die onderliggende faktore wat hedendaagse verspreiding dryf vas te stel. Met mtCOI het *"P. massiliensis"* drie geografies gestruktureerde bevolkings gehad wat geskei is by die Kaapse Punt en Kaap Agullhas filo-geoagrafiese breuke terwyl *Platynereis* B sp. nov. net twee goed gemengde plaaslike afstammelinge aandui wat geskei is by die Kaap Agullhas breuk. In kontras het die nDNS goed gemengde bevolkings aangedui vir beide spesies en die intrinsieke eieskappe van elke merker is gebruik om die verskille in patrone te verduidelik. *Pseudonereis podocirra* het 'n panmitiese meta-bevolking aangedui met 'n mitochondriese daastel en die is volhou selfs na die gebruik van 'n hoë-deurset ENP datastel. Ouderdom, en dus veerkragtigheid, was waarskynlik faktore wat bygedra het tot die kontrasterende patrone van struktuur en verbinding aangesien *P. podocirra* n. comb. demonstreer is as die ouer evolusionêre spesie. Wisselende temperature en paleo-toestande gedurende die Pleistoseen het waarskynlik gelei tot die suidoosterse uitbreidings van al drie spesies terwyl uitbreidings na die weskus net afgelei is vir *Platynereis* B sp. nov. en *P. podocirra*. *Platynereis* B sp. nov. en *"P. massiliensis"* het simpatriese spesiasie ondergaan as gevolg van reproduktiewe isolasie en temperatuur terwyl *"P. massiliensis"* en *P. dumerilii* waarskynlik allopatriese spesiasie ondergaan het. Alle *Platynereis* spesies toon bewyse van morfologiese stase ten spyte van hul antieke afwykingstye. *Pseudonereis podocirra* en *P. variegata* (Chile) het allopatriese spesiasie ondergaan en het morfologoese stase of konvergensie ondergaan. Historiese klimaat wisselinge, oseografiese strome, eko-streke en larwe ontwikkeling was faktore wat bygedra het tot die filo-geografiese struktuur terwyl allopatrie en simpatrie gekoppel met morfologiese stase identifiseer is as die mees waarskynlike modus en meganisme van kriptiese spesiasie van hierdie spesies in Suid-Afrika.

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Dedication

This thesis is dedicated to my parents, Ashok and Daisy Kara who have given me the greatest gift in life: Education

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Chapter One: General Introduction

Polychaete worms in the marine environment

Polychaete worms comprise a dominant component of almost all marine and estuarine benthic habitats, some of which are allogenic ecosystem engineers that form an integral part of their environment (Hutchings 1998; McHugh 2000; De Assis et al. 2012). As such, they modify the structure and topography of their environments, often providing a healthier system for other species to thrive in (Rilov et al. 2012). These include structural or habitat changes, such as the extensive calcium carbonate reefs created by serpulid worms, directly resulting in more available habitat for other species to settle on and indirectly controlling the availability of abiotic and biotic resources (Hutchings 1998; Currie et al. 2000; Rilov et al. 2012). On the other hand, burrowing worms play an important role in bioturbation in their environments resulting in the redistribution of organic matter trapped in sand pockets, aeration of anoxic sediment, regulation of water flow and nutrient mineralization (Hutchings 1998; Olsgard et al. 2003; Brett 2006; Ito et al. 2011). Polychaete worms also serve as food sources to birds, commercially important demersal fish and are the main diet preference of cuttle fish (Kalejta 1993; Brett 2006; Rivera and Rivera 2008).

In addition to these many ecosystem services, polychaetes serve as bait species for fishing activities and in the Indo-Pacific region the epitokes (sexually mature form) of the *Palola* Gray in Stair, 1847 worms are considered a delicacy and consumed by the (van Herwerden 1989; Bakken and Wilson 2005; Schulze and Timm 2012). Other polychaetes are used as pollution indicators for environmental monitoring, toxicological test animals for heavy metal contamination, bioassay organisms and model organisms to investigate evolutionary development and gene expression patterns (Grassle and Grassle 1976; van Herwerden 1989; Pocklington and Wells 1992; Reish and Gerlinger 1997; Arendt et al. 2002; Olsgard et al. 2003; Fischer and Dorresteijn 2004; de Rosa et al. 2005; Elías et al. 2006; Surugiu 2009; Fischer et al. 2010; Carr 2012; Garaffo et al. 2012). While such applications are important for the monitoring of disturbed and undisturbed marine environments and understanding the evolutionary development of invertebrates, these applications are only valuable if species have been identified correctly (Carr 2012).

Even though the polychaete group is very diverse with over 10, 000 described species from among an estimated total of 25, 000 – 30, 000 species worldwide, they have been routinely omitted from broad-scale studies investigating distribution (Hutchings 1998; Carr et al. 2011; Carr 2012). This has resulted in a significant gap in the knowledge of the diversity and distributional patterns of many polychaete species worldwide (Carr et al. 2011; Carr 2012). This knowledge gap in distribution patterns of polychaete worms has resulted from the high frequency of pseudocosmopolitan species (i.e. species that occur in more than one ocean basin that have a dubious taxonomic identity) for this group (Hutchings 1998; Carr et al. 2011; Carr 2012; Darling and Carlton 2018; Hutchings and Kupriyanova 2018).

The world-wide polychaete challenge

Cosmopolitan and cryptic species

The belief in cosmopolitanism in the polychaete group was prevalent throughout the 1900's (Salazar-Vallejo et al. 2017; Hutchings and Kupriyanova 2018). However, in recent years research has increasingly shown that perceived cosmopolitan distributions within this group are an artefact of the combination of several historical factors at play (Hutchings and Kupriyanova 2018). These factors include poor species descriptions, lack of type specimens to facilitate accurate species identifications, the European taxonomic bias and the conservative views of earlier influential taxonomists (Salazar-Vallejo et al. 2017; Hutchings and Kupriyanova 2018). Since the standard of descriptions in the 18th and 19th centuries were very different from present-day, occasionally only a name was assigned and type material rarely deposited (Hutchings and Kupriyanova 2018). In cases where a description was provided, it was brief and diagnostic characters were based solely on morphological differences that were often poorly illustrated (Westheide and Hass-Cordes 2001; Westheide and Schmidt 2003; Carr et al. 2011; Nygren 2014; Hutchings and Kupriyanova 2018). However, many polychaete worms have simple body plans such as those belonging to the family Amphinomidae Lamarck, 1818 (Barroso et al. 2009) and consequently have a limited number of visible morphological features that could have been incorporated into earlier species descriptions (Westheide and Schmidt 2003; Nygren 2014). Furthermore, thorough taxonomic identifications were

especially problematic for soft bodied polychaete worms because incorrect preservation methods resulted in the loss of appendages and changes in body colour which are important for the delineation of individual species (Costello et al. 2010; Nygren 2014; Hutchings and Kupriyanova 2018). In addition, this traditional taxonomic approach did not consider reproductive differences, physiological tolerances and adaptations to specialised habitats which do not present as visible morphological traits (Knowlton 1993; Klautau et al. 1999; Bickford et al. 2007; Nygren 2014).

Since the majority of these early descriptions were based on European fauna, as taxonomists were predominantly based there, polychaete taxonomy has a strong European bias (Hutchings and Kupriyanova 2018). Additionally, these taxonomists had conservative views which supported the concept of large morphological variation within a species and widespread and or cosmopolitan distributions (Hutchings and Kupriyanova 2018), despite the fact that contemporary natural gene flow among populations of species in geographically distant localities is almost impossible (Klautau et al. 1999; Geller et al. 2010). Thus, when specimens were collected from remote places such as the Caribbean, Japan, South America and Africa, taxonomic assignments were often made based on the existing names of European fauna despite substantial variation in morphology between species, thereby exacerbating the erroneous designation of poorly defined cosmopolitan species (Costello et al. 2010; Griffiths et al. 2010; Hutchings and Kupriyanova 2018). Nonetheless, with the advances in technology and ease of access to molecular methods, an increasing number of these perceived cosmopolitan species have dissolved into complexes of genetically distinct species that are either morphologically identical to one another (i.e. cryptic species) (e.g. Manchenko and Radashevsky 1998; Halt et al. 2009; Rius and Teske 2013; Lucey et al. 2015; Wäge et al. 2017) or morphologically similar (i.e. pseudocryptic species) (e.g. Glasby et al. 2013; Villalobos-Guerrero and Carrera-Parra 2015; Park and Kim 2017; Simon et al. 2017, 2018; Conde-Vela 2018). Such species are termed pseudocosmopolitan as their widespread distributions are actually a result of their dubious taxonomic histories (Darling and Carlton 2018). In many instances, the newly uncovered cryptic and pseudocryptic species were undescribed indigenous species (e.g. Lewis and Karageorgopoulos 2008; Clarke et al. 2010; Carr et al. 2011; Nygren and Pleijel 2011; Glasby et al. 2013; Tomioka et al. 2016; Simon et al. 2017, 2018; Zanol et al. 2017) and or incorrect

synonymisations of existing indigenous fauna (e.g. Villalobos-Guerrero and Carrera-Parra 2015; Conde-Vela 2018). However in other cases, they were alien species that went unnoticed in the introduced habitat (e.g. Sun et al. 2016) and have attained a widespread distribution as a result of the accidental anthropogenic transport around the world and are termed neocosmopolitan species (Darling and Carlton 2018).

Since the beginning of transoceanic travel in the early 1400's, marine biota were transported unknowingly between different places around the world (Carlton 2003, 2009; Rius et al. 2015). Even with the commencement of taxonomic studies in the late 18th and early 19th centuries, people were still unaware of the contribution that shipping was having on the movement of marine biota (Carlton 2009; Hutchings and Kupriyanova 2018). Due to the strong European taxonomic bias, many marine species were incorrectly classified as native to Europe when in fact they were alien, having arrived prior to collection and taxonomic description (Carlton 2009; Haydar 2010; Pineda et al. 2011; Rius et al. 2015; Sun et al. 2016). These species should at best be considered as cryptogenic due to the uncertainty surrounding their origin (Carlton 1996a, Klautau et al. 1999, Carlton 2009, Rius et al. 2015). Consequently many of these marine species may have been mislabelled as "widespread" or "cosmopolitan", when in fact this cosmopolitan distribution is an artefact of the extensive, unintentional, undocumented translocation of marine species around the world, and their status in Europe should actually be cryptogenic and or alien (Haydar 2010; Pineda et al. 2011; Pérez-Portela et al. 2013; Dijoux et al. 2014).

Mechanisms of cryptic speciation

Despite the increase in the number of cryptic species in current literature, the mechanisms contributing to such speciation are poorly understood (Beheregaray and Caccone 2007; Fišer et al. 2018; Struck et al. 2018). The speciation of cryptic taxa is considered to result from diversification without a change in gross morphology (Gittenberger 1991; Bickford et al. 2007). As a result, genes for reproductive incompatibility, which do not necessarily require a change to the morphological phenotype, are fixed throughout the population by either genetic drift and sexual selection or both (Bickford et al. 2007). Four hypotheses have been proposed to explain speciation of cryptic taxa

(Fišer et al. 2018; Struck et al. 2018). These are: 1) Recent divergence, when closely related cryptic species have diverged from one another relatively recenty, leaving very little time for any substantial morphological differences to develop; 2) Parallelism, when distantly related cryptic taxa have independently evolved from morphologically similar ancestors; 3) Morphological stasis, when closely related crypic species have diverged from one another over millions of years and 4) Morphological convergence, when distantly related species evolve to resemble one another due to the selection pressures imposed by their environments (Bickford et al. 2007; Fišer et al. 2018; Struck et al. 2018).

While the processes contributing to ecological speciation and thus morphologically distinct species are more commonly known, the mechanisms responsible for speciation resulting in identical species remains understudied (Fišer et al. 2018). Additionally, there is a growing need to understand the types of selection and or neutrality that contribute to the evolution of non-visible phenotypic traits in diversified species which will consequently shed light on whether they vary across geographic space and environmental gradients (Fišer et al. 2018). Furthermore, by concentrating research efforts on the mechanisms of cryptic speciation, taxonomists may thus provide evolutionary evidence for such cryptic species (Fišer et al. 2018). Since understanding the mechanisms contributing to cryptic speciation requires that multiple co-distributed species be compared in a phylogeographic dataset, results from this will provide important information for the discovery of biodiversity and management as they can potentially identify biodiversity hotspots (Beheregaray and Caccone 2007; Struck et al. 2018). To date, only one study has investigated the mechanisms responsible for cryptic speciation of polychaete worms (i.e. Struck et al. 2017), further demonstrating the gap in knowledge on the mechanisms contributing to cryptic speciation considering the prevalence of cryptic species in polychaete research.

Alien invasions

A species is considered alien when it has been transported outside its natural distributional range and establishes a breeding population in its introduced range (Carlton 1989, 2009; Hutchings et al. 2002; Crooks 2002; Blackburn et al. 2014; Robinson et al. 2016). As the cosmopolitan distribution of many polychaetes is a result of the extensive global transport (e.g. Bastrop et al. 1997), both

historical and contemporary (Pineda et al. 2011; Pérez-Portela et al. 2013; Dijoux et al. 2014; Rius et al. 2015; Hutchings and Kupriyanova 2018), many polychaetes are actually aliens. The presence of long-lived planktonic larvae observed for many taxa has facilitated their transportation to other regions via ship ballast water and the sedentary and filter feeding habits has facilitated the transport of tube dwelling worms on the hulls of ships (Carlton and Geller 1993; Carlton 1996; Mead et al. 2011a, b; Çinar 2012). Polychaetes such as *Polydora* spp. Bosc, 1802 bore into the shells of commercially cultivated molluscs and are frequently transferred with their hosts to other parts of the world (Simon and Sato-Okoshi 2015; Williams et al. 2016, 2017).

Alien species can be categorised as either naturalized or invasive in the introduced range depending on their impact on the surrounding fauna (Robinson et al. 2016). An alien species becomes invasive once it substantially expands its distributional range in the introduced range (Blackburn et al. 2014; Robinson et al. 2016). Invasive species may out compete native fauna for basic resources and some change the abiotic conditions in the introduced environment (ecosystem engineers) resulting in structural shifts in the native community (Crooks 2002; Blackburn et al. 2014; Robinson et al. 2016). Naturalised species on the other hand establish a breeding population at the point of introduction but do not spread to other places (Ray 2005; Blackburn et al. 2011; Robinson et al. 2016). Alien invasions are regarded as one of the most serious threats to biodiversity and the preservation of native species (Carlton and Geller 1993; Ricciardi and Rasmussen 1998; Stachowicz et al. 1999; Patti and Gambi 2001; Carlton 2009; Arias et al. 2013; Katsanevakis et al. 2014). Coupled with this is a further cause for concern; many invader species are known to consist of cryptic lineages that cannot be detected using morphological characters alone (Chapman 1988; Chapman and Carlton 1991; Bastrop et al. 1997; Carlton 2009; Sun et al. 2017a).

Detecting misidentified polychaete species and invasion pathways of alien species

Molecular tools are necessary in conjunction with taxonomic identifications for the detection of cryptic species, genetic diversity and distributional patterns of taxa (Knowlton 2000; Nygren 2014). This has been demonstrated for several cosmopolitan species that have since been shown to comprise complexes of species (e.g. Carr et al. 2011; Nygren 2014; Sun et al. 2016, 2017b, a; Sato-Okoshi et

al. 2017; Simon et al. 2017, 2018). In Nygren (2014), 87 nominal species were found to each comprise complexes of three or more cryptic species. Some of these cases are highlighted below.

The taxonomic reassessment of the cosmopolitan bearded fireworm *Hermodice carunculata* Pallas,1766*,* originally considered the only known species in its genus, revealed differences in the number of branchial filaments resulting in the re-instatement of its junior taxon *Hermodice nigrolineata* Baird, 1868 (Yáñez-Rivera and Salazar-Vallejo 2010). Similarly, since the designation of a neotypic specimen for *Marphysa sanguinea* (Montagu, 1815) (type specimen of Genus), studies investigating the taxonomic status of this cosmopolitan species and others belonging to the genus *Marphysa* (Quatrefages, 1865) have uncovered at least 11 pseudo-cryptic species with restricted distributions (Hutchings and Karageorgopoulos 2003; Lewis and Karageorgopoulos 2008; Glasby and Hutchings 2010; Idris et al. 2014; Katsiaras et al. 2014; Kurt Sahin 2014; Zanol et al. 2016, 2017; Lavesque et al. 2017; Elgetany et al. 2018), whilst three species regarded as junior synonyms of *M. sanguinea* from the Grand Caribbean were found to be incorrect synonymisations and consequently reinstated (Molina-Acevedo and Carrera-Parra 2015). In these examples, perceived cosmopolitanism was a result of incomplete and poor species descriptions of type specimens coupled with over-conservative taxonomic practices which had resulted in historically incorrect identifications and synonymisations of local species (Hutchings and Karageorgopoulos 2003; Lewis and Karageorgopoulos 2008; Idris et al. 2014; Molina-Acevedo and Carrera-Parra 2015; Zanol et al. 2016, 2017; Lavesque et al. 2017; Elgetany et al. 2018).

The application of molecular methods and taxonomic revisions of the cosmopolitan *Perinereis cultrifera* Grube, 1840 revealed that populations from adjacent marine and estuarine habitats actually represent morphologically and genetically distinct sister species and not a single species (Maltagliati et al. 2001). Using molecular methods, Barroso et al. (2009) and Arias et al. (2013) investigated the fireworm *Eurythoe complanata* Pallas, 1766 from the South Atlantic, Pacific and Caribbean basins and uncovered three cryptic species. They found that the apparent cosmopolitanism of *E. complanata* was a result of the simple body plans exhibited by species of Amphinomidae Lamarck, 1818 (Barroso et al. 2009; Arias et al. 2013). Similarly, *Capitella capitata* Fabricius, 1780 was thought to be a single species inhabiting organically rich environments until 1969 (Grassle and Grassle 1976;

Blake et al. 2009). Since then, at least 12 or 13 sibling species have been described based on differences in life histories, genetics and reproduction (Blake et al. 2009). Similarly, Bleidorn et al. (2006) revealed five genetically distinct lineages for *Scoloplos armiger* Muller, 1776, with two corresponding to the Pacific and three lineages specific to the North Atlantic. In these instances, perceived cosmopolitanism resulted from the lack of distinguishing features used for species differentiation (Westheide and Schmidt 2003; Barroso et al. 2009; Arias et al. 2013; Tomioka et al. 2016).

The aforementioned studies have demonstrated how unresolved taxonomy has led to incorrect identifications and synonymisations of species, resulting in a failure to recognise local indigenous species. This has not only led to the underestimation of regional indigenous species diversity, but has also led to the underestimation of alien species as many have gone undetected as they have been mistaken for widespread or cosmopolitan species (Carlton 2009; Griffiths et al. 2009; McGeoch et al. 2012; Nygren 2014). This misrepresentation of alien species on biodiversity inventories has serious implications for the conservation and management of these species as early warning of potential introductions, prevention and control measures rely on correct alien inventories (McGeoch et al. 2012), an effect that has been demonstrated several times before. For example, two spionids, *Marenzelleria viridis* Verrill, 1873 and *Marenzelleria wireni* Augener, 1913 were initially described from the North Sea but were both recognised as *M. viridis* in subsequent studies due to the lack of distinguishing morphological characters (Bastrop et al. 1997). However, populations analysed from the Baltic and North seas and Atlantic coast of America comprised two cryptic species (Bastrop et al. 1997). *Marenzelleria viridis* had in fact been introduced to the North Sea from Nova Scotia (North America) and subsequently spread to the Baltic Sea whereas the introduction of *M. wireni* into the Baltic Sea was suggested to have orignited from either Chesapeake Bay to Georgia or possibly from New Hampshire or the Arctic Ocean (Bastrop et al. 1997). Genetic studies revealed that neither species was indigenous to the Baltic and North seas and two independent introduction events were responsible for the presence of *Marenzelleria* Mesnil, 1896 species in Europe (Bastrop et al. 1997).

In another instance, the nereidid polychaete *Hediste diversicolor* (O.F Muller, 1776) was considered to be widespread across the Northeast Atlantic and Baltic Sea, the Mediterranean Sea, Black Sea,

Caspian Sea and the Northeastern Pacific (Scaps 2002; Audzijonyte et al. 2008; Virgilio et al. 2009; Einfeldt et al. 2014). However genetic analyses revealed that the Northeast Atlantic, Mediterranean, Black and Caspian Sea populations included three cryptic species (Virgilio et al. 2009). Additionally, the Northeastern Pacific individuals consisted of two cryptic species that were introduced independently at least three times from European populations (Einfeldt et al. 2014). Furthermore, the Baltic Sea population consisted of two cryptic species (Species A and B), of which species B further consisted of two genetically divergent lineages that resulted from two independent introductions from Quebec and the western Mediterannean, Black and Caspian seas (Audzijonyte et al. 2008).

Frequently alien species have significant impacts in their introduced ranges (McGeoch et al. 2012), and undetected aliens have the opportunity to become established which poses significant risks to biodiversity and ocean economies. For example *Sabella spallanzanii* Gmelin, 1791 is an invasive ecosystem engineer of Mediterranean and European Atlantic origin and became invasive in Australia and New Zealand (Currie et al. 2000; Patti and Gambi 2001; Bruschetti et al. 2009). *Sabella spallanzanii* created a dense calcium carbonate reef on the floor of Port Phillip Bay in Australia, directly affecting the local scallop fishery where fisherman found it time consuming to sort through worm-dominated dredge catches (Currie et al. 2000; Patti and Gambi 2001; Hewitt et al. 2004). Additionally, structural changes in the fish community were observed, including; an increased abundance of the little rock witing fish *Neoodax balteatus* (Valenciennes, 1840) and a decrease in native benthic species (Currie et al. 2000). Molecular analysis found that the native Mediterranean population further consisted of three sub-populations which differed genetically from the other native Atlantic population, and the alien Australian and New Zealand populations (Patti and Gambi 2001; Ahyong et al. 2017). The vector responsible for transporting *S. spallanzanii* to Australia and subsequently New Zealand was shipping (ballast water and hull fouling) (Patti and Gambi 2001; Ahyong et al. 2017). Similarly, the common biofouling *Hydroides dianthus* (Verrill, 1873) creates dense aggregates on aquaculture nets, seawater pipes, ship hulls and buoys which lead to significant financial burdens to the aquculture, navigation and shipping industries (Sun et al. 2017a). This species was thought to occur naturally along the east coast of the United States (US) with an

extended distribution range spanning North America to Florida and the Caribbean Sea and introduced to the Mediterranean Sea (Sun et al. 2017a). However, molecular analyses revealed that this species comprises two cryptic species of which one is actually native to the Mediterranean Sea and the other an alien along the east coast of US, Brazil and Asia (Sun et al. 2017a).

Due to the lack of historical records documenting the systematics and biogeographic distributions of various marine species prior to transoceanic travels, it has proven almost impossible for biologists to determine whether many species are actually native, introduced or truly cosmopolitan (Miura 2007; Geller et al. 2010; Lawson Handley et al. 2011; Rius et al. 2015). This, coupled with the fact that many cosmopolitan species represent cryptic divergent lineages (Nygren 2014; Rius et al. 2015; Hutchings and Kupriyanova 2018), has confounded our understanding of the global diversity of polychaete species and detection of alien species (Miura 2007; Carlton 2009; Geller et al. 2010; Haydar 2010). Furthermore, because many polychaete species are used as bioassay organisms, pollution detectors, model organisms for evolutionary investigations and toxicology indicators, the presence of undetected cryptic species may yield incorrect results and therefore cannot be implemented in management strategies (Nygren 2014).

South African polychaete worms

An underestimation of indigenous and alien polychaete diversity

The polychaete fauna of South Africa seem to be reasonably well resolved (Griffiths et al. 2010) due to the comprehensive work conducted by John Day which culminated in a monograph on the polychaetes of Southern Africa (Day 1967). For his monograph, Day undertook extensive field surveys in Southern Africa and included descriptions and illustrations of several species across multiple families (Day 1967; Hutchings and Kupriyanova 2018). Like many polychaete taxonomists in this time, John Day also happened to have rather conservative views, accepting large morphological variation within a species and widespread/cosmopolitan distributions and thus included many cosmopolitan European species in his monograph, such as *Marphysa sanguinea* and *Hydroides norvegica* Gunnerus, 1768 (Day 1967; Hutchings and Kupriyanova 2018). However, when

researchers started investigating the presence of European cosmopolitan polychaete species in South Africa, with particular reference to *M. sanguinea, Magelona papillicornis* (Műller, 1858), *Pseudopolydora antennata* (Claparède, 1869), *Rhynchospio glutaea* (Ehlers, 1897) and *Syllis armillaris* Műller, 1776 and *Syllis amica* Quatrefages 1866, across four different families, they found that all species actually represented indigenous species that were incorrectly identified (Lewis and Karageorgopoulos 2008; Clarke et al. 2010; Simon et al. 2017, 2018, Seddick 2018). Furthermore, Seddick (2018) and Simon et al. (2018) found that more than 50% of syllid and spionid polychaetes, in South Africa have cosmopolitan distributions and need to be revised. However, based on the number of indigenous species recorded in Day (1967), Awad et al. (2002) concluded that only 20% of the polychaetes recorded in South Africa are actually indigenous. Thus, if we do find that the remaining 80% of species have been misidentified and represent indigenous species, then the diversity of indigenous polychaete species according to Awad et al. (2002) have been severely underestimated.

Incorrect species identifications lead to an underestimation of the diversity of indigenous polychaete species and also hinders our ability to correctly identify alien species in South Africa, where the documentation of alien, invasive and cryptogenic species is a relatively recent avenue of research (Robinson et al. 2005, 2016, Griffiths et al. 2009, 2010, Mead et al. 2011b, a; Alexander et al. 2016). The first list of alien species compiled for South Africa by Griffiths et al. (1992) reported only 15 species. Taking into account South Africa's extensive shipping history and importation of molluscs for aquaculture, it was hypothesized that the number of alien and invasive species was significantly underestimated (Griffiths et al. 2009; Mead et al. 2011b). This resulted in the reassessment of the diversity and scale of marine alien and invasive species, increasing the overall numbers to 86 alien and 39 cryptogenic species (Griffiths et al. 2009; Mead et al. 2011b, a). Mead et al. (2011b, a) determined whether a species is cryptogenic or alien by assessing the grey and published literature and in only a select few cases were identities confirmed taxonomically. Species were regarded as alien in South Africa if they had been identified as alien in other climatically comparable regions such as Australia, New Zealand and South America (Mead et al. 2011b, a). Cryptogenic statuses were assigned to species with a discontinuous global distribution, but a reasonably resolved taxonomy

(Mead et al. 2011b, a). A questionable status is the third category used by Çinar (2012) for species that lack proper taxonomic resolution, have casual records for the region and have not been found in subsequent studies after the initial record. Additionally, Seddick (2018) defined a questionable species as one with multiple synonymies and type localities around the world (according to the World Polychaete Database, http://www.marinespecies.org/polychaeta/aphia.php?p=taxdetails&id=883) and a discontinuous global distribution and will be used in the present study. It should be noted that in addition to Mead et al. (2011b), Çinar (2012) also did not confirm the taxonomy of all the alien species they listed, and it is therefore possible that some species may have been incorrectly categorised while other alien species may have been overlooked. A more recent study revised the alien list for South Africa, which now includes 36 alien and 52 invasive species (Robinson et al. 2016). However, with the potential for indigenous and alien species to go unnoticed as they are mistaken for widespread and or cosmopolitan species as demonstrated in earlier examples, the native diversity of polychaete worms is most likely underestimated (e.g. Glasby et al. 2013; Simon et al. 2017; Sun et al. 2017a).

With particular reference to South African polychaete worms, 5 invasive, 3 alien and 1 naturalised species were recorded with 66% purportedly introduced to the region via ship hull fouling and ballast water discharge, demonstrating that shipping is an important vector in South Africa (Robinson et al. 2016). The remaining 33% was most likely introduced via importation of aquaculture species such as oysters and abalone (David and Simon 2014; Simon and Sato-Okoshi 2015; Robinson et al. 2016).

Since Mead et al. (2011b) and Çinar (2012) used similar methodologies when preparing alien polycahaete lists, it would be expected that if a non-indigenous species that occurs in South Africa is listed as alien by Çinar (2012), then it should, by extension, be on the Mead et al. (2011b) list. There are, however, some discrepancies between the two lists. For example, Çinar (2012) lists one additional alien polychaete (*Hydroides diramphus* Morch, 1863, designated by Bastida-Zavala (2008) as alien in South Africa) which was not reported in the Mead et al. (2011b) list. Furthermore, several polychaete species that occur in South Africa across the different polychaete families are classified as aliens elsewhere but have not been considered alien locally (Mead et al. 2011b; Çinar

2012). For example, in the family Eunicidae Berthold, 1827, six species originating from the Indo-Pacific, Mediterranean and Red Sea are classified as alien in other climatically similar regions (Çinar 2012), but not in South Africa where they occur in the subtropical and warm temperate regions (Day 1967). While the Mediterranean and the Red Sea are climatically comparable regions to South Africa, the potential alien status of these species were not considered locally.

Additionally, of the 10 alien species described for South Africa, 7 originated from localities in the Northern hemisphere, two from the Southern hemisphere and one species is considered cryptogenic (Mead et al. 2011b; Robinson et al. 2016; Simon unpub.). Such a high frequency of introduced species from the Northern Hemisphere compared to localities south of the Equator are congruent with the early transoceanic travels to and the subsequent colonisation of Africa. Chinese commercial shipping to the Indian Ocean began in the 10th century and European travels to Africa commenced in the 15th century (Rius et al. 2015). It is therefore possible that many species described as native European species, and consequently alien in South Africa, could actually represent native South African species that were transported during these expeditions to and from Africa, or vice versa (Carlton 2009; Griffiths et al. 2009; Rius et al. 2015). Conversely, South Africa has suffered from the presence of pseudo-indigenous species where introduced species have erroneously been identified as new species, leading to the incorrect assumption that they are indigenous (Carlton 2009; Griffiths et al. 2009). For example, in the case of the European gastropod *Myostella myosotis* (Draparnaud, 1801), this species was erroneously described as two native South African species, *Alexia acuminata* Morelet, 1889 and *Alexia pulchella* Morelet, 1889 (Carlton 2009). These issues all confound the overall estimations of native and marine alien diversity in South Africa.

To date, most invasion biology studies have focused on molluscs and gastropods with a handful of studies assessing the genetics and individual impacts of invasive species in South Africa (Alexander et al. 2016). The eleven studies published between 2006 and 2018, investigated taxonomy, reproduction, ecological impacts, invasion pathways and genetic structure of alien polydorid polychaetes on farmed species such as abalone and oysters and one other investigated the ecological interactions of a reef building polychaete (Simon et al. 2006, 2017, 2018; Simon and Booth 2007; David and Simon 2014; David et al. 2014; McQuaid and Griffiths 2014; Williams et al. 2016,

2017). Even though polychaete taxonomy has a rich history in South Africa owing to Day's invaluable monograph (Day 1967), the taxonomy of cosmopolitan species requires further revision and updating.

A reassessment of nereidid polychaetes

Nereididae Blainville, 1818 represents one of the most species rich families in the polychaete group (Dean 2001; Bakken and Wilson 2005; Santos et al. 2005; Glasby et al. 2013). Species in this family exhibit a variety of reproductive and feeding strategies and tolerances to various environmental conditions allowing them to colonise rocky shore habitats, estuaries, deep ocean floor, freshwater habitats and rain water puddles in terrestrial environments (Bakken and Wilson 2005). Many nereidids are commercially important, such as *Hediste diversicolor* and *Pseudonereis variegata* Grube, 1857 that are harvested and sometimes sold to use as bait for recreational fishing or food for aquaculture species (van Herwerden 1989; Scaps 2002; Santos et al. 2005; Bakken 2007). Other species such as *Alitta succinea* Leuckart, 1847 are used in various environmental monitoring tests as indicator species for detection of pollution and heavy metal contamination (Rhee et al. 2007; Villalobos-Guerrero and Carrera-Parra 2015) and *Platynereis dumerilii* Audouin & Milne Edwards, 1834 is used as a model organism for investigating evolutionary development, gene expression patterns and general ecological experiments (Pocklington and Wells 1992; Arendt et al. 2002; Fischer and Dorresteijn 2004; Hui et al. 2007; Zantke et al. 2014). Furthermore, all these species enjoy wide distribution ranges although there is increasing evidence to indicate that populations in all of these localities do not represent a single species. This suggests that global polychaete diversity, distribution patterns and invading alien species have been severely underestimated.

Despite the recognised importance of nereidid species, their taxonomy is not well resolved. For example, several large genera are described for the family with species containing highly variable individuals commonly referred to as "species groups" within species complexes (Bakken 2006; Glasby et al. 2013). The majority of variation between individual species groups has been attributed to differences in paragnath morphology on the pharynx (Figure 1.1 A – C), morphology of parapodial lobes (Figure 1.1 D, E), morphological differences between reproductive forms (Figure 1.1 F, G) and

body colouration and patterning (Figure 1.1 H – K) (Bakken and Wilson 2005; Glasby et al. 2013). Despite the large morphological variability, individuals in the different groups were still classified as members of a single species with cosmopolitan distributions. With the development of molecular techniques and thorough taxonomic re-assessments, many of the groups within these cosmopolitan species were found to represent independent species (Sato and Masuda 1997; Scaps 2002; Virgilio et al. 2009; Glasby et al. 2013; Villalobos-Guerrero and Carrera-Parra 2015). For example, three species from Australia, *Nereis denhamensis* Augener, 1913, *Pseudonereis anomala* Gravier 1900 and *Perinereis suluana* Horst, 1924 were thought to have widespread distributions (Glasby et al. 2013). Nonetheless, genetic analyses revealed that each species is part of a separate cryptic species complex; *N. denhamensis* and *P. anomala* each contain two additional cryptic species and *P. suluana* contains one additional cryptic species (Glasby et al. 2013). In other cases, researchers uncovered the presence of multiple cryptic species after molecular examination of a highly variable species (Sato and Masuda 1997; Audzijonyte et al. 2008; Reish et al. 2014); since species names are not provided for individual taxa, these are still referred to as species complexes. Such a problem results from the lack of characters that can be used to distinguish genetically distinct species and since the differences between them are purely genetic. Taxonomists therefore find it a difficult task to provide species descriptions based on nucleotide sequences (Darling and Carlton 2018). A commonly known example is the cosmopolitan *Neanthes acuminata* (Ehlers, 1868) species complex which comprise at least four genetically distinct species but still only one valid species name exists because they are morphologically identical (Reish et al. 2014).

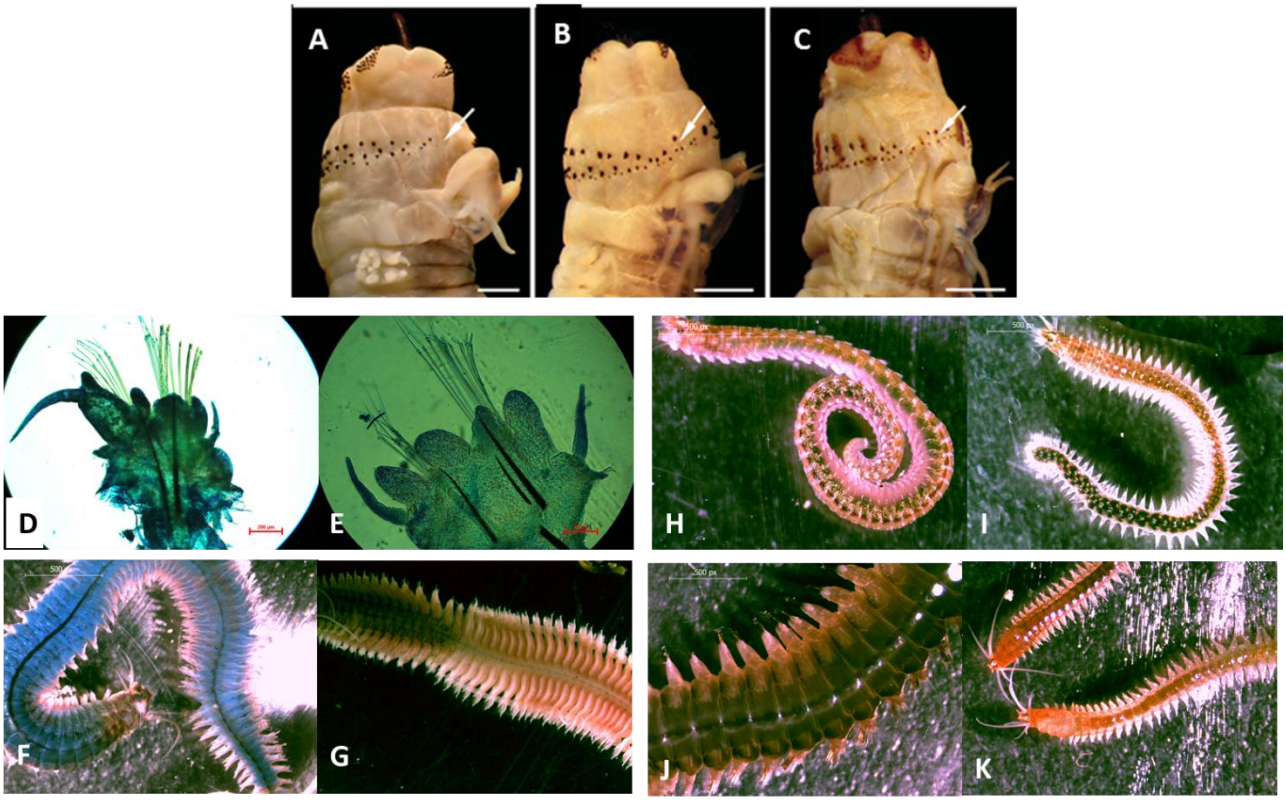

Figure 1.1: Morphological differences of nereidid worms A-C: Differences in paragnath arrangement. Adapted from Villalobos-Guerrero and Carrera-Parra (2015), D & E: differences in size and shape of parapodial lobes, F & G: differences in body colour and modification of segments in the heterenoreid stage (modification for reproduction) and H-K: differences in body colouration and pattern.

Nereididae in South Africa

According to Day (1967) Nereididae from the southern African region include 50 species belonging to 19 genera. Of these, only eleven species are reported as indigenous (Table 1.1), 18 have distributions outside of South Africa and disjunct global distributions after the synonymisations of multiple taxa (Table 1.2). The remaining species were only recorded from Mozambique, Madagascar and Namibia (not presented here).

Table 1.1: A summary of nereidid polychaete species that are indigenous to South Africa. Where appropriate, alien distributions, taken from Day (1967), Mead et al. (2011b), Çinar (2012) and Zenetos et al. (2012), are provided. All species names were checked to ensure they represent the current revised taxa and the current regional distributional ranges were extracted from the World Polychaete Database.

Species name	**Type locality**	**Current distribution**
Ceratonereis keiskama Day, 1953	Keiskama Estuary	Saldanha Bay to Port St. Johns. Found at the top reaches (low salinity) of the estuary
Dendronereis zululandica Day, 1951	St. Lucia	Richards Bay to Mozambique in tropical muddy estuaries
Micronereis capensis, Day, 1963	Agulhas Bank	Around the Cape
Neanthes papillosa Day, 1963	2km west of Cape Town, Atlantic Ocean	2km west of Cape Town, Atlantic Ocean
Neanthes willeyi Day, 1934	Table Bay	Lamberts Bay to Table Bay and Port Elizabeth Confirmed alien in the Mediterranean Sea
Neanthes agulhana Day, 1963	Agulhas Bank	Table Bay to Knysna and East London Confirmed alien in Portugal, Spain and the Mediterranean
Nereis fusifera Quatrefages, 1865	Table Bay	Table Bay
Nereis gilchristi Day, 1967	Agulhas Bank	Cape Agulhas to Port Elizabeth Confirmed alien in the Mediterranean Sea
Perinereis capensis, Kinberg, 1866	Cape of Good Hope	False Bay to Richards bay
Perinereis falsovariegata, Monro, 1933	Still Bay	Table Bay to Durban and Delgoa Bay
Platynereis calodonta Kinberg, 1866	Cape of Good Hope	False Bay to Port Shepstone in rocky shore algal beds

Çinar (2012) listed five species (*Platynereis australis* Schmarda, 1861*, Platynereis dumerilii, Nereis jacksoni* Kinberg, 1866*, Nereis persica* Fauvel, 1911 and *Alitta succinea*) that have been recorded from South Africa (Day 1967) and that are also considered alien in other parts of the world (Table 1.2). However, of these only one, *A. succinea*, was on the list of aliens prepared by Mead et al. (2011b) for South Africa. *Alitta succinea* was classified as an alien here because it is a recognised alien species in other regions of the world that have a similar climate to South Africa and has a discontinuous global distribution (Mead et al. 2011b). However, 13 species representing type localities in 8 type regions have been synonymised with *A. succinea* thus it is considered as a

pseudocosmopolitan species in the present study due to its dubious taxonomic history (Read and Fauchald 2018a). The remaining species are established aliens (*Nereis jacksoni* and *N. persica*), considered questionable (*P. australis*) in the Mediterranean and cryptogenic in Brazil (*P. dumerilii*) (Çinar 2012). Thus, since the Mediterranean Sea, Brazil and South Africa are considered climatically comparable regions (Mead et al. 2011a, b; Smit et al. 2013), they should probably also have been classified as alien in South Africa, given that climatic similarity was used as a criterion when classifying species here. One additional species is considered in the present study, *P. variegata* (Table 1.2). It has never been considered as alien elsewhere in the world, yet it has an apparently widespread distribution suggesting it might be alien in at least part of its distributional range. However, 14 species described from 10 type localities were synonmised as *P. variegata* suggesting that its identification in South Africa might be questionable. Nonetheless, considering that *N. jacksoni, N. persica, P. dumerilii, P. australis* and *P. variegata* have multiple synonymised names and discontinuous global distributions (Table 1.2) they too should be considered pseudocosmopolitan (Darling and Carlton 2018) and their status classified as questionable in South Africa.

Priority species for further investigation

From among the pseudocosmopolitan nereidid species recorded in South Africa, *Platynereis dumerilii, Platynereis australis, Pseudonereis variegata* and *Alitta succinea* were prioritised for revision owing to their reported statuses as alien, questionable and cryptogenic, multiple synonymised names, global disjunct distributions and widespread distributions across the coast of South Africa.

Alitta succinea was recorded as “fairly common” in multiple harbours and estuaries in South Africa, including Mossel Bay, Plettenberg Bay, Port Elizabeth, Durban Harbour, Witsand Estuary, Gouritzmond Estuary, Gansbaai Harbour and Lamberts Bay (Day 1967). However, specimens conforming to its description could not be located at any of these sites despite extensive sampling (Kara, unpublished data), while the species did not appear on inventories generated for St Helena Bay, Saldanha Bay and Durban Bay Harbour from 2015 – 2017 by Anchor Environmental (Rogan

Harmer, pers. comm.). Together, this casts doubt on the presence of this species in South Africa. As a result, the current research will only focus on *P. dumerilii, P. australis* and *P. variegata.*

Table 1.2: A summarised checklist of nereidid species recorded from South Africa according to collections by Day (1967). The current worldwide distributional ranges, number of synonyms and type localities were extracted from the World Polychaete Database. *(? and Questionable, requires further investigation)*. Records in bold represent those species that will be investigated in the present study.

Species	Type locality	South African distribution	Worldwide distribution	Number of synonyms	Status/reference
Alitta succinea Leuckhart, 1847	Cuxhaven	Plettenberg Bay, Cape St. Francis, Port Elizabeth, Richards Bay and Durban	Massachusetts to Gulf of Mexico, Uruguay, Belgium, English Channel, France, North Sea and South Africa	12	Alien in Hawaii, Japan, Australia, Brazil, Argentina and South Africa (Mead et al. 2011b; Çinar 2012)
Ceratonereis (Composetia) hircinicola Eisig, 1870	Mediterranean Sea	St. Lucia	Mediterranea Sea, North Atlantic, Spain and South Africa	5	?
Dendronereis arborifera Peters, 1854	Mozambique	Port Elizabeth and Richards Bay	Mozambique, Madagascar and South Africa	0	?
Namalycastis indica Southern, 1921	Chilka Lake, India	Richards Bay to St. Lucia and Mozambique	India, South Africa, Mozambique, Andamans and Nicobar Islands	0	?
Namanereis quadraticeps Blanchard, 1849	Chile	Saldanha Bay	Southern California, North Carolina, Caribbean Sea, North Atlantic, New Zealand, Mexico, Japan and South Africa	0	?
Nereis coutieri Gravier, 1899	Red Sea	Durban, Richards Bay and Kosi Bay	Suez Canal, Red Sea, Mozambique and South Africa	0	?
Nereis eugeniae Kinberg, 1866	Tierra del Fuego, Argentina	Port Nolloth, Lamberts Bay and Saldanha Bay	Chile, Falkland Islands, Argentina, Kerguelen and South Africa	1	?
Nereis falcaria Willey, 1905	Gulf of Mannar	Saldanha Bay, False Bay and Cape Agulhas	Mozambique, New Zealand, North Atlantic, South Africa,	2	?

Nereis falsa Quatrefages, 1865	Mediterranean Sea	East London and St. Lucia	France, Morocco, North Carolina, Mediterranean Sea, Madagascar, Caribbean Sea, Gulf of Mexico, South Africa, Venezuela	2	?
Nereis jacksoni Kinberg, 1866	Port Jackson, Australia	Cape St. Francis, Port Elizabeth, East London and Durban	Red Sea, South Australia, New South Wales, Chatham Islands, Caribbean Sea, Cuba, Gulf of Mexico, North Atlantic Ocean, Madagascar, Mozambique, New Zealand and South Africa	1	Alien in the Mediterranean (Çinar 2012)
Nereis lamellosa Ehlers, 1868	Adriatic Sea	Lamberts Bay, Plettenberg Bay and East London	Morocco, Senegal, Gulf of Mexico, Mediterranean (Greece), North Atlantic, Spain and South Africa	0	?
Nereis pelagica Linnaeus, 1758	Western Europe	Saldanha Bay	Bay of Fundy, Caribbean Sea, English Channel, France, Gulf of Mexico, Ireland, Mozambique, North Atlantic, North Sea, Norway, Spain, Trinidad, United Kingdom, Venezuela and South Africa	15	Questionable (This study)
Nereis persica Fauvel, 1911	Bahrain	Bashee and Richards Bay	Red Sea, North Atlantic, Mozambique, Madagascar, Mediterranean Sea and South Africa	2	Alien in the Mediterranean (Çinar 2012)
Perinereis cultrifera Grube, 1840	Gulf of Naples	Table Bay, Port Elizabeth, Durban and Richards Bay	Senegal, Mediterranean Sea, English Channel, France, Gulf of Mexico, Ireland, Madagascar, North Atlantic Ocean, South Africa, Spain and United Kingdom	7	?
***Platynereis australis* Schmarda, 1861**	Auckland, New Zealand	Port Nolloth to Hermanus	Japan, South Georgia, Falkland Islands, Kerguelen, Ross Sea, Chile and South Africa	0	Questionable in the Mediterranean (Çinar 2012) Questionable in South Africa (this study)

***Platynereis dumerilii* Audouin & Milne Edwards, 1833**	La Rochelle, France	Table Bay to Port Shepstone	Belize, Caribbean Sea, Cuba, English Channel, France, Gulf of Mexico, Ireland, Mediterranean Sea, Mozambique, North Atlantic Ocean, Norway, Panama, Red Sea, South Africa, Spain, Trinidad & Tobago and United Kingdom	23	Cryptogenic in Brazil (Çinar 2012) Questionable (This study)
***Pseudonereis vareigata* Grube, 1857**	Valparaiso, Chile	Lamberts Bay to Port Shepstone	Caribbean Sea, Mozambique, Panama, Red Sea and South Africa	14	Questionable (This study)
Simplisetia erythraeensis Fauvel, 1918	Madagascar	Lamberts Bay, Saldanha Bay and Richards Bay	Madagascar, Japan, Mozambique, Red Sea, South Africa and Pacific Ocean	3	?

The usefulness of investigating genetic structure, diversity and demographic history for South African marine species

Understanding polychaete diversity

The increase in molecular studies have shown that many cosmopolitan polychaetes dissolve into complexes of local cryptic species (Carr et al. 2011; Glasby et al. 2013; Nygren 2014; Simon et al. 2017). In addition to teasing out cryptic species, phylogeography has proven useful in determining the historical evolutionary and ecological processes that have shaped present day geographical distribution, population structure and connectivity of species across its distributional range (Avise et al. 1987; Bermingham and Moritz 1998; Avise 2000; Teske et al. 2011a; Bowen et al. 2014; Villamor et al. 2014). In turn, the phylogeographic patterns can be used in comparative studies with multiple sympatric species to identify the processes that govern the overall biological diversity of a region (Bermingham and Moritz 1998; Taberlet et al. 1998; Avise 2000; Teske et al. 2011a; Bowen et al. 2014). In South Africa, a proper understanding of biodiversity and distribution of marine species has been confounded by incorrect species identifications and anthropogenic marine invasions (as outlined in earlier sections). Phylogeography thus, serves as an important tool for uncovering areas of endemism, diversity and patterns that govern a species' distribution. Consequently, this information can be used to implement proper management practices that protect and maintain biological diversity (Bermingham and Moritz 1998). Additionally, in systems that have been impacted by anthropogenic activites, population genetic investigations serve as an important tool to understanding the origin, diversity and whereabouts of the introduced species (Mead et al. 2013; Rius et al. 2015).

Oceanographic currents and ecoregions of South Africa

The unique shape of the South African continental shelf together with the contrasting ocean and current systems has been found to influence dispersal and consequently the phylogeography of many marine species (Griffiths et al. 2010; Teske et al. 2011a). The coast of South Africa is governed by two major current systems; the warm Agulhas current in the Indian Ocean on the east coast and

the cold Benguela current in the Atlantic Ocean on the west coast (Figure 1.2) (Lutjeharms et al. 2001; Griffiths et al. 2010). The continental shelf along the east coast of the country is narrow, widens extensively in the south forming the shallow Agulhas Bank and becomes narrow up the west coast (Lutjeharms et al. 2001; Griffiths et al. 2010). Due to the narrowing of the shelf in the west, the coast is dominated by two features, the dynamic wind-driven upwelling inshore system, and the sluggish offshore system flowing towards the equator, forming the eastern component of the South Atlantic subtropical gyre (Lutjeharms et al. 2001; Griffiths et al. 2010). The Agulhas current in the east brings in tropical nutrient poor water flowing southward from the equator (Griffiths et al. 2010). The narrow shelf off the northern coast of KwaZulu-Natal influences the close movement of the current along the shelf, but as the shelf widens off Durban, the current flows more offshore (Griffiths et al. 2010). Following the wide edge of the Agulhas Bank south of East London, the current moves well offshore and retroflects southward carrying pockets of cooler inshore water towards the north parallel to the coast (Griffiths et al. 2010). The area between Cape Agulhas and Port Elizabeth forms the South coast that experiences a wind driven upwelling system. The area between Cape Point and Cape Agulhas is an overlap region where the south and west coast current systems meet (Griffiths et al. 2010). Anticyclonic eddies, commonly known as Agulhas rings are formed and flow into the south Atlantic at the area where the Agulhas current retroflects (Griffiths et al. 2010). The unique and dynamic coastline of South Africa is therefore known to support an astonishing diversity of marine species with richness decreasing from the warm Indian Ocean to the cold Atlantic in the west thus dividing the inshore coast into multiple ecoregions (Turpie et al. 2000; Lombard et al. 2004; Griffiths et al. 2010; Sink et al. 2012).

The two oceanic systems that govern the coast of South Africa give rise to six major marine ecoregions based on varying temperatures, nutrients and productivity (Griffiths et al. 2010; Sink et al. 2012). Four of these ecoregions refer to the inshore regions whilst the remaining two comprise the upper and lower bathyal zones and the abyss (Sink et al. 2012). The five inshore ecoregions will be discussed in greater detail as many of the species discussed in the following sections refer to rocky shore species occurring along these inshore regions. These are: the Southern Benguela ecoregion from Port Nolloth to Cape Point, the Agulhas ecoregion extending from False Bay to Wild

coast, the Natal ecoregion occurring from Wild coast to Cape Vidal and the Delagoa ecoregion occurring from Cape Vidal to Mozambique (Figure 1.2) (Sink et al. 2012).

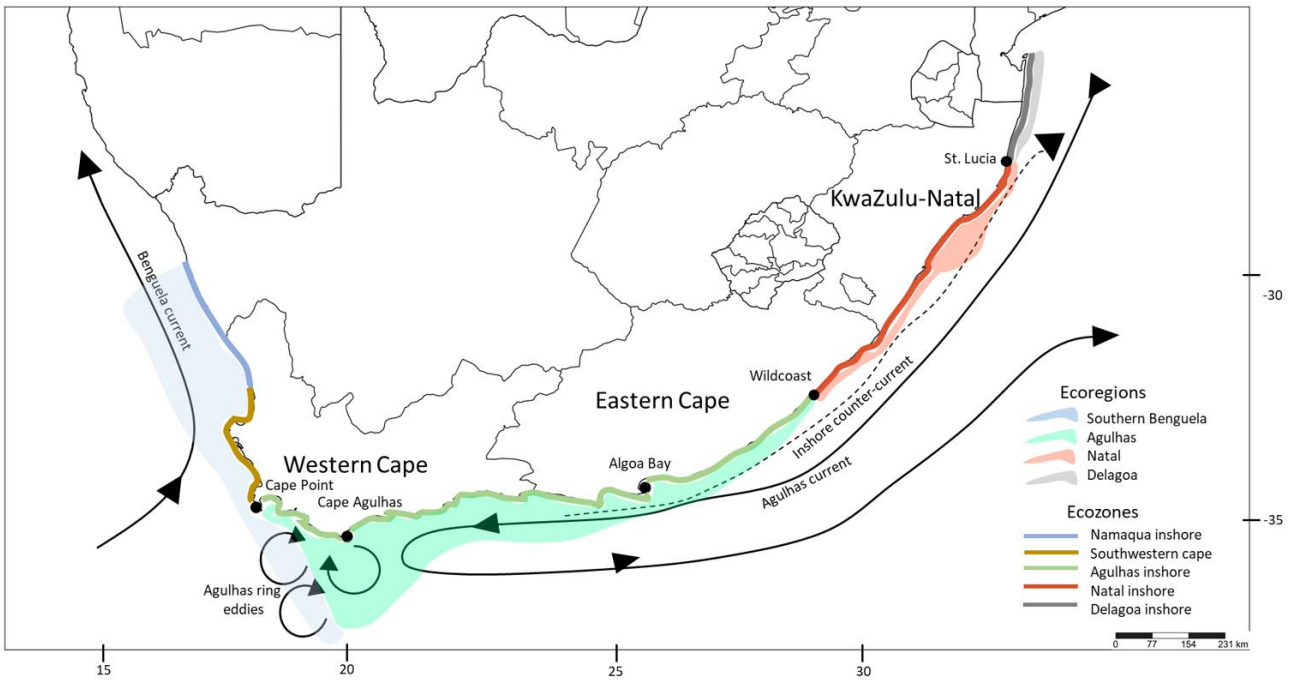

Figure 1.2: Map of South Africa displaying the two major oceanographic currents, the warm Agulhas on the east coast and the cold Benguela current on the west coast (Lutjeharms and van Ballegooyen 1988; Lutjeharms et al. 2001). These give rise to the four ecoregions: the Southern Benguela, the Agulhas, the Natal and Delagoa ecoregions with their respective inshore zones (Sink et al. 2012). Four phylogeographic breaks (black dots); Cape Point, Cape Agulhas, Algoa Bay and St. Lucia commonly found separating lineages of a species (Teske et al. 2011a). Also depicted are the Agulhas ring eddies and the Agulhas inshore counter current (Lutjeharms and van Ballegooyen 1988; Lutjeharms et al. 2001).

The boundaries between the ecoregions roughly coincide with changes in temperatures and phylogeographic breaks that form genetic barriers between populations of species resulting in divergent lineages across its ranges (Teske et al. 2011a, b, 2018b; Smit et al. 2013). Several important phylogeographic breaks have been identified (Teske et al. 2011a). The Cape Point and Cape Agulhas breaks are along the south-west coast, with the region between them forming a transitional zone separating the Southern Benguela and Agulhas ecoregions (Teske et al. 2011a; Sink et al. 2012). The transitional zone between these two breaks is known to contain multiple

species endemic to this zone (Teske et al. 2011a). In the south-east coast region, two other breaks have been detected; the Algoa Bay and Wild coast breaks with the intervening area serving as a second transitional zone separating the Agulhas and Natal ecoregions (Teske et al. 2011a; Sink et al. 2012). An additional third break has been found along the north-eastern coast of KwaZulu-Natal at St. Lucia separating the Natal and Delagoa ecoregions (Teske et al. 2011a; Sink et al. 2012).

Patterns on population structure and connectivity of marine species

The population structures of many coastal species with wide distribution ranges are found to be influenced by the interplay between larval development mode, oceanographic currents, frontal systems and the ecoregions (Lombard et al. 2004; Teske et al. 2011a; Sink et al. 2012). The large number of phylogeographic studies on marine species in South Africa have revealed that species that generally exhibit effective dispersal mechanisms such as *Upogebia africana* (Ortmann, 1894) and *Perna perna* (Linnaeus, 1758) tend to display two well-mixed regional lineages that are separated by the Cape Point or Cape Agulhas barrier (Teske et al. 2007a; Zardi et al. 2007). In other cases, where species have poor dispersal capabilities such as *Hymenosoma orbiculare* Demarest, 1823 and *Exosphaeroma hylecoetes* Barnard, 1940 populations are split into three regionally structured lineages that are separated by the Cape Point and or Cape Agulhas and Algoa Bay barriers (Teske et al. 2007a). In contrast, a handful of studies demonstrated that species share genetic material and are not structured (Teske et al. 2011a). In these cases dispersal is either influenced by oceanic currents and frontal systems (e.g. Teske et al. 2007b; Neethling et al. 2008; von der Heyden et al. 2010, 2013) or by human-mediated activities that would largely influence dispersal across the genetic breaks (e.g. Williams et al. 2016). Alternatively, lack of genetic structure may have been dictated by range expansions in response to historical glacial fluctuations; in particular, the Pleistocene and Last Glacial Maximum served as important climatic events that influenced population expansions of many marine species (e.g. Gopal et al. 2006; Matthee et al. 2006; Neethling et al. 2008; Muller et al. 2012; von der Heyden et al. 2013; Reynolds et al. 2014; Mmonwa et al. 2015; Muteveri et al. 2015).

In contrast to the plethora of phylogeographic studies on taxonomic groups such as molluscs, crustaceans, echinoderms and chordates (elasmobranchs and bony fish) (Gopal et al. 2006; Teske et al. 2007b, 2011a; Muller et al. 2012; von der Heyden et al. 2013; Mmonwa et al. 2015; Bester-van der Merwe et al. 2017; Hewitt et al. 2018), polychaete phylogeography in South Africa is poorly understood. To date, only four species have been investigated. Of these, *Marphysa corallina* Kinberg, 1865 and *Polydora hoplura* Claparède, 1868 displayed well-connected populations where in the former case dispersal is assumed to be natural whereas in the latter case, it was the consequence of movement of aquaculture species (Kara 2015; Williams et al. 2016). The remaining two species, *Boccardia polybranchia* (Haswell, 1885) and *Arenicola loveni* Kinberg, 1866 exhibited two regionally structured lineages corresponding to the Cape Point phylogeographic barrier (Williams et al. 2016; Naidoo 2017). Of these, only *P. hoplura* is a confirmed alien (Read and Fauchald 2018g) and *A. loveni* (Read and Fauchald 2018f) indigenous whereas the taxonomic status of the remaining species are doubtful due to the multiple synonymised names and global disjunct distributions (Kara 2015; Williams et al. 2016), further demonstrating the lack of phylogeographic studies of indigenous and alien polychaete species in South Africa.

Molecular markers and Next generation Sequencing

Molecular markers are used to determine the patterns of differentiation and diversity between populations of species (Grapputo et al. 2005). In recent years, several studies have demonstrated the usefulness of the cytochrome c oxidase subunit 1 gene (herein referred to as mtCOI) as a barcode for separating closely related polychaete species (Hebert et al. 2003; Carr et al. 2011; Glasby et al. 2013; Nygren 2014; Villalobos-Guerrero and Carrera-Parra 2015). This mtCOI barcode allows for the rapid identification of overlooked species and has proven useful in uncovering patterns of biodiversity and distribution of multiple species (Hebert et al. 2003). This is because mitochondrial markers are maternally inherited, fast evolving, single locus genes with conserved structure and universal primers are available that can be used for various marine invertebrate taxa (Sakai et al. 2001; Grapputo et al. 2005; Muirhead et al. 2008; de Jong et al. 2011; Teske et al. 2011a; Giska et al. 2015). Thus, mtCOI remains the common category of markers when addressing issues of

possible cryptic speciation (Carr et al. 2011; Nygren 2014). Furthermore, the development of the barcoding initiative has provided a good online resource as sequence data here are often linked to a museum voucher specimen so that researchers can confidently identify a species (Hebert et al. 2003; Carr et al. 2011a). Nuclear markers (nDNA) on the other hand, are bi-parentally inherited, retain genetic variability and are more representative of effective population size than mtDNA (Grapputo et al. 2005).

In invasion biology, mtDNA has been useful in distinguishing between source/founding populations and identifying the occurrence of multiple introduction events. This is because mtDNA is under strong genetic drift resulting in a loss of variability during bottlenecks because of its single mode of inheritance as opposed to nDNA (Muirhead et al. 2008; Giska et al. 2015). Conversely, while many earlier studies have used mtDNA to infer phylogeographic patterns, it has been deemed unsuitable for investigating hybridisation and reproductive isolation among genetic lineages due to the maternal mode of inheritance (Teske et al. 2011a, 2018a). Bowen et al. (2014) still believes that it is one of the most useful tried and tested methods that will show population structuring because of the small effective population sizes. Nonetheless, several studies have shown dissimilar population structuring of mtDNA and nDNA therefore suggesting that future studies employ both gene fragments to arrive at more accurate conclusions (Bowen et al. 2014; Giska et al. 2015; Teske et al. 2018a).

Studies investigating phylogeographic patterns in South Africa primarily used mtDNA and nDNA markers which limited investigations to that of genetic breaks and cryptic speciation across the unique temperature gradient along the coast (Teske et al. 2006, 2007a, 2011a; Zardi et al. 2007). Nonetheless, these markers are not sensitive enough to address more complex scenarios that address historical and more recent evolutionary histories (Last Glacial Maximum) that may shed light on present day distributions, genetic structure and diversity of species (Teske et al. 2011a). Additionally, with an increase in anthropogenic effects and climate change altering the distribution and abundances of species assemblages, there is a need to investigate changes to genetic patterns driven by fishing pressure and climate change (von der Heyden 2009; Teske et al. 2011a). As a result, the development of a high-density single nucleotide polymorphism dataset is the most useful when addressing more complex hypotheses regarding historical and evolutionary histories of a

species (Brumfield et al. 2003; Emerson et al. 2010; Kumar et al. 2012; Defaveri et al. 2013; Reitzel et al. 2013; Mesak et al. 2014).

Single nucleotide polymorphisms also known as SNPs are point source mutations that result in single base pair differences between sequences and or chromosomes (Brumfield et al. 2003; Kumar et al. 2012). They are bi-parentally inherited, are biallelic and therefore highly polymorphic and are found in abundance throughout the genome (Brumfield et al. 2003; Kumar et al. 2012; Mesak et al. 2014). Their untility has been demonstrated in several phylogeographic, population genomic and phylogenetic studies (e.g. Emerson et al. 2010; Czesny et al. 2012; Zakas et al. 2012; Zarraonaindia et al. 2012) as they possess functional importance, and have stable inheritance. Furthermore, the development of a new method known as Restriction Site-Associated DNA sequencing (RAD-seq), has allowed for thousands of SNPs to be isolated from non-model organisms (Brumfield et al. 2003; Davey and Blaxter 2010; Kumar et al. 2012; Defaveri et al. 2013; Reitzel et al. 2013; Toonen et al. 2013). RAD-seq effectively reduces the size and complexity of the genome by sequencing fragments of DNA that have been cut by restriction endonucleases producing high coverage of homologous genomic regions from multiple individuals (Baird et al. 2008). While several RAD-seq strategies have been developed (reviewed in Andrews et al. (2016)), ezRAD remains one of the easier and superior strategies as it is compatible with a wide range of restriction enzymes and uses a standard Illumina TruSeq library preparation with agarose gel or an SPRI bead size selection step, thus making it a flexible and scalable approach to be used by any laboratory (Toonen et al. 2013). Furthermore, the size selection step is integral for the isolation of loci as inconsistencies between sizes of genomic fragments across libraries can lead to differences in the numbers of loci obtained therefore making downstream intraspecific comparisons inaccurate (Andrews et al. 2016).

In the past, isolating loci was an expensive and tedious task as it involved several processes from cloning, sequencing, marker development and application (Reitzel et al. 2013). However, Next Generation Sequencing (NGS) provides a cheaper and faster alternative by identifying thousands of loci in non-model organisms at a reduced cost (Hohenlohe et al. 2010; Pool et al. 2010; Davey et al. 2011; Kumar et al. 2012; Fumagalli et al. 2013; Reitzel et al. 2013). In the past, the Roche 454 platform was preferred over the Illumina platform for the development of loci due to larger fragment

sizes obtained (Rothberg and Leamon 2008; Shokralla et al. 2012). This in turn allows for the effective design of primer pairs for subsequent amplification (Rothberg and Leamon 2008; Liu et al. 2012; Shokralla et al. 2012). However, due to the recent improvements in Illumina technology, longer read lengths and paired-end sequencing is possible together with an increase in the number of reads per run (Willing et al. 2011; Liu et al. 2012; Shokralla et al. 2012; da Fonseca et al. 2016). NGS methods are able to detect minute genetic differences between individuals by scanning thousands of loci across the genome providing fine scale results (Liu et al. 2012; Mesak et al. 2014; da Fonseca et al. 2016).

Rationale for the study

Polychaete worms are dominant in bentic habitats thus forming a large part of the overall diversity of their environment (Hutchings 1998). Several polychaete worms are regarded as ecosystem engineers as they contribute to the health of the system in many ways such as aerating anoxic sediments, redistribution of organic material and the regulation of water flow (Hutchings 1998). Due to their nature of being ecosystem engineers, invading species have the ability to alter ecosystems drastically such as changing food webs and restructuring of benthic habitats (Pettengill et al. 2007; Çinar 2012; Sun et al. 2017a). Additionally, alien invasions are also known to severely impact the aquaculture industry such as that demonstrated by Simon et al. (2006, 2009a); Simon and Sato-Okoshi (2015).

Since polychaete taxonomy is in a state of flux due to its convoluted taxonomic history, this has led to many indigenous and alien species going unnoticed as they are mistaken for widespread or cosmopolitan species (Nygren 2014; Hutchings and Kupriyanova 2018). Not only do incorrect identifications underestimate regional diversity and areas of endemism of indigenous species, it also hinders our ability to implement proper management measures for the early detection, prevention and control of alien species (Awad et al. 2002; Carlton 2009; McGeoch et al. 2012; Fišer et al. 2018). Incorrect species identifications also result in the incomplete understanding of phylogeographic patterns of indigenous marine invertebrate species which are used in comparative analyses to

determine the underlying historical processes that have shaped their contemporary distribution, connectivity and population structure (Bermingham and Moritz 1998; Taberlet et al. 1998; Teske et al. 2007b, 2011a) in South Africa. The results from phylogeographic studies of multiple co-distributed species can then be used in regional and global management programs to conserve the processes that govern the overall biological diversity (Teske et al. 2011a; Bowen et al. 2014).

Since ~50% of the nereidid fauna recorded for South Africa have type localities outside of the region, multiple synonymised names and disjunct global distributions, it is possible that species in this family that have been categorised as alien or cosmopolitan in much of its range because they have been incorrectly identified in the past. This has been demonstrated for other polychaete species of the region where many that were considered comspolitan were actually historical misidentifications of indigenous species (Lewis and Karageorgopoulos 2008; Clarke et al. 2010; Simon et al. 2017, 2018; Seddick 2018). Furthermore, Seddick (2018) and Simon et al. (2018) have determined that > 50% of the syllid and spionid species recorded for the region need taxonomic revision due to their dubious taxonomic statuses, thus reinforcing the need to revise all pseudocosmopolitan polychaetes in South Africa.

Teske et al. (2011a) identified gaps in our knowledge of phylogeography which further hinder our understanding of the biodiversity in South Africa. The first knowledge gap is the phylogeography of neglected taxa (such as polychaetes). As demonstrated above, phylogeographic studies on South African polychaete worms are severely lacking as to date only four species have been investigated in this regard (Kara 2015; Williams et al. 2016; Naidoo 2017). On a global scale, Carr et al. (2011) and Carr (2012) demonstrated that due to the “cosmopolitan syndrome” many polychaete worms were intentionally omitted from large scale biogeographic studies and thus are poorly studied in several regions. As such, representatives from South Africa are frequently absent from large-scale global taxonomic revisions that attempt to challenge the ‘cosmopolitan paradigm’ (e.g. Read 2007; Sun et al. 2016, 2017b, a; Salazar-Vallejo et al. 2017), resulting in an incomplete understanding of global polychaete diversity and distribution patterns. The second gap in knowledge highlighted by Teske et al. (2011a) is the need to generate multispecies and multilocus data sets. In South Africa we are aware of only three studies that have developed SNP databases to provide genomic

resources (Franchini et al. 2011) and detect SNP loci under selection (Bester-van der Merwe et al. 2011) on abalone, *Haliotis midae* Linnaeus, 1758, whilst a third investigated phylogeographic patterns of two non-model rocky shore species, *Parechinus angulosus* (Leske, 1778) and *Scutellastra granularis* (Linnaeus, 1758) (Nielsen et al. 2018). This study attempts to help address these knowledge gaps by investigating the taxonomic and genetic issues surrounding understudied polychaete taxa (particularly family Nereididae) and identify the phylogeographic patterns of multiple closely related species using multiple genes (mtDNA and nDNA) and a multilocus dataset (SNPs).

Therefore, the overarching aims of this study are to:

1) Determine whether the three selected nereidid polychaete species in South Africa are indigenous to the region and

2) Determine factors contributing to the population genetic patterns of these three sympatric species across their distributional ranges in South Africa to identify the historical and contemporary processes that have governed their present-day distributions.

To achieve these aims, the following research objectives will be addressed in the individual chapters:

- Chapter Two addresses the taxonomic revision and genetic identity of the questionable *Pseudonereis variegata,*
- Chapter Three addresses the taxonomic and genetic status and genetic structure of two congeneric nereidids, *Platynereis dumerilii* and *Platynereis australis* with questionable identities in South Africa,
- Chapter Four addresses the population structure of the nominal *P. variegata* (in context of the results from Chapter Two) using a high-throughput SNP dataset,
- Chapter Five investigates the phylogeography of the three nereidid polychaetes in a comparative context and examines potential speciation mechanisms of cryptic species,
- Chapter Six provides an integrated overview of the taxonomy and phylogeography of three widespread nereidid species in South Africa.

Chapter Two:

Integrative taxonomic methods reveal an incorrect synonymisation of the South African *Pseudonereis podocirra* (Schmarda, 1861) as the widespread *Pseudonereis variegata* (Grube, 1866) from Chile.

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Introduction

The idea that polychaete worms have widespread or cosmopolitan distributions (i.e., occurring in more than one ocean basin) was common from the 1900's (Salazar-Vallejo et al. 2017; Hutchings and Kupriyanova 2018). This was mainly because high morphological variation amongst conspecific individuals was considered normal, resulting in a single species name unwittingly being applied to several species with widespread distributions (Knowlton 1993; Wilson and Glasby 1993; Chen et al. 2002; Glasby et al. 2013; Sampertegui et al. 2013; Hutchings and Kupriyanova 2018). Furthermore, most early taxonomists were based in Europe and produced monographs of European taxa, and researchers in other parts of the world later assigned European names to specimens there (e.g. Fauvel 1921; Augener 1934; Hartman 1959) because no other species names were available. Also, species from remote locations were also frequently synonymised into a single, widespread species (e.g. Villalobos-Guerrero and Carrera-Parra 2015). These errors can frequently be attributed to the lack of type material and poor and inaccurate species descriptions which hamper accurate identifications. This perceived cosmopolitan nature of polychaete distribution gained traction because strong support came from influential taxonomists such as Pierre Fauvel, Olga Hartman and John Day (Hutchings and Kupriyanova 2018).

A consequence of this generally accepted cosmopolitanism is that regional biodiversity is severely underestimated (Carlton 2009). For example, 69% of the spionid polychaetes recorded in South Africa were not originally described in the region (Day 1967). Since then, the cosmopolitan distributions of more than three of these species have been rejected and represent new species (e.g. Sikorski and Pavlova 2016; Simon et al. 2017, 2018). It is therefore probable that this also applies to other taxa such as Nereididae Blainville, 1818. Of the 50 species across 19 genera of Nereididae reported for South Africa, 40% have type localities outside of South Africa with disjunct global distributions and 65% have more than 1 synonymised name (Day 1967; Read and Fauchald 2018b). This suggests that the native diversity of South African nereidid polychaete worms has also been underestimated and need to be investigated. One of these species, *Pseudonereis variegata* (Grube, 1857) is a suitable candidate for further investigation because it includes 14 synonymised species

from 10 type localities and consequently has a discontinuous distribution including the Caribbean Sea, Mozambique, Panama, the Red Sea, Namibia and South Africa (Read 2018).

Pseudonereis variegata was first described from Chile and Peru in South America as *Nereilepas variegata* by Grube (1857) but the description was very confusing and vague and did not include drawings of diagnostic characters. Fauvel (1921) later referred *N. variegata* to *Pseudonereis* Kinberg, 1865 due to the presence of conical-, bar- and pectinate-shaped paragnaths, characters unique to the genus. This description included details of only the paragnath arrangement in areas on the pharynx and the expanded notopodial ligules in posterior parapodia but did not include drawings and was based on material collected from Fènèrive in Madagascar. Rozbaczylo and Bolades (1980) later produced a very good description of the species that included the paragnath arrangement, changes in parapodial morphology along the body and chaetal morphology complete with drawings, based on specimens collected from Iquique in Chile. Bakken (2007) produced revised descriptions of *Pseudonereis* and of *P. variegata* in particular and expanded on two new diagnostic characters to the genus: p- and shield shaped bars on the pharynx. Like the description by Fauvel (1921) this description was not based on material from Chile, but instead on the syntypes of *Nereis ferox* Hansen, 1882, from Rio de Janeiro, another species that had previously been synonymised with *P. variegata* (Ehlers, 1901). Fauvel (1921) later examined *N. ferox* and noted that it differed from *P. variegata* by the different lengths of tentacular cirri and the larger expansion of the posterior ligules but accepted the synonymy based on the premise that the differences represent morphological variation within the species.

To date, no studies have investigated the taxonomic status of *P. variegata* outside of Chile and Peru. In South Africa, specimens that conform to the general description of *P. variegata* are commonly known as mussel worms and are popularly used as bait for recreational fishing (van Herwerden 1989). Three of the synoynyms for *P. variegata* are from South Africa. The oldest is *Nereis mendax* Stimpson, 1856, found in tubes common in the circumlittoral zone from the Cape of Good Hope (Stimpson 1856). The description of this species is, however, vague and does not include information regarding paragnath types, arrangement or any drawings. *Mastigonereis podocirra* Schmarda, 1861 was described from Table Bay in the Western Cape. This description was detailed but nonetheless

confusing; it included drawings of the parapodial and chaetal morphologies but not of the type and arrangement of the paragnaths. Finally, the description of *Nereis (Nereilepas) stimpsonis* Grube, 1866, also from Table Bay is comprehensive, but does not include drawings. Fauvel (1921) synonymised *M. podocirra* with *P. variegata* based on a drawing by McIntosh that included the arrangement of paragnaths on the pharynx. Later Day (1967) synonymised *Nereis mendax* and *N. stimpsonis* with *P. variegata*, without a formal explanation. Considering the poor taxonomic history of what is known as *P. variegata* from South Africa, the following scenarios are proposed: a) *N. mendax, M podocirra* and *N. (Nereilepas) stimpsonis* are valid species, b) *P. variegata* in South Africa is a new species, c) *P. variegata* in South Africa is either *N. mendax, M podocirra* or *N. (Nereilepas) stimpsonis,* or d) All four represent a single species. To resolve this taxonomical conundrum, type material of the three synonymised species from South Africa need to be examined in conjunction with newly collected material and molecular tools need to be used in an integrative framework to determine whether newly collected material is different to specimens from Chile.

The first aim of the study was therefore to determine whether newly collected material from South Africa are any of the four proposed species in the scenarios mentioned above by conducting thorough morphological examinations. The second aim was to investigate the status of this species in South Africa in comparison to the specimens purported to be the same species from elsewhere in the world using the mitochondrial cytochrome c oxidase subunit 1 (mtCOI) gene together with species delimitation analyses. mtCOI has a high evolutionary rate and is relatively easy to amplify (Hebert et al. 2003) making it a commonly used marker that has also proven useful for separating closely related nereidid species (Brett 2006; Carr et al. 2011; Villalobos-Guerrero and Carrera-Parra 2015). mtCOI sequences were already available for *P. variegata* from its type locality in Chile and other places such as China and South Korea and therefore were used in this study.

Materials and Methods

Sample collection

Seventy-seven specimens were collected from eleven sites along the South African coast from November 2015 to March 2017 (Table 2.1, Figure 2.1). Specimens were collected from the lower intertidal rocky shores at low tide at each site. They were collected from among *Perna perna* (Linnaeus, 1758) and *Mytillus galloprovincialis* Lamarck, 1819 beds and in *Gunnarea gaimardi* (Quatrefages, 1848) sand tubes and stored in bags of seawater for processing in the laboratory. There, specimens were anesthetized with 7% $MgCl_2$ diluted in distilled water, photographed and thereafter preserved in 100% ethanol and stored at room temperature for detailed morphological and molecular analysis. In addition to collected material, specimens identified as *P. variegata* lodged at the Iziko South African Museum (SAMC-A20742 and SAMC-A089956) and the two junior synonyms *N. (Nereilepas) stimpsonis*, Wroclaw Museum in Poland (MNHW-317) and *M. podocirra,* Natural History Museum in Vienna (NHMW-3Zoo-20503 and NHMW-3Zoo-2179) were examined. Type material of *Nereis mendax* was not available as Stimpson's collection was lost in the great Chicago fire (https://siarchives.si.edu/collections/siris_arc_217251). Due to the vague species description and absence of type material of this species, this name is considered indeterminable.

Morphological analysis

Specimens were measured and the total number of chaetigers counted. Diagnostic characters such as the number of tentacular cirri, palps and antennae on the prostomium, the number and arrangement of paragnaths on the pharynx and the morphology of chaetae and parapodia along the length of the body were examined following previous studies (Grube 1857; Fauvel 1921; Day 1967; Rozbaczylo and Bolados 1980; Bakken 2007; Sampertegui et al. 2013). Photographs were taken using a Leica DM1000 light microscope with an attached Leica EC3 camera and with an Olympus TG5 camera on macro setting. Sections of the chaetigers along the length of the body were made. Line drawings were done to scale with a drawing tube attached to a microscope and from tracings of high-quality photographs. Adobe Photoshop CC v6.3 was used to process images and create plates. Specimens collected were deposited at the Iziko Museum, South Africa under catalogue numbers SAMCA089962 – SAMCA089965.

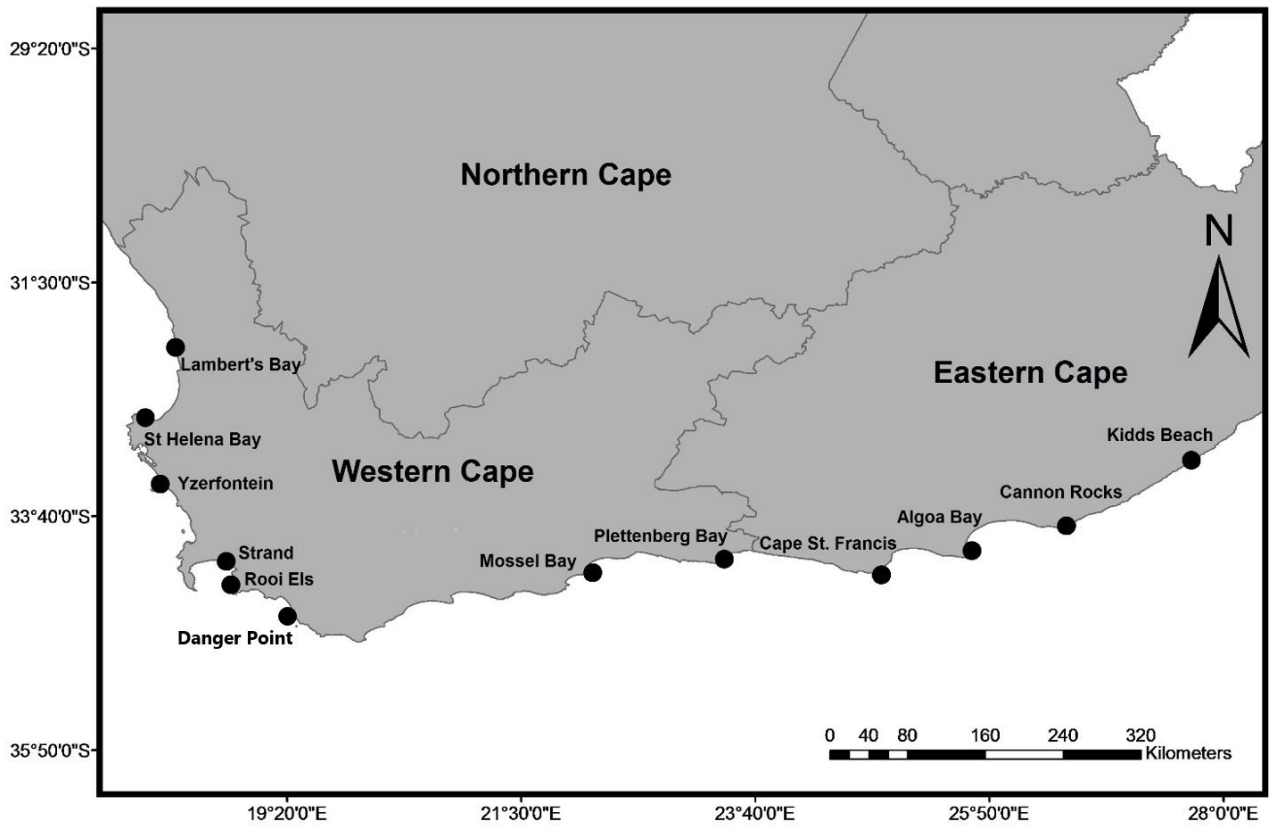

Figure 2.1: Twelve sampling localities of *Pseudonereis podocirra* n. comb. along the South African coast.

Molecular analysis

DNA was extracted from tissue samples following the instructions of the ZR Genomic DNA Tissue MiniPrep Kit. The isolated genomic DNA was stored at -80 °C for the application in Polymerase Chain Reaction (PCR). Isolated genomic DNA was amplified with universal mitochondrial primers: LCO1490 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer et al. 1994). PCR amplifications were conducted using 12,5 µl of *OneTaq* Quick-Load Master Mix (New England BioLabs), 9.5 µl of molecular biology grade water, 0.50 µl of forward and reverse primer at 10 µM concentration, 0.5 µl of 1% Bovine Serum Albumin and 1 µl of template DNA to make up a total reaction volume of 25 µl. Thermal cycling conditions were an initial denaturation of 95 ºC for 3 minutes, followed by 35 cycles of 94 ºC for 30 seconds, 45 ºC for 30 seconds, 72 ºC for 1 minute, followed by a final extension of 72 ºC for

7 minutes. PCR amplicons were sequenced at the Central Analytical Facility at Stellenbosch University using the two primers mentioned above.

Table 2.1: Sampling localities *of Pseudonereis podocirra* n. comb. from twelve sample sites along the South African coast with geographic co-ordinates of sample sites and Genbank accession numbers.

Sample Site	Site Code	Date collected	Co-ordinates	mtCOI accession numbers
Mossel Bay	MB	27 – 10 – 2015	34º18'63.87"S 22º15'92.86"E	MH766319
Rooi Els	RE	03 – 05 – 2016	34º29'78.46"S 18º81'47.40"E	MH766236-MH766329
Danger Point	DP	22 – 06 – 2016	34°62'48.01"S 19°32'13.65"E	MH766320-MH766322
Lamberts Bay	LB	14 – 10 – 2016	32º10'19.82"S 18º30'30.05"E	MH766314-MH766318
Strand	S	17 – 10 – 2016	34º11'88.18"S 18º82'49.51"E	MH766330-MH766331
Yzerfontein	YZ	19 – 10 – 2016	33º36'49.48"S 18º15'97.64"E	MH766341-MH766342
St. Helena Bay	SHB	15 – 11 – 2016	32º75'34.99"S 18º02'16.60"E	MH766340-MH7766353
Cape St. Francis Bay	SFB	27 – 02 – 2017	34º20'65.48"S 24º83'44.86"E	MH766332-MH766334
Plettenberg Bay	PB	28 – 02 – 2017	34º06'18.02"S 23º37'97.76"E	MH766323-MH766325
Algoa Bay	AB	28 – 03 – 2017	33º98'25.13"S 25º66'91.64"E	MH766298-MH766303
Cannon Rocks	CR	29 – 03 – 2017	33º75'15.08"S 26º54'58.36"E	MH766304-MH766308
Kidds Beach	KB	30 – 03 – 2017	33º14'71.54"S 27º70'32.59"E	MH766310-MH766313

Phylogenetic methods

Sequences were edited individually using BioEdit (v7.2.6) (Hall 1999), aligned using the ClustalW multiple alignment method and trimmed to a length of 650bp. Sequences were blasted on Genbank using the BLAST algorithm, however no highly similar sequences were found. Apart from the newly generated sequences, sequences of the following species belonging to genus *Pseudonereis* namely *P. anomala, P. pseudonoodti* and *P. gallapagensis*, were also included in the phylogenetic analysis in addition to *P. variegata* from Chile, South Korea and China (Table 2.2). The closely related *Perinereis aibuhitensis* was used as an outgroup (Accession number: GU362686).

PartitionFinder2 (Guindon et al. 2010; Lanfear et al. 2017) was used to determine the best-fit model of evolution using the Akaike Information Criterion (AIC), which selected the HKY+G model and was used in subsequent Bayesian Inference and Maximum Likelihood analyses. Bayesian inference (BI) method was used to construct a phylogenetic tree using Mr Bayes 3.1.2 (Ronquist et al. 2012). The Bayesian tree was calculated using 4 Markov chains of 6 000 000 generations sampled simultaneously, with every 1000th tree sampled. The first 25% of trees were discarded as burn in, and the remaining were used to construct a 50% majority-rule consensus tree with Bayesian posterior probability support for each clade. Convergence between runs (stationarity of parameters) was investigated using Tracer v1.5 (Rambaut and Drummond 2013). The mixing quality of all parameters was verified by analysing 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 was observed for all parameters and thus considered as good mixing. Maximum Likelihood trees were computed in MEGA7 (Kumar et al. 2016).

Haplotype data files were generated for the dataset using DnaSP v5 (Librado and Rozas 2009). An unrooted TCS network was constructed using PopArt (Leigh and Bryant 2015) with a haplotype data file containing only individuals from South Africa. The TCS algorithm follows the statistical parsimony criterion with a 95% probability.

Species delimitation methods

Fasta aligned files were used as input for Automatic Barcode Gap Discovery method and the online webserver http://wwwabi.snv.jussieu.fr/public/abgd/ was used for the analysis. The following parameters were used for the dataset: Pmin (the minimum intraspecific pairwise distance) was 0.001, Pmax (the maximum intraspecific pairwise distance) was 0.157. Pmax and Pmin values were calculated using MEGA7. X (the barcode gap threshold) was set to 1.5 and the Kimura 2 parameter model was used and run for 20 steps. A high Pmax value was used to avoid the detection of putative species groups that could potentially represent structuring within the South African clade. A binary rooted Bayesian phylogenetic tree was used as input for mPTP analysis using the online webserver http://mptp.h-its.org/#/tree. Bayesian analysis was run for 6 000 000 MCMC generations. Convergence of parameters was assessed using Tracer v1.5. Genetic

distances were calculated within and between clades estimated from ABGD and mPTP analyses in MEGA7 using the Kimura 2 Parameter (K2P) model with complete deletion of gaps and run for 500 bootstraps.

Table 2.2: Genbank accession numbers, locality and reference data of COI sequences for specimens belonging to four species of the genus *Pseudonereis* used for phylogenetic analyses.

Species	**Accession number COI**	**Location**	**Reference**
P. anomala	JX420278	Australia	Glasby *et al.* 2013
P. anomala	JX420271	Australia	Glasby *et al.* 2013
P. gallapagensis	JF293311	Colombia	Hererra *et al.* 2016 [1]
P. gallapagensis	JF293309	Colombia	Hererra *et al.* 2016 [1]
P. pseudonoodti	JF293313	Colombia	Hererra *et al.* 2016 [1]
P. pseudonoodti	JF293312	Colombia	Hererra *et al.* 2016 [1]
P. variegata	HQ705198	Chile	Sampertegui *et al.* 2013
P. variegata	HQ705199	Chile	Sampertegui *et al.* 2013
P. variegata	HQ705183	Chile	Sampertegui *et al.* 2013
P. variegata	KY129881	China	Chen *et al.* 2016 [2]
P. variegata	KY129880	China	Chen *et al.* 2016 [2]
P. variegata	KC800622	China	Deng 2014 [3]
P. variegata	KC800621	China	Deng 2014 [3]
P. variegata	JX503029	South Korea	Kim *et al.* 2012 [4]
P. variegata	JX503027	South Korea	Kim *et al.* 2012 [4]
P. variegata	JX503028	South Korea	Kim *et al.* 2012 [4]

[1]Hererra,L., Gonzalez,F.L., Cantera,J.R. and Barreto,G. Mitochondrial DNA sequences of Nereididae (Annelida: Polychaeta) from the Colombian Pacific. Unpublished
[2]Chen,Y., Yao,R., Xie,F. and Yan,A. Construction of DNA barcode reference library for intertidal polychaetous annelids commonly found in the East China Sea. Unpublished
[3]Deng,Y. Direct Submission
[4] Kim,S., Kim,W. and Koo,H. DNA Barcoding of Marine Intertidal Invertebrates in Korea. Unpublished

Results

Taxonomy

Family **NEREIDIDAE** Blainville, 1818

Subfamily **NEREIDINAE** Blainville 1818

Genus ***Pseudonereis*** Kinberg, 1865

Type species*. Pseudonereis gallapagensis* Kinberg, 1865

Pseudonereis podocirra*,* reinst., n. comb.

(Figs 2.2 – 2.4)

Mastigonereis podocirra Schmarda 1861: 108, fig. 217

Nereis (Nereilepas) stimpsonis Grube 1866: 176

Pseudonereis variegata Day 1967:331, figs 14.12 A – F, (NOT Grube, 1857), from Day (1967)

Material Examined:

Syntypes. Mastigonereis podocirra Schmarda, 1861, South Africa, Western Cape, Cape of Good Hope, 2 specimens, examined specimen in poor condition (NHMW-3Zoo-20503), unexamined specimen complete and in good condition (NHMW-3Zoo-2179), coll. LK Schmarda. *Nereis (Nereilepas) stimpsonis* Grube 1866, South Africa, Western Cape, Table Bay, 2 specimens in very poor condition (MNHW-317), coll. E Grube.

Topotypes. Pseudonereis variegata Grube, 1857, South Africa, Western Cape, Melkbosstrand, 3 specimens in good condition (SAMC-A20742), coll. JH Day from under algae and barnacles.

Non-type material. Namibia, Torra Bay, 3 specimens in good condition (SAMC-A089956), coll. JH Day in 1973 from under algae and barnacles. South Africa, Western Cape, Yzerfontein, 1(SAMC-A089962) 33º36’49.48”S 18º15’97.64”E, 0 m, coll. J. Kara, 19.x.2016 from among *Perna perna* and *Mytilus galloprovincialis* beds in the lower intertidal zone. One large complete specimen. South Africa, Western Cape, Yzerfontein, 1(SAMC-A089963), 33º36’49.48”S, 18º15’97.64”E 0 m, coll. J

Kara, 19.x.2016 from *P. perna* and *M. galloprovincialis* beds, Lamberts Bay, 1(SAMC-A89964) 32º10’19.82”S, 18º30’30.05”E 0 m, coll. J. Kara, 14.x.2016 from under algal mats and sand tubes, Mossel Bay, 1(SAMC-A089965) 34º18’63.87”S, 22º15’92.86”E 0 m, coll. J. Kara, 27.x.2015 from *P. perna* and *M. galloprovincialis* beds. Specimens all complete and in good condition.

Additional specimens. South Africa, Western Cape, St. Helena Bay, 25, 32º75’34.99”S, 18º02’16.60”E 0 m, coll. J. Kara, 15.xi.2015 from *P. perna* and *M. galloprovincialis* beds, Strand, 20, 34º11’81.8”S, 18º82’49.51”E 0 m, coll. J. Kara, 17.x.2016 from *Gunnarea gaymardi* tubes, Rooi Els, 10, 34º29’78.46”S, 18º81’47.40”E 0 m, coll. J. Kara, 3.v.2016 from *P. perna* and *M. galloprovincialis* beds, Plettenberg Bay, 6, 34º06’18.02”S, 23º37’97.76”E 0 m, coll. J. Kara, 28.ii.2017 from *P. perna* and *M. galloprovincialis* beds. Eastern Cape, Cape St. Francis, 10, 34º20’65.48”S, 24º83’44.86”E 0 m, coll. J. Kara, 27.ii.2017 from *P. perna* and *M. galloprovincialis* beds, Algoa Bay, 19, 33º98’25.13”S, 25º66’91.64”E 0 m, coll. J. Kara, 28.iii.2017 from *P. perna* and *M. galloprovincialis* beds, Cannon Rocks, 30, 33º75’15.08”S, 26º54”58.36”E 0 m, coll. J. Kara, 29.iii.2017 from *P. perna* and *M. galloprovincialis* beds, Kidds Beach, 27, 33º14’71.54”S, 26º54’58.36”E 0 m, coll. J. Kara, 30.iii.2017 from *P. perna* and *M. galloprovincialis* beds.

Since no holotype was designated, we have designated NHMW-3Zoo-20503 from the syntype material as the Lectotype to stabilise *Pseudonereis podocirra* n. comb. Specimens were collected from the Cape of Good Hope in the Western Cape of South Africa by L.K Schmarda.

Lectotype description.

Body colour and pigmentation patterns indeterminable. Body length 67–104 mm for 84-92 segments. Uniform in width after apodus segment, tapering in posterior. Prostomium longer than wide. Pair of frontal antennae, cirriform. Pair of palps, swollen base and rounded distal ends. Four pairs of tentacular cirri, longest cirrus extending back to chaetiger 1. Pharynx, dark brown/black jaws, 6 teeth. Paragnaths on pharynx, Area VI: large shield shaped bar, most broken off in other areas. Dorsal notopodial ligule stout rounded, as long as ventral ligule in chaetiger 4, 14-15, expanded in chaetiger 73. Dorsal cirri simple, lack basal cirriphores, twice length of ventral notopodial ligule, basally attached in chaetiger 4, 14-15, subterminally attached in chaetiger 73. Ventral notopodial ligule stout

rounded in chaetiger 4, 14-15, digitiform in chaetiger 73. Neuropodial superior ligule small, rounded. Neuropodial inferior ligule rounded, prominent. Ventral neuropodial ligule rounded in chaetiger 4, 14-15, digitiform in chaetiger 73. Ventral neuropodial ligule half length of acicular neuropodial ligule. Notochaetae only homogomph spinigers, blades finely serrated. Homogomph spinigers in superior neuropodial position, blades curved and finely serrated, heterogomph falcigers, small concave serrated blades. Inferior neuropodial fascicle, heterogomph falcigers, small concave serrated blades.

Re-description based on topotypic and non-type material.

Body colour and pigmentation visible when alive and shortly after preservation in 100% ethanol thereafter body colour fades but pigmentation patterns are retained. Body colour variable on dorsum: greenish brown, greyish brown and medium brown (Fig. 2.2 A, B and C). White pigmentation around both eye spot pairs on prostomium, absent in greyish brown variant (Fig. 2.2 B). Thick pigmented bar from chaetiger 3, more distinct from chaetiger 7 (Fig. 2.2 A, B and D). Black pigment spots along midpoint of segment boundaries from chaetiger 14 to posterior (Fig. 2.2 A–D).

Robust species 15–110 mm long for 36–102 chaetigers (n = 217). Uniform in width after apodous segment, tapering in posterior. Prostomium longer than wide (Fig. 2.3 A). Pair of frontal antennae, cirriform (Fig. 2.2 A and Fig. 2.3 A). Pair of palps, swollen base and rounded distal ends (Fig. 2.3 A). Four pairs of tentacular cirri, distinct cirriphores, longest cirri extend back to chaetiger 3 – 6 (Figure 2.2 A, C, E and Fig. 2.3 A). Pharynx, dark brown/black jaws, 5 or 6 teeth (Fig. 2.3 H, I). Paragnaths on pharynx in distinct areas (Fig. 2.3 H and I, Fig. 2.4), mix of conical paragnaths, circular base, tapering apex, others shield-shaped and p-bars, rectangular base, blunt. Area I: 1 conical paragnath (Fig. 2.4 A), II: 15–17 conical paragnaths in wedge shape (Fig. 2.4 A), III: 22 conical paragnaths in 3 rows (Fig. 2.4 B), IV: 27–32 conical paragnaths and p-bars in a closely spaced arc shape (Fig. 2.4 B), V: 1 conical paragnath (Fig. 2.4 A), VI: large shield shaped bars (Fig. 2.4 A) and VII-VIII: 40 conical paragnaths and p-bars alternating in 2–4 rows (Fig. 2.3 H and I, Fig. 2.4 B and C).

Parapodia of chaetigers 1 and 2 uniramous, thereafter biramous. Dorsal notopodial ligule stout rounded, equal in length to ventral ligule in anterior chaetigers (Fig. 2.3 C). Notopodial prechaetal lobe absent. Notopodial ligule increasing in length and width from chaetiger 13 to posterior (Fig. 2.3

D - G). Dorsal cirri simple, lack basal cirriphores, twice the length of ventral notopodial ligule. Dorsal cirri basally attached to dorsal notopodial ligule in anterior chaetigers (Fig. 2.3 C), subterminally attached from chaetiger 20 to posterior (Fig. 2.3 E – G). Ventral notopodial ligule stout rounded in anterior chaetigers, digitiform in posterior chaetigers (Fig. 2.3 C – G). Neuropodial superior ligule small, rounded. Neuropodial inferior ligule rounded, prominent. Neuropodial postchaetal lobe rounded, projecting beyond acicular ligule in anterior chaetigers, pointed and lower than acicular ligule from chaetiger 50 to posterior (Fig. 2.3 F – G). Ventral neuropodial ligule rounded in anterior chaetigers, triangular from chaetiger 13, and digitiform from chaetiger 30. Ventral neuropodial ligule half length of acicular neuropodial ligule along the length of the body. Ventral cirrus half length of neuropodial acicular ligule. Notochaetae only homogomph spinigers, blades finely serrated (Fig 2.3 K). Homogomph spinigers in superior neuropodial position (Fig. 2.3 L), blades curved and finely serrated, heterogomph falcigers, small concave serrated blades (Fig. 2.3 J). Inferior neuropodial fascicle, heterogomph falcigers, small concave serrated blades (Fig 2.3 J). Pygidium, one pair of anal cirri, reaching back five chaetigers (Fig. 2.2 B, D and F, Fig. 2.3 B).

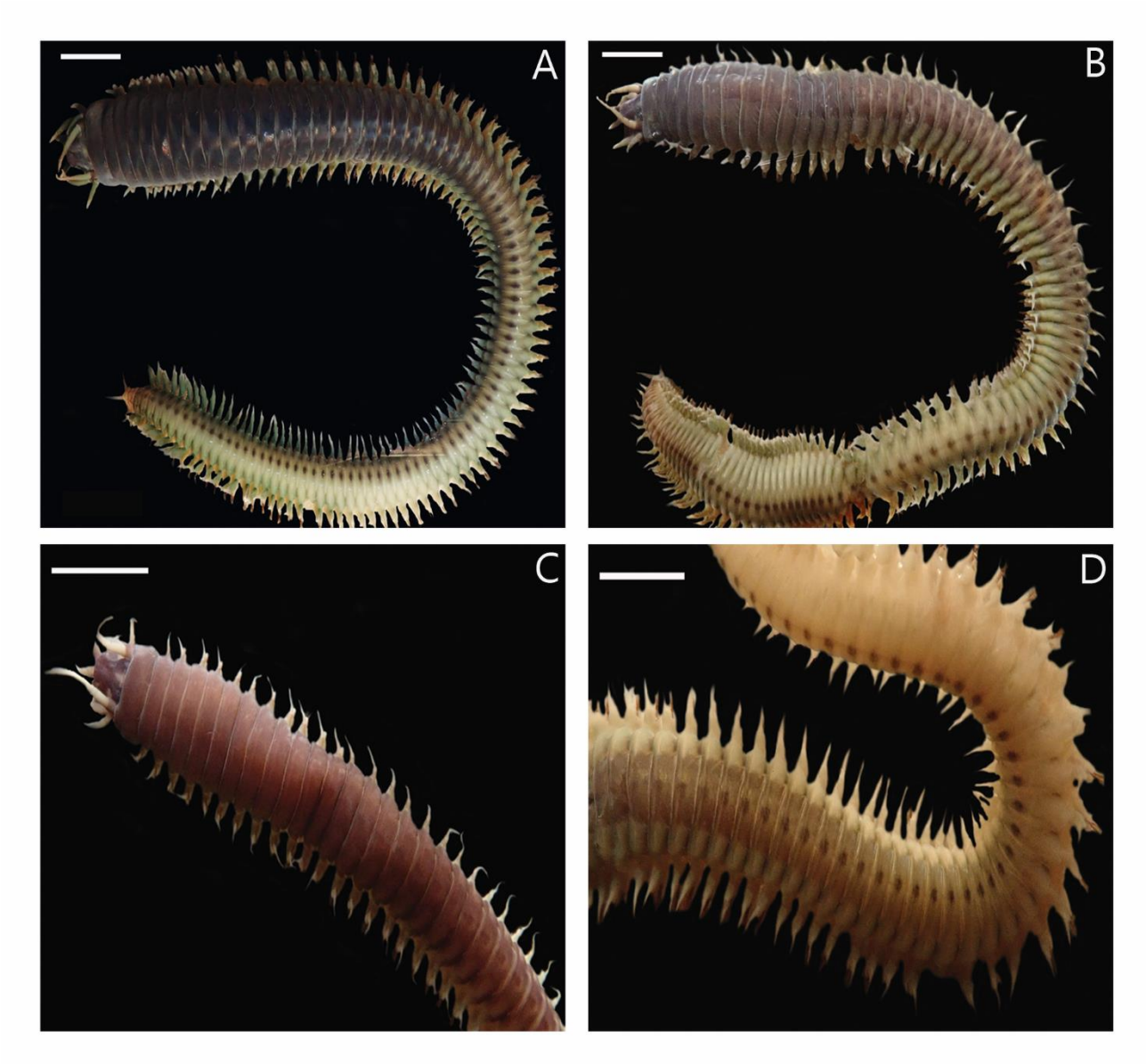

Figure 2.2: Three colour variants of *Pseudonereis podocirra* n. comb. A: greyish brown, B: greenish brown and C – D: brown variants. A and B are the dorsal view, C is the dorsal anterior view and D is the dorsal posterior region. Scale bar in A – D: 5mm.

DNA Barcode. Yzerfontein, Western Cape, South Africa (MH766342). 604 bp fragment of the Universal mitochondrial cytochrome oxidase subunit 1 gene, Primer pair: LCO1490 and HCO2198 (Folmer et al. 1994).

Habitat: Very common in the lower intertidal zone among *P. perna* and *M. galloprovincialis* mytilid beds, living in abandoned tubes of *G. gaimardi* and under abandoned barnacle shells.

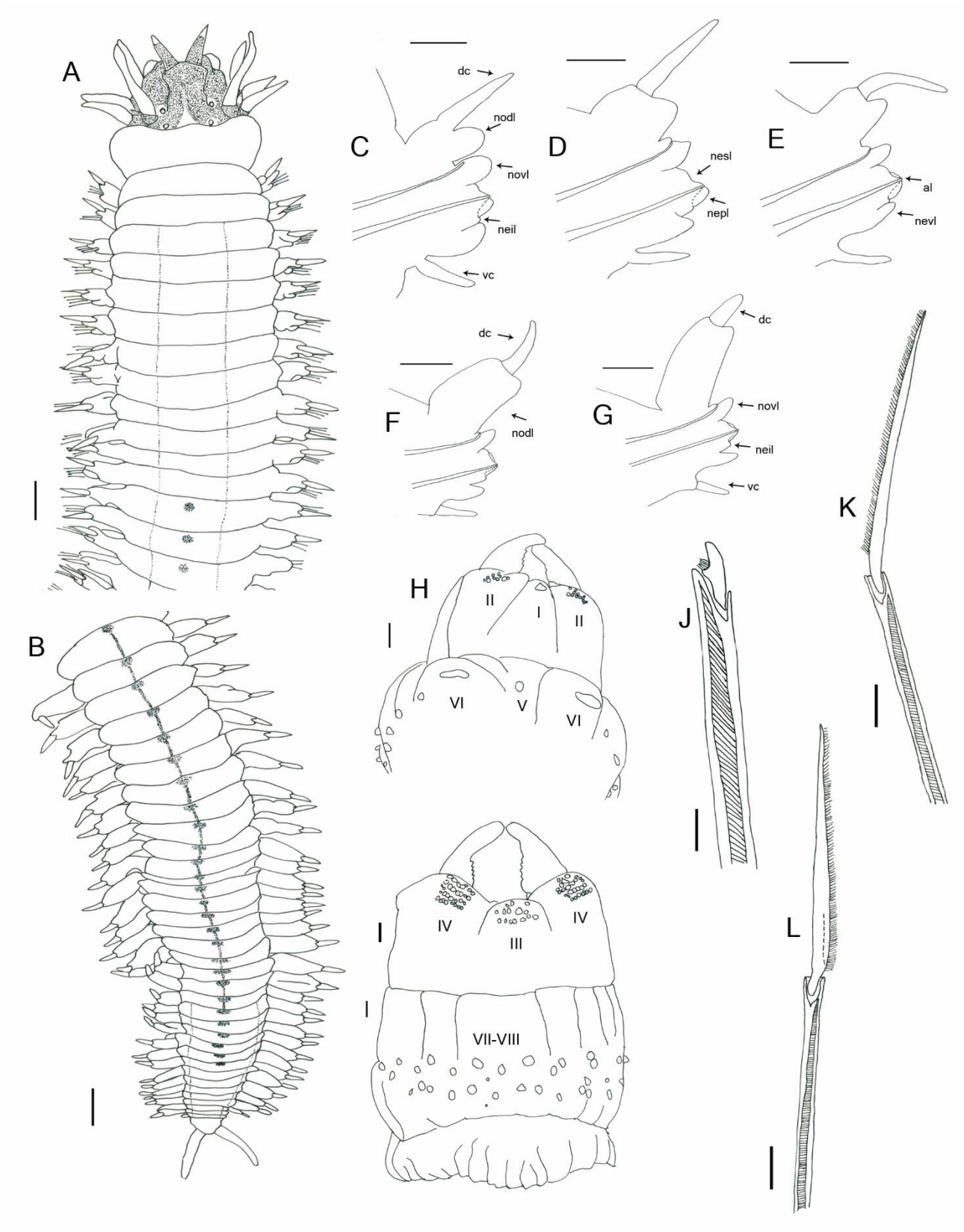

Figure 2.3: *Pseudonereis podocirra* n. comb. A: dorsal anterior, B: dorsal posterior, C: 5th chaetiger, posterior view, D: 13th chaetiger, posterior view, E: 30th chaetiger, posterior view, F: 50th chaetiger, posterior view, G: 65th chaetiger, posterior view, H: dorsal everted pharynx, I: ventral everted pharynx, J: heterogomph falciger neuropodial superior and inferior fascicle, K: homogomph spiniger

notopodium, L: homogomph spiniger neuropodial dorsal fascicle. Scale bar in A – B and H – I: 1mm, C – G: 0.5mm, J – L: 12.5µm. nodl: notopodial dorsal ligule, novl: notopodial ventral ligule, nesl: neuropodial superior ligule, neil: neuropodial inferior ligule, nepl: neuropodial postchaetal ligule, al: acicula ligule, nevl: neuropodial ventral ligule, vc: ventral cirrus.

Distribution. From Lamberts Bay in the Western Cape to Kidds Beach in the Eastern Cape of South Africa. Day (1967) and Penrith and Kensley (1970) also recorded the species in KwaZulu-Natal in South Africa, Namibia and Mozambique.

Remarks:

Specimens identified as *P. variegata* by Day (collected in South Africa and Namibia) and those collected in our study were similar with respect to their size, arrangement of paragnaths, changes in parapodial morphology along the length of the body and the absence of heterogomph spinigers and most likely represent a single species (Table 2.3). These specimens also resemble *M. podocirra* and *N. stimpsonis*, although specimens of both these species were in a very poor condition, making it impossible to accurately assess certain diagnostic characters. *Nereis stimpsonis* was severely shrunken which may have led to an underestimation of total body length (60 mm) (Table 2.3). This also made it difficult to examine the changes in parapodial morphology along the length of the animal. For *M. podocirra* the paragnath arrangement for regions II – V and VII – VIII could not be determined because some were loose, and others had broken off and again the changes in parapodial morphology along the body could not be examined. Nonetheless, *N. stimpsonis* and *M. podocirra* were identical to the specimens collected by Day and in this study in terms of the expanded notopodial ligules, and subterminal attachment of the dorsal cirri in posterior parapodia and the absence of heterogomph spinigers in the inferior neuropodial fascicle (Table 2.3). Additionally, the total body length of *M. podocirra* and the numbers of paragnaths for *N. stimpsonis* fell within the same ranges as for the specimens from South Africa examined here (Table 2.3). One of the two *N. stimpsonis* specimens examined had 2 conical paragnaths on region V whereas all freshly collected specimens from South Africa only ever had 1 in this region (Table 2.3) this may be an aberration. As a result, there are not enough compelling differences between freshly collected South African specimens, *N. stimpsonis* and *M. podocirra* to designate them as separate species. As the oldest description, *Pseudonereis podocirra* n. comb. is therefore reinstated and *N. stimpsonis* considered

its junior synonym and the specimens collected by JH Day from Melkbosstrand (SAMC-A20742) are designated as its topotypic material. Additionally, *P. variegata* from South Africa is also considered as *P. podocirra* n. comb. with a DNA barcode for a specimen from Yzerfontein (60.7 km north of where the topotypic material had been collected) to represent the species molecularly.

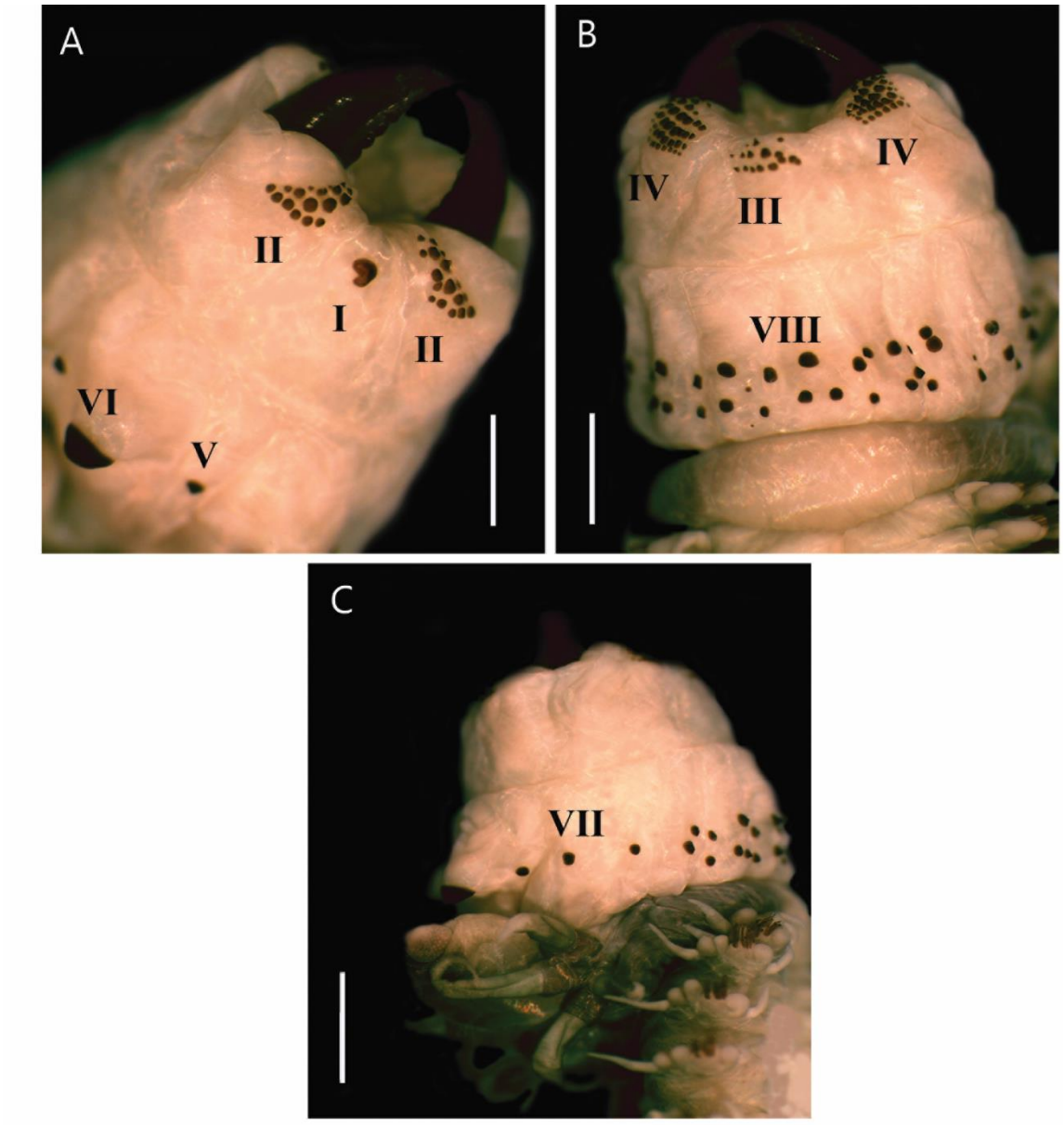

Figure 2.4: *Pseudonereis podocirra* n. comb. Paragnath arrangement on pharynx. A: anterior dorsal view, B: anterior ventral view, C: anterior lateral view. Roman numerals represent regions designated on the pharynx. Scale bars in A – C: 1mm.

Table 2.3: Comparison of diagnostic characters of *Pseudonereis podocirra* n. comb. from South Africa, *Pseudonereis variegata* from Chile, Rio de Janeiro, South Africa and *Nereis (Nereilepas) stimpsonis* and *Mastigonereis podocirra* from South Africa. HS – Homogomph spinigers, HeS- Hetergomph spinigers, HeF – Heterogomph falcigers. (–) indicate no data available.

	***Pseudonereis podocirra* n. comb.**	***P. podocirra* n. comb. (as *P. variegata*)**	***M. podocirra***	***P. podocirra* n. comb. (as *N. (Nereilepas) stimpsonis*)**	***P. variegata***	***P. variegata***	***P. variegata***
Reference / Museum ID	This study	Collected by Day 1951 and 1953 SAMC – A20742 SAMC – A089956	NHMW-3Zoo-2179 NHMW-3Zoo-20503	MNHW-317	Rozbaczylo and Bolades 1980	Sampertegui *et al.* 2013	Bakken 2007
Locality	South Africa	South Africa	South Africa	South Africa	Chile	Chile	Rio de Janeiro
Depth (m)	0 (intertidal)	0 (intertidal)	0 (intertidal)	0 (intertidal)	-	-	-
Length (mm)	15 – 110	18 – 90	67 – 104	60	51	42 – 43	26 – 46
Number of segments	36 – 102	59 – 92	84 – 92	93	130	61 – 123	64 – 94
Number of paragnaths, Areas:							
I	1	0 – 1	1	1	1	1 – 2	1 – 2
II	11 – 26	8 – 20	-	12 – 17	23 – 26	13 – 33	13 – 34
III	8 – 48	16 – 35	-	22 – 25	72	61 – 87	59 – 76
IV	19 – 53	26 – 45	-	30 – 32	-	56 – 90	63 – 87
V	1	1	-	1 – 2	1	1	1

VI	1 shield shaped bar	1 shield shaped bar	1 shield shaped bar	1 shield shaped bar	1 shield shaped bar	1 shield shaped bar	1 shield shaped bar
VII – VIII	29 – 48	38 – 44	-	39 – 44	39 – 41	37 – 46	18 – 21
Anterior parapodia							
Notochaetae	HS only	HS only	HS only	HS only	HS only	HS only	HS only
Neurochaetae **Superior fascicle** **Inferior fascicle**	 HS and HeF HeF only	 HS and HeF HeF only	 HS and HeF HeF only	 HS and HeF HeF only	 HS and HeF HeF and HeS	 HS and HeF HeF and HeS	 HS and HeF HeF and HeS
Posterior parapodia							
Notochaetae	HS only	HS only	HS only	HS only	HS only	HS only	HS only
Neurochaetae **Superior fascicle** **Inferior fascicle**	 HS and HeF HeF only	 HS and HeF HeF only	 HS and HeF HeF only	 HS and HeF HeF only	 HS and HeF HeF and HeS	 HS and HeF HeF and HeS	 HS and HeF HeF and HeS
Notopodial ligule	Expanded and elongate	Expanded and elongate	Expanded and elongate	Expanded and elongate	Expanded and elongate	Elongated	Expanded and elongate
Dorsal cirrus attachment	Sub-terminal	Sub-terminal	Sub-terminal	Sub-terminal	Sub-terminal	Sub-terminal	Terminal

Pseudonereis podocirra n. comb. closely resembles *P. variegata* from Chile and Brazil (Rozbaczylo and Bolados 1980; Bakken 2007; Sampertegui et al. 2013), but several minor morphological differences became clear once more detailed analyses were conducted (Table 2.3). *Pseudonereis podocirra* n. comb. have fewer paragnaths in areas II, III and IV compared to *P. variegara* from Chile and Brazil (Rozbaczylo and Bolados 1980; Bakken 2007; Sampertegui et al. 2013). By contrast, it has fewer paragnaths in areas VII & VIII compared to *P. variegata* specimens from Brazil (Bakken 2007) (Table 2.3). *Pseudonereis podocirra* n. comb. has an expanded dorsal notopodial ligule from chaetiger 13 whereas this occurs much later, from chaetiger 30 onwards, for the Brazilian specimens of *P. variegata*. Additionally, the dorsal cirrus is attached basally in anterior chaetigers and subterminally from chaetiger 20 onwards in *P. podocirra* n. comb. but is attached basally in anterior chaetigers, subterminally from chaetiger 30 and terminally in the posterior in *P. variegata* from Brazil. *Pseudonereis podocirra* n. comb. is similar to specimens of *P. variegata* from Chile in terms of the subterminal attachment of the dorsal cirrus in posterior chaetigers. The most obvious difference between *P. podocirra* n. comb. and specimens of *P. variegata* from Chile and Brazil is the absence of heterogomph spinigers in the neuropodial inferior fascicle (Table 2.3). *Pseudonereis podocirra* n. comb. is also bigger (15 – 110mm, average = 48mm) than individuals from Chile and Brazil (26 – 51mm and 42 – 43mm, respectively) (Table 2.3). Additionally, none of the specimens examined (newly collected and museum) have a hard plate-like basement of paragnaths which has been reported for *Alitta acutifolia* (Ehlers, 1901)*, Neanthes pachychaeta* (Fauvel, 1918) and *N. thysanota* (Ehlers, 1920) (Glasby et al. 2011; Villalobos-Guerrero and Carrera-Parra 2015). Instead *P. podocirra* n. comb. and *P. variegata* displayed individual hardened paragnaths that either arose from a circular base (conical paragnaths) or a rectangular base (shield-shaped bars and p-bars). Both these shapes were also observed in an epitokus form from Lamberts Bay and is considered as taxonomically informative (Bakken et al. 2009; Glasby et al. 2011). Even in museum specimens where the paragnaths had fallen off, no basement-like plate or membrane was observed, confirming that at least for *P. podocirra* n. comb. and *P. variegata,* they entirely lack this structure.

Phylogenetic analysis and haplotype network

The mtCOI dataset contained 77 sequences with a final length of 605 bp, with 52 haplotypes and 267 informative characters. The Bayesian and Maximum Likelihood trees recovered similar topologies and a monophyletic group of *Pseudonereis* with strong support (Figure 2.5). However, one specimen of *P. variegata* from Chile does not fall within the ingroup and its position close to the outgroup suggests that this specimen was misidentified. Two sister clades, A and B were recovered with maximum support in Bayesian analysis (Figure 2.5). Clade A contained only *P. podocirra* n. comb. from South Africa and was supported with maximum support in both phylogenetic analyses (Figure 2.5). Clade A contained two divergent sub-clades (1–2). Sub-cluster 1 contained individuals from Plettenberg Bay and Cape St. Francis from the South coast and had maximum support in both phylogenetic analyses. Sub-cluster 2 contained individuals from Lamberts Bay, Yzerfontein, Strand, Rooi Els and Mossel Bay and had high posterior probability support (Figure 2.5). However, Sub-cluster 2 and other populations did not exhibit any apparent geographic structure. Clade B consisted of three sub-clades (1–3) (Figure 2.5). Sub-clade 1 included *P. anomala* from Australia and *P. gallapagensis* from Colombia with very strong posterior probability support (1.0). Sub-clade 2 also had high posterior probability and maximum likelihood support (1.0, 100) and contained *P. variegata* from China and South Korea. Lastly, sub-clade 3 included *P. variegata* from Chile forming a sister cluster to *P. pseudonoodti* from Colombia, although with a weak support (Figure 2.5).

The unrooted mtCOI haplotype network in Figure 2.6 comprises 40 haplotypes that were sampled from 77 individuals across 12 sampling localities in South Africa spanning the West, South west, South and South east coasts. The network is characterised by a central dominant haplotype (Haplotype 2, *n*=10, 5 localities) surrounded by four other dominant haplotypes (Haplotype 4–7) and several private haplotypes connected to it or via unsampled haplotypes that differ by 1 mutation step. Haplotypes 2 (*n*=10), 4 (*n*=4), 6 (*n*=3) and 7 (*n*=4) contained individuals from the West coast (St. Helena Bay, Lamberts Bay and Yzerfontein), South west coast (Strand and Danger Point), South coast (Mossel Bay and Cape St. Francis) and South east coast (Cannon Rocks and Kidds Beach), whereas Haplotype 5 comprised only individuals from the South (Plettenberg Bay and Cape St. Francis) and South east coast (Cannon Rocks). Sub-clade A1 from the phylogenetic tree was

recovered by the network analysis whereas Sub-clade A2 was not recovered and instead connected to dominant haplotypes 4 and 6 via unsampled haplotypes. Sub-clade A1 differed from Haplotype 23 by 26 mutation steps and the highest mutation steps connecting two haplotypes was 41 (Plettenberg Bay and an unsampled haplotype).

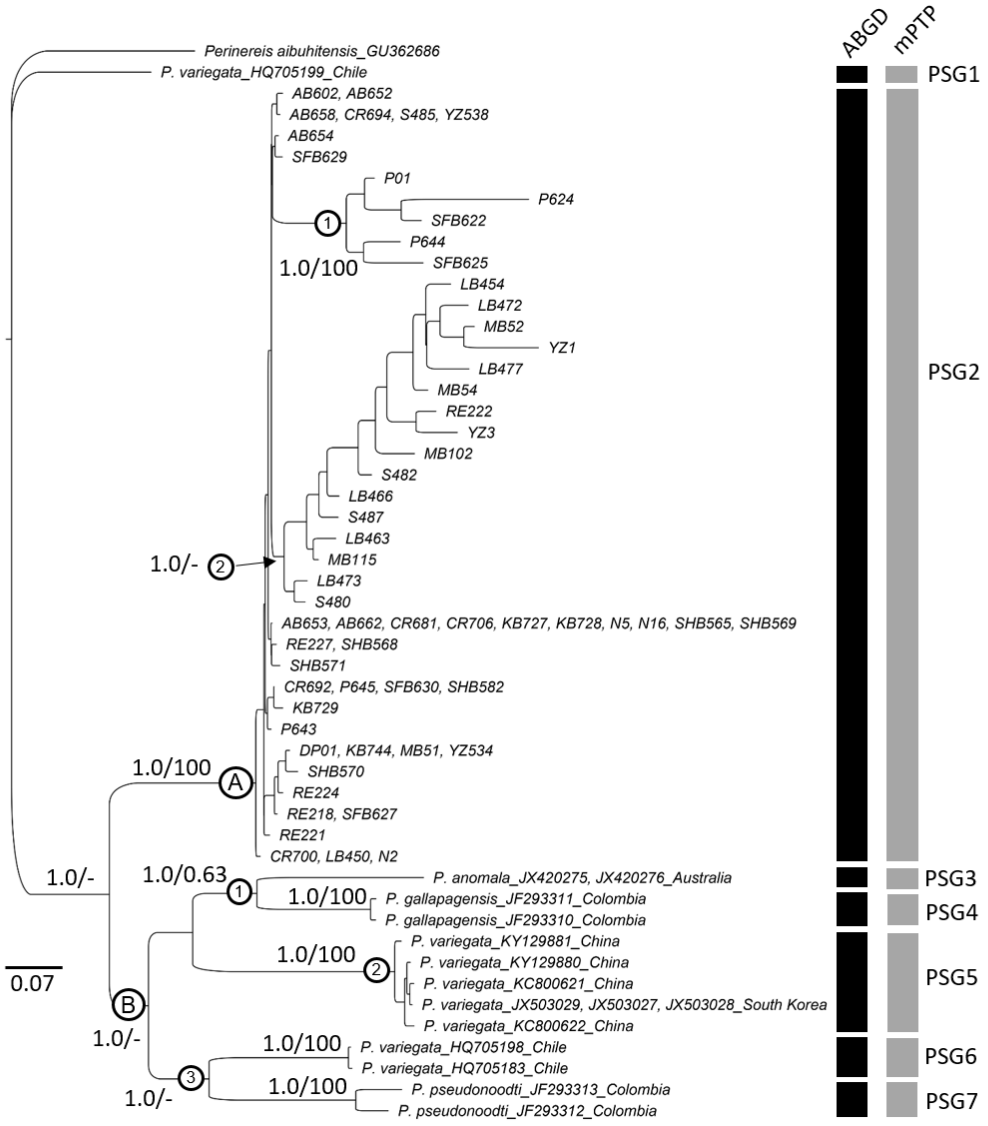

Figure 2.5: Phylogenetic tree based on COI alignment of *Pseudonereis* spp. indicating Bayesian probabilities and Maximum Likelihood bootstrap (right) support, at each respective node. Hyphens indicate nodes that were not recovered by analyses. Results from two species delimitation models are presented to the right of the tree. Automatic Barcode Gap Discovery (ABGD) results are in black and multi-rate Poisson Tree Process (mPTP) results are in grey. Species delimitation analyses grouped species into Putative Species Groups (PSG), PSG1 – *P. variegata* (Chile), PSG2 – *P. podocirra* n. comb. (South Africa), PSG3 – *P. anomala* (Australia), PSG4 – *P. gallapagensis* (Colombia), PSG5 – *P. variegata* (China and South Korea), PSG6 – *P. variegata* (Chile) and PSG7

– *P. pseudonoodti* (Colombia). Clade A contains *P. podocirra* n. comb. from South Africa (locality data as in Table 1) and Clade B contains three sub-clades of *Pseudonereis* species from elsewhere in the world (Table 2). Sub-clade B1 represents *P. anomala* (Australia) and *P. gallapagensis* (Colombia), B2 contains *P. variegata* (China and South Korea) and B3 comprises *P. variegata* (Chile) and *P. pseudonoodti* (Colombia).

Species delimitation and genetic distances

ABGD analysis of mtCOI revealed seven putative species groups (PSG) using the Kimura 2 Parameter model (Figure 5). mPTP analysis also recovered seven putative species groups (Figure 2.5). Two putative species groups (PSG1 and PSG6) were recovered for *P. variegata* from Chile (Figure 2.5). PSG1 contains a single (presumably incorrectly identified) individual that has an unresolved placement basal to other *Pseudonereis* species. By contrast, PSG6 forms a monophyletic grouping among other *Pseudonereis* species (Figure 2.5). PSG1 and PSG6 differ by 25% (Table 2.4, Figure 2.5). PSG2 contains all *P. podocirra* n. comb. and differs from PSG1 and PSG6 by 27% (0.031) and 26% (0.031), respectively (Table 2.4). PSG3 contains *P. anomala* from Australia, PSG4 contains *P. gallapagensis* from Colombia while PSG7 contains *P. pseudonoodti* from Colombia. Finally, PSG5 contains *P. variegata* from China and South Korea and differs from *P. podocirra* n. comb. by 27% (0.032) and from *P. variegata* from Chile (PSG6) by 25% (0.033) (Table 2.4, Figure 2.5). The seven putative species groups correspond to the seven putative species clusters recovered by Bayesian and Maximum Likelihood analyses (Figure 2.5). Additionally, the interspecific genetic distance for *Pseudonereis* species ranges between 25-27% (Table 2.4).

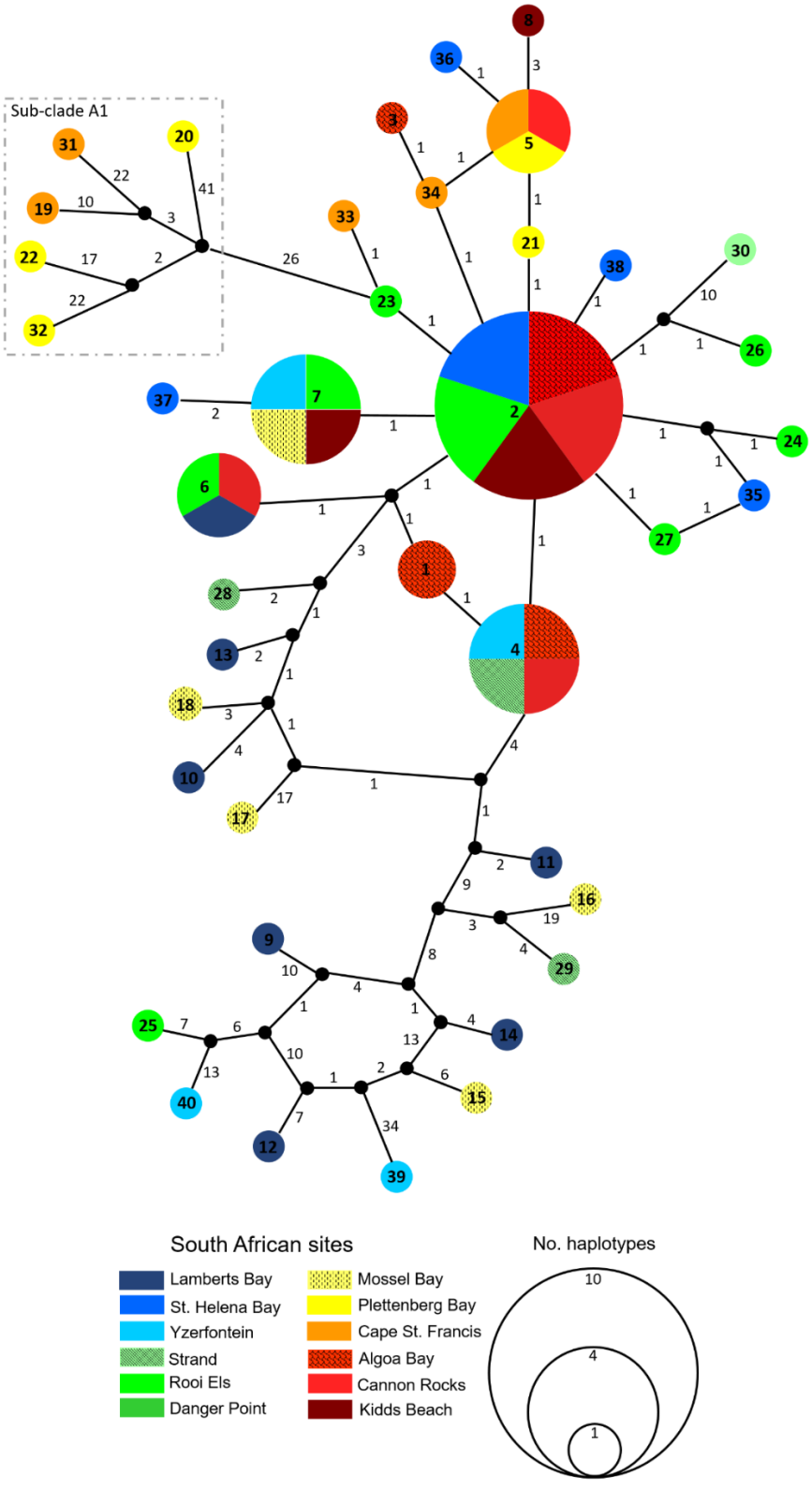

Figure 2.6: Unrooted mtCOI haplotype network for *Pseudonereis podocirra* n. comb. Haplotype numbers are in each circle. Numbers on branches connecting each haplotype represent mutational steps. The size of each circle is proportional to the number of individuals present in that haplotype. Small black dots connecting two haplotypes represent unsampled haplotypes. Each site within a region is represented by a different colour. Sub-clade A1 represents a divergent haplogroup.

Table 2.4: Interspecific and intraspecific distances of the COI gene between the seven putative species groups. *Pseudonereis variegata* (Chile), *Pseudonereis podocirra* n. comb. (South Africa), *Pseudonereis anomala* (Australia), *Pseudonereis gallapagensis* (Colombia), *Pseudonereis variegata* (China and South Korea), *Pseudonereis variegata* (Chile) and *Pseudonereis pseudonoodti* (Colombia). Kimura 2 Parameter distances are below the diagonal with standard error estimates above the diagonal. Intraspecific distances are along the diagonal in bold for each species-group with the standard error estimates after parentheses.

	***P. variegata* Chile**	***P. podocirra* n. comb. South Africa**	***P. anomala* Australia**	***P. gallapagensis* Colombia**	***P. variegata* China and South Korea**	***P. variegata* Chile**	***P. pseudonoodti* Colombia**
***P. variegata* Chile**	-	0.031	0.036	0.033	0.035	0.033	0.031
***P. podocirra* n. comb. South Africa**	0.275	**0.045±0.005**	0.037	0.032	0.032	0.031	0.028
***P. anomala* Australia**	0.294	0.301	**0.000±0.000**	0.028	0.034	0.031	0.031
***P. gallapagensis* Colombia**	0.266	0.256	0.219	**0.004±0.003**	0.032	0.031	0.030
***P. variegata* China and South Korea**	0.268	0.271	0.295	0.264	**0.002±0.002**	0.033	0.033
***P. variegata* Chile**	0.255	0.266	0.272	0.228	0.258	**0.041±0.010**	0.027
***P. pseudonoodti* Colombia**	0.259	0.241	0.246	0.241	0.275	0.224	**0.006±0.003**

Discussion

This is the first study to interrogate the widespread status of *Pseudonereis variegata.* Morphological examination showed that not only is the species in South Africa different to that from Chile, but that at least one of the synonymies has to be reversed and that the synonymisation of *N. ferox* with *P. variegata* should be investigated further. Additionally, molecular data confirm the independent status of *P. podocirra* n. comb. from South Africa, but also highlights another in Asia that requires further investigation.

Early nereidid descriptions rarely followed a standardised method resulting in some taxonomists following Kinberg's (1865) emphasis on paragnath type, form and number whereas others focused on parapodial morphology, as initiated by Malmgren (1867), when describing new species. Since *M. podocirra* and *N. stimpsonis* were described in 1861 and 1866, respectively, these important diagnostic characters were not considered when synonymies were made simply because they had never been documented (Fauvel 1921; Hartman 1959; Day 1967). Thus, the original description of *P. podocirra* n. comb. superficially conforms to the generalised description of *P. variegata* from Chile. However, our closer morphological examination highlights subtle but distinct and consistent differences, indicating that *P. podocirra* n. comb. and *P. variegata* are probably separate species. These differences include the consistent absence of the heterogomph spinigers and the fewer numbers of paragnaths. Furthermore, *P. ferox* differs from *P. variegata* by several characters such as the numbers of paragnaths on the pharynx, changes in parapodial morphologies and the attachment of dorsal cirri in posterior parapodia. This, in addition to the fact that *P. ferox* is not from the same ocean basin as *P. variegata,* suggests that it probably represents a different species.

Three of the six species clades recovered by phylogenetic reconstruction corresponded to *P. podocirra* n. comb. and *P. variegata* from Chile and Asia, respectively, with very strong support. This provides strong evidence that these are three separate species and is further supported by additional distance and coalescent based species delimitation analyses. Testing species boundaries using single locus species delimitation analyses such as ABGD and mPTP does not require species to be morphologically defined *a priori* (Pons et al. 2006; Leliaert et al. 2014). Thus the delimitation analyses could also identify cryptic species that are not evident from morphological observations, or dissolve

apparently cosmopolitan species (Carr et al. 2011). The utility of species delimitation analysis was demonstrated by Carr et al. (2011) who successfully split nereidid species into their predefined morphological groups using mtCOI species delimitation analyses. However, a specific genetic distance threshold for mtCOI has not been established for polychaete worms due to overlap of intraspecific and interspecific genetic distances (Brett 2006; Kvist 2014). For example, broad-based studies on annelids suggested average interspecific distances of 16.5% and 29.97%, but for nereidids in particular, interspecific distances are incredibly variable and can be as low as 3.7% and as high as 27% (Brett 2006; Carr et al. 2011; Glasby et al. 2013; Kvist 2014; Villalobos-Guerrero and Carrera-Parra 2015; Wäge et al. 2017). As such, the interspecific distances found for *Pseudonereis* (25–27%) in our study are well within the range published for other nereidid polychaetes. Genetic distances thus supported the separation of *P. podocirra* n. comb. and *P. variegata* from Chile and Asia into three species. As a result, *P. variegata* from Asia needs to be revised as it clearly represents an independent species.

The structure of *P. podocirra* n. comb. was characterised by a high number of private haplotypes without any clear geographic structuring and with high divergences between haplotypes and mixing of haplotypes between distant populations. Additionally, the three colour morphs observed did not correlate to any particular locality or substrate type. This absence of geographic structure has been observed for other marine invertebrates that have a pelagic larval dispersal stage such as molluscs, eunicid polychaetes and brittle stars (Iannotta et al. 2007; Muths et al. 2009; Fernández et al. 2015). The reproductive strategy of *P. podocirra* n. comb. is unknown, but it may have a long pelagic larval dispersal phase as described in the closely related *P. anomala* (Hamdy et al. 2014). The lack of mtCOI structure could also be attributed to the anthropogenic movement of bait worm (Arias et al. 2013) as *P. podocirra* n. comb. is commonly used as a bait species in South Africa (van Herwerden 1989). It is common practice for recreational fisherman to discard unused bait worm into the water (Arias et al. 2013). In South Africa in particular, many fishermen do not fish in the same place they had collected bait worm (Kyle Smith, pers. comms. South African National Parks), making it possible that the lack of genetic structure observed for *P. podocirra* n. comb. could be due to the movement of bait worms used for fishing. However, the impacts of such a scenario have not been investigated

in South Africa before and therefore needs to be explored further. Alternatively the lack of structure could be a result of diversifying selection acting on the gene or due to past demographic history such as recent sudden expansions (Hart and Marko 2010; Marko and Hart 2011). The former scenario seems unlikely in the case of *P. podocirra* n. comb. since diversifying selection would act to produce regionally structured lineages as a result of the different local inshore currents and other environmental discontinuities (Teske et al. 2007a; Fernández et al. 2015). The latter scenario is more plausible as the haplotype network produced an overall star-like phylogeny that is consistent with recent sudden population expansions. Additionally, the sharing of haplotypes between distant populations (e.g., Haplotype 2 is shared between St. Helena Bay and Cannon Rocks that are 979.5 km apart) may be a consequence of an ancestral polymorphism that was retained in individuals originating from a refugial population (de Jong et al. 2011; Zhang et al. 2014). This may have resulted in a high genetic similarity of the newly expanding populations, falsely implying extensive gene flow (Maggs et al. 2008). The divergent haplotypes from two West coast populations (Lamberts Bay and Yzerfontein), three South-west coast populations (Strand, Rooi Els and Danger Point) and three South coast populations (Mossel Bay, Plettenberg Bay and Cape St. Francis) could be a result of the admixing of multiple divergent lineages in secondary contact (Fernández et al. 2015) once populations started expanding out of refugia. However, these hypotheses can be tested more effectively using larger sample sizes and further analysis using Next Generation Sequencing datasets.

Our analyses indicate that the synonymisation of *N. stimpsonis* and *P. podocirra* n. comb. in South Africa with *P. variegata* was incorrect and it is recommended that all junior synonyms outside of Chile are revised, particularly *P. ferox* from Brazil. Consequently, a revision of *P. variegata* based on material from its type locality is warranted. With the aid of an integrated framework approach, this study provides a redescription and the reinstatement of a native South African species that had erroneously been synonymised with an apparently cosmopolitan species. This demonstrates the importance of thorough taxonomic examination and molecular identification prior to naming and synonymising species. In addition, we have demonstrated that one of the 19 nereidid species with type localities outside of South Africa, actually represents a native species, and that the diversity of

Pseudonereis has been underestimated in the region. It is therefore probable that other nereidid species with several type localities outside of South Africa and several synonymised names may also represent indigenous species and therefore require further investigation.

Addendum

Since this article was accepted for publication, two studies that further confirm my findings were published. One rejected the synonymisation of *Nereis ferox* with *Pseudonereis variegata* and reinstated it to full species (Conde-Vela 2018), whilst the other, Park (2018) found that the Asian species previously identified as *P. variegata* is actually a misidentification of an undescribed local species.

Chapter Three:

Molecular and taxonomic methods reveal a true cryptic polychaete species complex in South Africa, with comments on population structure and the description of a new *Platynereis* Kinberg, 1865 species.

Introduction

The previous chapter demonstrated how a pseudocompolitan species actually represented an incorrect synonymisation of an indigenous species in South Africa. This, reinforces the idea that the same might apply to the two *Platynereis* species that were also prioritised for further investigation owing to their dubious taxonomic classifcations (Chapter 1).

Challenging the cosmopolitan paradigm using molecular techniques combined with thorough morphological examination in an integrated framework has revealed that a significant portion of polychaete diversity harbour a number of cryptic and pseudocryptic species (Knowlton 1993, 2000; Glasby et al. 2013; Nygren 2014; Simon et al. 2017, 2018). Pseudocryptic species complexes are the consequence of over-conservative taxonomic practices that clumped together several genetically distinct but morphologically similar species into a single species with an artificial cosmopolitan distribution spanning more than one ocean basin (Glasby et al. 2013; Kawauchi and Giribet 2014; Achurra et al. 2015; Villalobos-Guerrero and Carrera-Parra 2015; Johnson et al. 2016; Álvarez-Campos et al. 2017; Simon et al. 2017; Sun et al. 2017a). By contrast, true cryptic species are morphologically identical to one another and can only be distinguished by non-visual characters such as reproductive strategies, genetic data and ecological preferences (Manchenko and Radashevsky 1998; Drake et al. 2007; Bickford et al. 2007; Halt et al. 2009; Rius and Teske 2013; Struck et al. 2017). In these cases, species complexes are hypothesised to be a result of evolutionary forces such as genetic drift coupled with morphological stasis, constrained evolution and or convergent evolution (Bickford et al. 2007; Smith et al. 2011; Derycke et al. 2016; Struck et al. 2017, 2018; Fišer et al. 2018).

Unravelling these species complexes can help to uncover distribution patterns, regional biodiversity and areas of endemism of previously overlooked polychaete species which could have management and conservation implications (Bickford et al. 2007, Nygren 2014). South Africa in particular is considered a biodiversity hotspot as its dynamic coastline, governed by two major ocean current systems (the warm Agulhas current along the east coast and the cold Benguela current along the west coast), is known to support a diverse number of marine species (von der Heyden 2009; Griffiths et al. 2010; Teske et al. 2011a). The different temperature profiles of the current systems resulted in

the classification of four major inshore ecoregions: the Southern Benguela, Agulhas, Natal and Delagoa ecoregions and the boundaries between them roughly coincide with phylogeographic breaks that serve as sudden barriers to gene flow (Figure 1.2) (Griffiths et al. 2010; Teske et al. 2011a; Sink et al. 2012). Consequently, each biogeographic province supports its own assemblages of marine species (Griffiths et al. 2010; Teske et al. 2011a).

Even though South Africa harbors such a large diversity of marine species (Griffiths et al. 2010), it is evident that our knowledge on native polychaete diversity has been underestimated as one eunicid and several spionid polychaetes that were classified as cosmopolitan actually represent local species that were historically misidentified (Lewis and Karageorgopoulos 2008; Sikorski and Pavlova 2016; Simon et al. 2017, 2018). This also applies to taxa belonging to Nereididae Blainville, 1818 where it was found that *Perinereis namibia* Wilson and Glasby 1993 is an independent species from *Perinereis nuntia vallata* (Lamarck, 1818) that was recorded from Southern Africa (Wilson and Glasby 1993) and *Pseudonereis podocirra* n. comb. (Schmarda, 1861) which was previously incorrectly synonymised as the cosmopolitan *Pseudonereis variegata* (Grube, 1866) due to morphological similarities (Chapter 2). From among the pseudocosmopolitan nereidid species recorded for the region, two species, *Platynereis dumerilii* (Audouin and Milne Edwards, 1833) and *Platynereis australis* (Schmarda, 1861) serve as ideal candidates for further investigation as the former species has several synonymised names and both the species have discontinuous global distributions (Read and Fauchald 2018c, e). Additionally, both *P dumerilii* and *P. australis* are known to harbour complexes of cryptic species (Read 2007; Wäge et al. 2017). In the Mediterranean, *P. dumerilii* has been demonstrated to comprise a complex of four species (Calosi et al. 2013; Lucey et al. 2015; Valvassori et al. 2015; Wäge et al. 2017), while a second complex was hypothesised to occur in Brazil (Santos C. and Halanych K., pers. comm.). Similarly, *P. australis* in New Zealand comprises a complex of four species (Read 2007). This therefore suggests that the South African records of both *P. dumerilii* and *P. australis* are most likely incorrect.

Platynereis dumerilii inhabits temporary tubes and is abundant in shallow rocky reefs covered mainly by brown algae (Gambi et al. 2000; Giangrande et al. 2002). It was first described from the French Atlantic coast and was found to be widespread throughout the Mediterranean (Audouin and Milne

Edwards 1833; Fauvel 1921; Giangrande 1988; Gambi et al. 2000; Fischer and Dorresteijn 2004; Read 2007; Fischer et al. 2010). Thereafter it was recorded in many locations outside of the Mediterranean ranging from the Gulf of Mexico, Cuba, the English Channel, Norway, the Black Sea, Mozambique and South Africa resulting in its status as a cosmopolitan species (Day 1967; de Leon-Gonzalez et al. 2001; Fischer and Dorresteijn 2004; Read 2007; Popa et al. 2014). *Platynereis dumerilii* has 23 synonyms (Hartman (1944, 1948, 1959) was responsible for 20 synonymisations), from 17 localities and three sub-species (Read and Fauchald 2018d). Eleven of the synonyms are from regions beyond its natural distributional range and since there is no evidence that *P. dumerilii* was accidently transported to other regions via human mediated pathways, this could mean that valid, previously described species were erroneously synonymised as Hartman was known to synonymise names without providing a formal explanation (Read 2007).

The cryptic diversity among several species within the *Platynereis* complex has been demonstrated by the existence of different reproductive strategies (Just 1914, 1915; Fischer and Dorresteijn 2004; Fischer et al. 2010; Lucey et al. 2015). Although different reproductive strategies may be a flexible trait within some polychaete families (Levin 1984), recent studies have found that it may be indicative of independent species (Read 2007; Valvassori et al. 2015). This applies to *Platynereis dumerilii*, where differences in reproductive strategies highlighted the presence of a complex of morphologically identical species. On the east coast of United States of America, *Platynereis megalops* (Verrill,1873) was incorrectly synonymised by Hartman (1944) as *P. dumerilii*. However, reproductive studies by Fischer and Dorresteijn (2004) confirmed that the two species are independent due to differences in reproductive strategies. *Platynereis dumerilii* is a gonochoristic species where males and females metamorphose into sexually mature heteronereids that undergo a mass spawning event triggered by lunar cycles; after fertilisation takes place, both male and female die (Just 1929; Fischer and Dorresteijn 2004; Fischer et al. 2010). *Platynereis megalops* in contrast, shows direct sperm transfer which is shortly followed by oviposition (Just 1914, 1915). Nothing further is known about the development of larvae.

In the Mediterranean, *Platynereis massiliensis* (Moquin-Tandon, 1869) was commonly mistaken as *P. dumerilii* (but never considered a synonym) due to their identical morphologies at the non-

reproductive adult stage (Valvassori et al. 2015). However, *P. massiliensis* is a protandrous hermaphrodite that broods eggs that hatch into lecithotrophic larvae that lack epitokus transformation and swimming larval stages (Schneider et al. 1992) which differs from *P. dumerilii* as described above. Females die after oviposition, males then fertilise the eggs inside the brood tube and continue to ventilate and protect the developing embryos until development into young worms (Lucey et al. 2015). Thereafter the male changes sex and the process is repeated (Lucey et al. 2015). The scarce literature, vague species descriptions and identical morphologies of *P. dumerilii* and *P. massiliensis* led to their taxonomic confusion resulting in the widespread occurrence of *P. dumerilii* throughout Europe (Valvassori et al. 2015). Nonetheless, ocean acidification studies in the Mediterranean revealed two genetically and reproductively different forms of *Platynereis*, one with a free spawning habit and the other with brooding and semi-direct developing larvae coinciding with *P. dumerilii* and *P. massiliensis*, respectively (Calosi et al. 2013; Lucey et al. 2015).

Studies by Calosi et al. (2013), Lucey et al. (2015) and Wäge et al. (2017) did not only confirm the presence of *P. massiliensis* in the Mediterranean but they also revealed that each form comprised a species complex with structured populations that coincided with their respective reproductive habits. Accordingly, Wäge et al. 2017 identified two geographically structured brooding clades, with high nucleotide diversities typical for species lacking planktonic larvae. However, Wäge et al. (2017) could not conclude with certainty which clade represents the real *P. massiliensis* as their analysis did not include specimens from the type locality of this species but assigned the name to the clade that included samples from close to its type locality. By contrast, the two remaining clades displayed less geographic structure with low nucleotide diversities, typical for species with planktonic larvae; one of these clades included samples from Arcachon, 180km from *P. dumerilii*'s type locality in La Rochelle, France, leading Wäge et al. (2017) to confidently conclude that these specimens represent the real *P. dumerilii.*

Day (1953, 1967) recorded the presence of both *P. australis* and *P. dumerilii* in South Africa even though he considered them to be morphologically similar. *Platynereis australis* is also a tube dweller that is found in shallow subtidal zones in sand and mud substrates (Estcourt 1967; Read 2007). It was first described from Auckland Harbour in New Zealand and is reported to have a temperate

distribution that extends to South Africa and Namibia (Day 1967, Read 2007). In its type locality, this species is known to be a gonochoristic broadcast spawner (Read 2007). *P. australis* was found to comprise a complex of four species; *P. australis* and the newly described *P. mohanga* Read, 2007*, P. kau* Read, 2007 and *P. karaka* Read, 2007 which had almost identical paragnath arrangements and chaetal morphologies, but differed in pigmentation patterns and morphology in the heteronereid stage (Read 2007). Thus, Read (2007) suggested that all *P. australis* occurring outside of New Zealand need taxonomic revision.

To date, no studies have investigated the taxonomic status of *P. dumerilii* and *P. australis* in South Africa. *Platynereis dumerilii* has a South African distribution extending across the Southern Benguela and Natal ecoregions in South Africa, from Table Bay in the Western Cape to Port Shepstone in KwaZulu-Natal (Day 1953, 1967). Two of its synonyms were described from Table Bay in South Africa, *Mastigonereis quadridentata* (Schmarda, 1861) and *M. striata* (Schmarda, 1861) and were differentiated from one another by numbers of paragnaths, body colouration and pattern and the absence of a notopodial falciger (Schmarda 1861). *Mastigonereis quadridentata* was synonymised by Augener (1913) as *P. australis* due to similarities in paragnath numbers on the pharynx and morphology of the parapodia. However, Day (1953) did not agree with this synonymy due to the presence of a notopodial falciger in Schmarda's (1861) figure and therefore considered it as the "common" *P. dumerilii. Mastigonereis striata* was synonymised by Hartman (1959) but there was no indication as to why this placement was made. *Platynereis australis,* on the other hand, was reported from Port Nolloth to Hermanus in the Western Cape (Day 1953, 1960), occurring along the Southern Benguela and Agulhas ecoregions. However, since then no studies conducted for the region have reported the occurrence of *P. australis*. The species descriptions prepared by Day (1967) for South African *P. australis* and *P. dumerilii* are similar to one another and do not include features of the heteronereid stages. The only distinguishing characters are the absent notopodial falcigers and the longer notopodial lobes that are swollen basally in *P. australis* and dorsal cirri from the posterior feet (Day 1953, 1967). Read's (2007) description of *P. australis* from New Zealand mainly includes differences in the heteronereid stages of the worm. Nonetheless, the description of the non-

reproductive form by Read (2007) reveal no striking differences when compared to that of Day (1967).

Since both species are known to comprise complexes of morphologically similar species in their type localities (Read 2007; Calosi et al. 2013; Lucey et al. 2015; Valvassori et al. 2015; Wäge et al. 2017), it is hypothesised that South African records most likely represent historical misidentifications. Furthermore, since both species' distributions cover more than two ecoregions in South Africa, it is expected that these two species will also comprise regionally structured lineages as demonstrated for several other marine invertebrate species in South Africa (Teske et al. 2007a, 2011a; Toms et al. 2014; Williams et al. 2016). The type of structuring patterns are mostly dictated by reproductive habits, larval dispersal mechanisms and past demographic events of the region (Teske et al. 2011a). It is expected that species with long larval dispersal phases will display two regionally structured lineages that are separated most commonly by the Cape Point barrier whereas species having poor larval dispersal capacities will have more than two regionally structured lineages separated by two or more genetic barriers (e.g. Teske et al. 2007, 2011). When species do not display structured populations that correlate to any genetic barrier or biogeographic provinces, their contemporary distributions have usually been influenced by past demographic events such as vicariance events or climatic oscillations (Reynolds et al. 2014; Toms et al. 2014).

The first aim of the study is therefore to determine whether newly collected material of the putative *P. dumerilii* and *P. australis* from South Africa represent their morphologically identical congeners from France and New Zealand, respectively, by conducting thorough morphological comparisons and by reconstructing phylogenetic relationships using one mitochondrial (universal mtCOI) and one nuclear (Internal Transcribed Spacer Region 1) marker. The second aim was to compare the two South African specimens in a morphometric comparison to determine whether the diagnostic characters used by Day (1967) are reliable enough to separate these species. The third aim was to investigate whether the two species have geographically structured populations along the coast of South Africa using both mtCOI and ITS1 markers.

Materials and Methods

Sample collection

Specimens identified as *P. dumerilii* and *P. australis* according to Day (1967) were collected from 14 sites along the South African coast from November 2015 to March 2017 (Figure 3.1, Table 3.1). Both species were found in sand-mucus tubes among *Ulva* sp. Linnaeus, 1753, *Jania ahaerens* J.V. Lamouroux, 1816, *Jania verrucosa* J.V. Lamouroux, 1816, *Amphiroa ephedraea* (Decaisne, 1842) and *Corralina officinalis* Linnaeus, 1758. Patches of algae were collected at low tide and stored in bags of seawater for processing in the laboratory. There, algae were processed under a Leica MZ75 dissecting microscope and individual worms were removed and placed in separate bowls. Worms were anesthetized with 7% Magnesium Chloride in distilled water, photographed and four individuals per species per site were preserved in 4% formalin in seawater and the rest in 100% ethanol for detailed morphological and molecular analysis, respectively. Type material of *Mastigonereis quadridentata* and *M. striata* that were previously collected from South Africa could not be located for morphological comparisons and thus their names are considered indeterminable.

Morphology preparation and analysis

Specimens were examined using a Leica DM1000 light microscope and Leica MZ75 dissecting microscope with a Leica EC3 camera attached. Species were identified according to diagnostic characters used in previous studies (Audouin and Milne Edwards 1833; Schmarda 1861; Day 1967; Read 2007). Photographs of the specimens and their diagnostic characters were taken. Sections of the chaetigers were made along the length of the body. The following characters were measured and counted for the morphometric analysis: total number of chaetigers, total body length, longest tentacular cirri which was measured in relation to the chaetiger number it extended to, rod-like paraganths in tight rows in areas III, IV, VI and VII-VIII on the pharynx and lastly the presence/or absence of the notopodial falciger. A principal component analysis was used to explore the phenotypic variation within samples based on a standardised correlation matrix of the eight characters in SPSS version 22. Components with an eigenvalue of > 1 were retained. The results were illustrated by plotting the component scores on a scatterplot against species groups. Line

drawings were done to scale with a drawing tube attached to the light microscope. Adobe Photoshop CC v6.3 was used to process images and create plates.

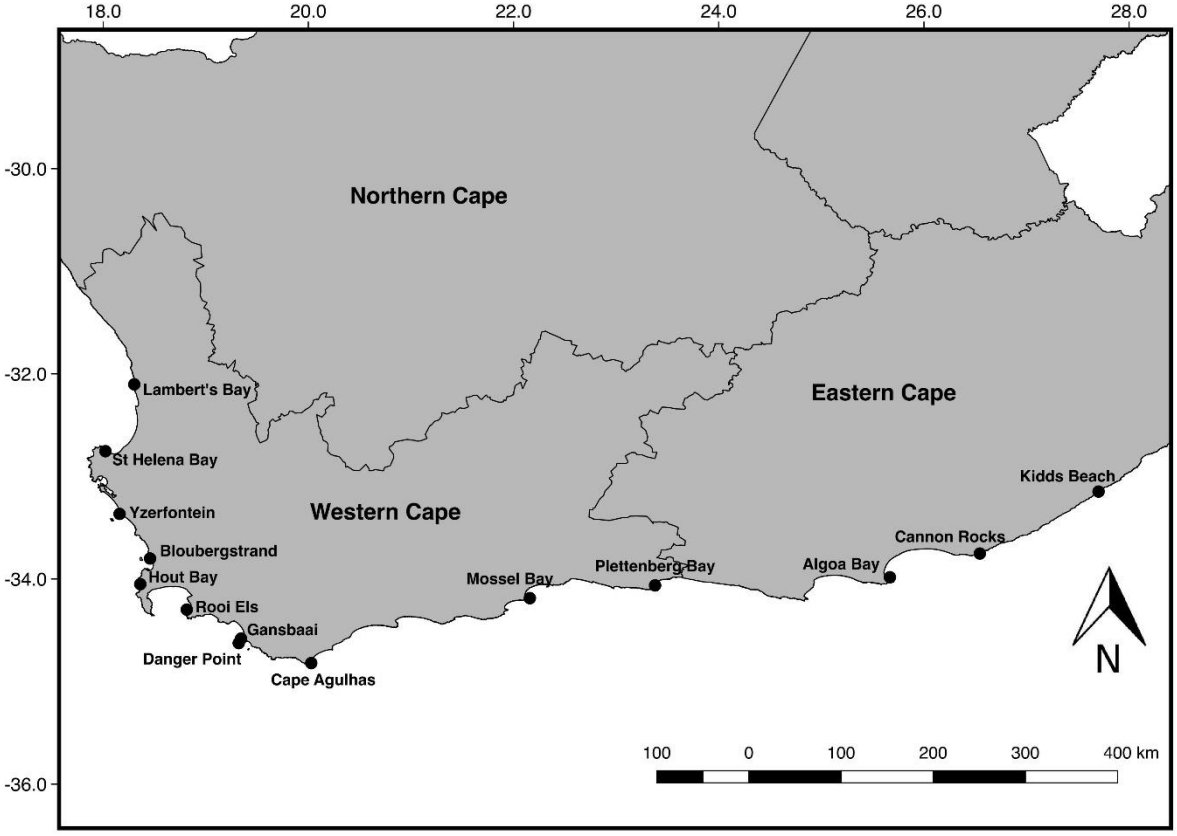

Figure 3.1: Fourteen sampling localities of *Platynereis* sp. along the South African coast.

Table 3.1: Numbers of *Platynereis dumerilii* and *Platynereis australis* from fourteen sample sites, arranged from west to east along the South African coast. Dates of collection and geographic co-ordinates of sites also included. Numbers in brackets are those examined for morphological analysis.

Sample Site	Site Code	Date collected	Co-ordinates	*Platynereis dumerilii*	*Platynereis australis*
Lamberts Bay	LB	14 – 10 – 2016	32°10'19.82"S 18°30'30.05"E	23 (11)	9 (9)
St. Helena Bay	SHB	15 – 11 – 2016	32°75'34.99"S 18°02'16.60"E	27 (25)	3 (3)
Yzerfontein	YZ	19 – 10 – 2016	33°36'49.48"S 18°15'97.64"E	30 (30)	0
Bloubergstrand	BB	02 – 09 – 2016	33°79'98.38"S 18°45'68.46"E	24 (15)	2 (2)
Hout Bay	HB	06 – 2017	34°04'98.51"S 18°36'14.8"E	5 (5)	0
Rooi Els	RE	03 – 05 – 2016	34°29'78.46"S 18°81'47.40"E	12 (9)	28 (14)
Danger Point	DP	22 – 06 – 2016	34°62'48.01"S 19°32'13.65"E	7 (8)	21 (21)
Gansbaai	GB	22 – 06 – 2016	34°58'08.75"S 19°34'34.88"E	0	31 (17)
Cape Agulhas	CA	04 – 07 – 2016	34°81'94.05"S 20°02'81.53"E	3 (3)	20 (11)
Mossel Bay	MB	27 – 10 – 2015	34°18'63.87"S 22°15'92.86"E	14 (14)	0
Plettenberg Bay	PB	28 – 02 – 2017	34°06'18.02"S 23°37'97.76"E	4 (4)	6 (6)
Algoa Bay	AB	28 – 03 – 2017	33°98'25.13"S 25°66'91.64"E	5 (5)	8 (8)
Cannon Rocks	CR	29 – 03 – 2017	33°75'15.08"S 26°54'58.36"E	2 (2)	12 (12)
Kidds Beach	KB	30 – 03 – 2017	33°14'71.54"S 27°70'32.59"E	10 (10)	22 (22)

DNA extraction, amplification and sequencing

The ZR Genomic DNA Tissue MiniPrep Kit was used to extract DNA from tissue samples from five – eight specimens per species per site as per manufacturer's instructions. The isolated DNA was stored at -80 ºC for application in Polymerase Chain Reaction (PCR). Genomic DNA was amplified using the universal mitochondrial primer pair: LCO1490 5'- GGTCAACAAATCATAAAGATATTGG -3' and HCO2198 5'- TAAACTTCAGGGTGACCAAAAAATCA -3' and the ITS1 primer pair used were ITS18SFPoly 5'- GAGGAAGTAAAAGTCGTAACA -3' and ITS5.8SRPoly 5'- GTTCAATGTGTCCTGCAATTC -3'. PCR amplifications were conducted using 12.5 µl of *OneTaq* Quick-Load Master Mix (New England BioLabs), 9.5 µl of molecular biology grade water, 0.50 µl of forward and reverse primer at 10 µm concentration, 0.5 µl of 1% bovine serum albumin (BSA) and 1 µl of template DNA to make up a total volume of 25 µl. Thermal cycling conditions were an initial denaturation of 95 ºC for 3 minutes, followed by 35 cycles of 94 ºC for 30 seconds, 45 ºC for 30 seconds, 72 ºC for 1 minute, followed by a final extension of 72 ºC for 7 minutes. Cycling conditions for ITS1 were an initial denaturation of 94 ºC for 2 minutes, followed by 25 cycles of 94 ºC for 30 seconds, 48 ºC for 10 seconds, 72 ºC from 1 minute, followed by a final extension of 75 ºC for 5 mins. PCR products of mtCOI and ITS1 were run on a 1% agarose gel using 3 µl of PCR product and 5 µl of Quick-Load Purple 100bp DNA ladder. Images were taken using a fluorescent gel imaging system. PCR amplicons were sequenced at the Central Analytical Facility at Stellenbosch University using just the forward primers (LCO1490 and ITS18SFPoly).

Phylogenetic methods

Mitochondrial COI and ITS1 sequences were already available for *P. dumerilii* from France, Italy, Spain and India whereas only mtCOI sequences were available for *P. australis* from New Zealand, which were all used in this study. Additionally, sequences of morphologically similar species to *P. dumerilii* were also included. Mitochondrial COI and ITS1 sequences were aligned using the ClustalW multiple alignment method, edited and then trimmed using BioEdit (v.7.2.6) (Hall 1999). The mtCOI dataset was trimmed to a length of 574bp and ITS1 to 754bp. The BLAST algorithm was used to search for highly similar sequences on Genbank. Sequences included in the analysis were

P. australis from New Zealand, *P. dumerilii, P. massiliensis* and *Platynereis* sp. from Europe, India, United Kingdom and lab cultures, *P. calodonta* (Kinberg, 1866) from South African and *P. bicanaliculata* (Baird, 1863) from Canada (Table 3.2). The closely related *Nereis pelagica* Linnaeus, 1758 and *Nereis zonata* Malmgren, 1867 were used as outgroups to root the mtCOI phylogenetic trees (Accession numbers: KR916896 and HQ024405) and *Perinereis aibuhitensis* (Grube, 1878), *Perinereis floridina* (Ehlers, 1868) and *Perinereis nuntia* (Lamarck, 1818) were used to root the ITS1 tree (Accession numbers: AF332153, AF332152 and EF545152, respectively, Table 3.2).

Haplotype data files were generated for each dataset using DnaSP v5 (Librado and Rozas 2009). MrModelTest 2.3 (Nylander 2004) was used to calculate the best fit model of evolution for mtCOI and ITS1. The Aikaike Information Criterion (AIC) selected the HKY+G model for the mtCOI dataset and the GTR+G model for the ITS1 dataset and were used in subsequent analysis.

Bayesian Inference (BI) method was used to construct phylogenetic trees for mtCOI and ITS1 using MrBayes 3.1.2 (Ronquist et al. 2012). Bayesian trees were calculated using 4 Markov Chains of 6 000 000 generations sampled simultaneously with every 1000th tree sampled for both datasets. A 50% majority rule consensus tree for both datasets with Bayesian posterior probability support for each clade was constructed by discarding the first 25% trees as burn in. Tracer v1.5 (Rambaut and Drummond 2009) was used to investigate the convergence between runs (stationarity of parameters). The mixing quality of all parameters was verified by analysing the plot of the log likelihood versus the sampled trees and the effective sample sizes (ESS) for all parameters calculated in Tracer. ESS values greater than 200 was observed for all parameters and thus considered as good mixing. Maximum Likelihood trees with bootstrap support for clades were computed in MEGA7 (Kumar et al. 2016) for both mtCOI and ITS1. An unrooted TCS network was constructed using PopArt (Leigh and Bryant 2015) with a haplotype data file containing only individuals belonging to the *Platynereis dumerilii* species complex. These comprised of individuals from Clades A – C3 for mtCOI and Clade A1 – B for ITS1. The TCS algorithm follows the statistical parsimony criterion with a 95% probability. Considering the significant genetic divergence among species clades, haplotype networks were constructed separately for each.

Table 3.2: Genbank Accession numbers, locality, phylogenetic clade numbers and reference data of mtCOI and ITS1 specimens of the genus *Platynereis* used for phylogenetic analysis.

Species	Accession number COI	Location	Marker	mtCOI phylogeny/network clade	ITS1 phylogeny/network clade	Reference
Platynereis bicanaliculata	HM473593 – HM473598	Canada	COI	E	-	Carr et al. 2011
Platynereis dumerilii	KR916915 – KR916917	Portugal	COI	A	-	Lobo et al. 2016
	KT124703	France	COI	C1	-	Wäge et al. 2017
	KT124708	France	COI	C1	-	Wäge et al. 2017
	KT124707	France	COI	C1	-	Wäge et al. 2017
	KT124706	France	COI	C1	-	Wäge et al. 2017
	KT124672	France	COI	C1	-	Wäge et al. 2017
	KT124671	France	COI	C1	-	Wäge et al. 2017
	KT124670	France	COI	C1	-	Wäge et al. 2017
	KT124669	France	COI	C1	-	Wäge et al. 2017
	KT124668	France	COI	C1	-	Wäge et al. 2017
	KT124692 - KT124701, KT124709	Italy	COI	C1	-	Wäge et al. 2017
	KP1247954	Italy	COI	C1	-	Lucey et al. 2015
	KT124675 - KT124679	Spain	COI	C1	-	Wäge et al. 2017
	KU714775 - KU714780	Spain	COI	C1	-	Miralles et al. 2016
	KF737174	India	COI	C1	-	Singh et. al. 2013 [1]
	KF815726	India	COI	C1	-	Singh et. al. 2013 [2]
Platynereis massiliensis	KP127953	Italy	COI	A	-	Lucey et al. 2015
	KT124683 - KT124682, KT124680 - KT124681	Italy	COI	A	-	Lucey et al. 2015
Platynereis dumerilii Heteronereid	KT124691,	Italy	COI	C1	-	Wäge et al. 2017

[1] Singh, R., Sahu, S.K., Thangaraj, M and Rajasekaran, R. Direct Submission
[2] Singh, R., Sahu, S.K. and Thangaraj, M. Molecular taxonomy of polychaetes. Unpublished

	KT124688 - KT124690					
Platynereis sp.	KT124709 - KT124715, KT124717	Vulcano, Italy	COI	C3	-	Wäge et al. 2017
	KT124702, KT124704, KT124705	Stareso, France	COI	C2	-	Wäge et al. 2017
Platynereis australis	-	New Zealand	COI	D2	-	Santos et al. 2016 [3]
	-		COI	D2	-	Santos et al. 2016 [3]
	-		COI	D2	-	Santos et al. 2016 [3]
Platynereis dumerilii	KC591946	Italy	ITS1	-	A2	Calosi et al. 2013
	KC591936	Italy	ITS1	-	A2	Calosi et al. 2013
	KC591943 - KC591945	Italy	ITS1	-	A1	Calosi et al. 2013
	KC591939 - KC591941	Italy	ITS1	-	A1	Calosi et al. 2013
	KC591934 KC591935	Italy	ITS1	-	A1	Calosi et al. 2013
	EF117899 - EF117902, EF117906	Lab cultures	ITS1	-	A1	Hui et al. 2007
	KC591949 - KC591950	UK	ITS1	-	A1	Calosi et al. 2013
	KF850505	India	ITS1	-	A1	Singh et al. 2013 [2]

[3] Santos, C.S.G and Halanych, K. Pers. comms.

Results

A total of 150 individuals were identified as *P. dumerilii* with 75% of the specimens collected from sites along the West coast whereas 90% of the 141 individuals identified as *P. australis* were collected from sites along the South-west and South coasts (Table 3.1).

Sequence variation

For both mtCOI and ITS datasets consisted of 5-8 sequences per species per site selected randomly from a total of 291 specimens.

The mtCOI dataset had a total of 100 sequences from South Africa (52 sequences of *P. dumerilii* and 48 sequences of *P. australis*). The remainder of the dataset comprised 67 sequences of *P. dumerilii* from France, Portugal, Italy, Spain and India*, P. massiliensis* from Italy*, P. australis* from New Zealand*, P. calodonta* from South Africa*, P. bicanaliculata* from Canada and *Platynereis* sp. from Italy and France (Table 3.2). The dataset included two outgroups, *N. pelagica* and *N. zonata* which were used to root the phylogenetic trees. The final aligned and edited dataset consisted of 81 haplotypes with a total of 574 sites. Of these, 178 sites were monomorphic and 205 were polymorphic. The dataset contained 336 mutations, 25 singleton variable sites and 180 parsimony informative sites. Haplotype diversity was 0.959 with a standard deviation of 0.007 and nucleotide diversity was 0.0165 with a standard error of 0.153.

The ITS dataset had a total of 107 South African sequences (*P. dumerilii* = 54 and *P. australis* = 53). The remaining dataset consisted of 19 sequences of *P. dumerilii* from the Mediterranean, United Kingdom, India and laboratory cultures (Table 3.2). Three outgroups, *Perinereis aibuhitensis, Perinereis floridina* and *Perinereis nuntia* were used as outgroups to root the phylogenetic trees. The final aligned and edited data file was 790bp long and consisted of 65 haplotypes with a total of 709 sites. There were 22 monomorphic sites, 210 polymorphic sites, 424 mutations, 16 singleton variable sites and 194 parsimony informative sites. Haplotype diversity was 0.948 with a standard error of 0.203 and nucleotide diversity was 0.203 with a standard deviation of 0.336.

Phylogenetic analyses, genetic diversities and networks

Mitochondrial COI Bayesian and Maximum likelihood trees produced five distinct species clades, labelled A – E, that were strongly supported by posterior probability and maximum likelihood support (Figure 3). Species clades A and B together with *P. dumerilii* (Clade C1, Figure 3.2) and *Platynereis* sp. (Clade C2 and C3, Figure 3.2) form a strongly supported monophyletic group that comprises individuals that morphologically resemble each other and in some cases are indistinguishable, forming the *Platynereis dumerilii* species-complex. Within this complex, Clade A has high posterior probability and maximum likelihood support and comprises Species A from South Africa with *P. dumerilii* from Portugal and *P. massiliensis s.l.* from Italy nesting among the SA species (Figure 3.2). South African specimens in Clade A comprised mostly individuals that were morphologically identified as *P. australis* and genetically, Clade A differed from *P. australis* from New Zealand by 26% (±0.028) (Table 3.3). Clade B has high posterior probability and maximum likelihood support and comprises mostly specimens from South Africa that were morphologically identified as *P. dumerilii* (Figure 3.2). Clade B genetically differed from its congener from France (Clade C1, the real *P. dumerilii* according to Wäge et al. (2017)) by 27% (±0.029) (Table 3.3). Thus, South African specimens from Clades A and B constitute different species and will be henceforth referred to as Species A and Species B, respectively. Even though Species A and B from South Africa formed two well-supported clades (Figure 3.2) that genetically differed from each other by 27% (±0.030) (Table 3.3), these species clades did not match the morphological groupings with 100% accuracy. Twenty-five percent of the individuals from the South coast (Algoa Bay, Plettenberg Bay and Kidds Beach) initially identified as *P. dumerilii* grouped within Species Clade A and 15% of the individuals from West coast and South coast (Lamberts Bay, St. Helena Bay and Cannon Rocks) identified as *P. australis* grouped within Species Clade B. This strongly indicates that morphology alone cannot be used to distinguish between Species A and B from South Africa.

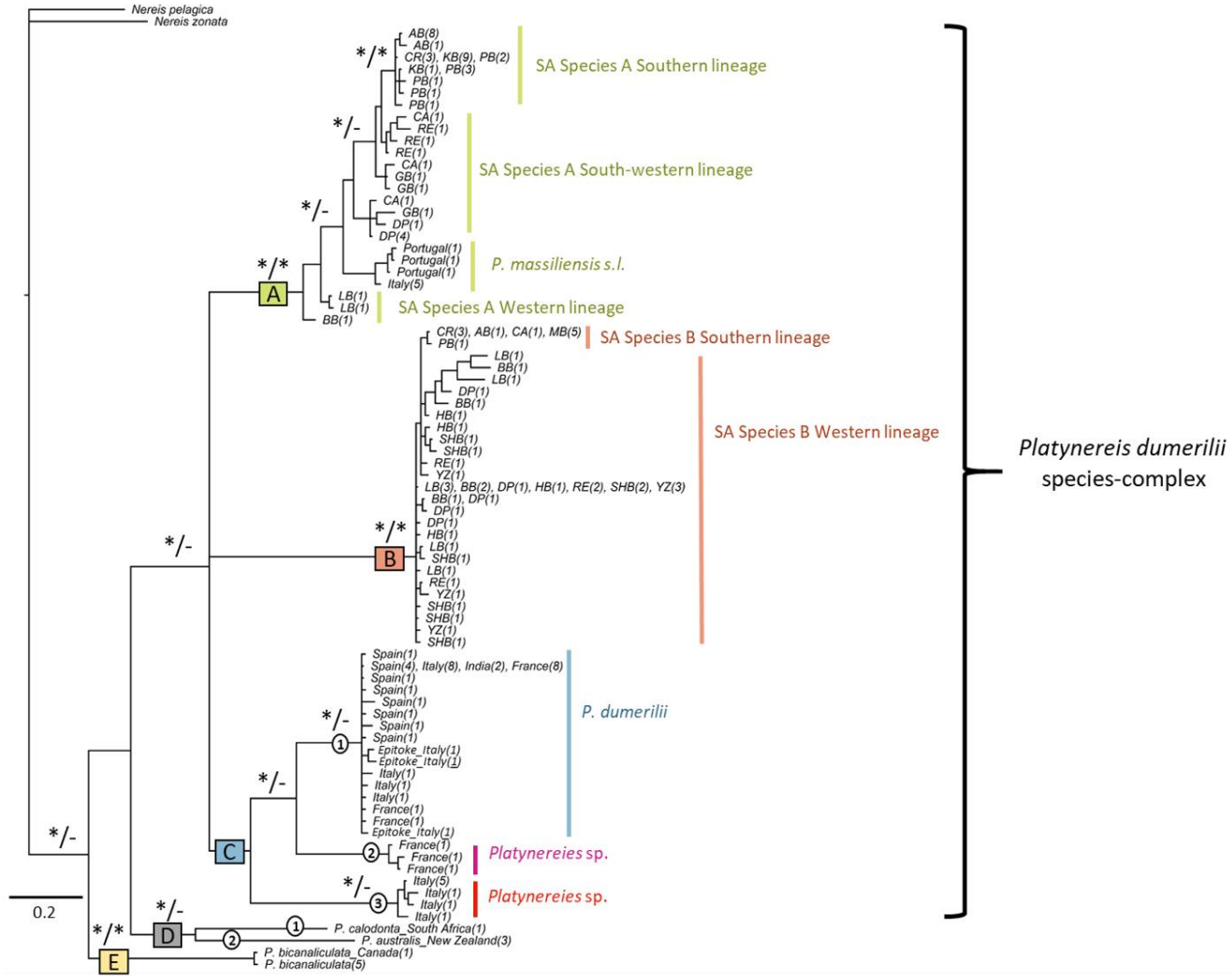

Figure 3.2: Mitochondrial COI tree based on Bayesian Inference and Maximum Likelihood analyses. Asterisks at nodes indicates clades supported significantly by Bayesian posterior probability (>0.95), right side of slash and Maximum Likelihood support (>90%) on left side of slash. Clade A (green) and B (peach) contain sequences of Species A and B from South Africa (locality data in Table 3.1) and Clade C (blue, pink and red), D (gray) and E (yellow) contain *Platynereis* sequences from South Africa and elsewhere in the world (locality and reference data in Table 3.2).

Clade C contains three sub-clusters: Sub-cluster C1 has strong posterior probability and contains the real *P. dumerilii* from the Mediterranean and India and has a low intraspecific distance of 0.5% (±0.001, Figure 3.2, Table 3.3). Sub-cluster C2 contains *Platynereis* sp. from the Stareso vents in France (*P. dumerilii* clade - Clade C3 in Wäge et al. (2017)) (Figure 3.2). Sub-cluster 3 has high posterior probability and contains *Platynereis* sp. from the Vulcano vents in Italy (Clade C2 in Wäge et al. (2017)) (Figure 3.2). Both these clades have low intraspecific distances of 1.1% (±0.004, C2)

and 0.7% (±0.002, C3) (Table 3.3). Species B from South Africa differs from Clade C2 and C3 by 27% (±0.030) and 26% (±0.029) respectively, also suggesting that Species B is independent of the others. *Platynereis calodonta* from South Africa and *P. australis* from New Zealand, form Clade D with high posterior probability support. They differed from one another by 23% (±0.027) and Clade D2 containing three *P. australis* individuals from New Zealand had an intraspecific variability of 0% (±0.000) (Table 3.3). Lastly Clade E is well-supported by posterior probability and maximum likelihood analysis and comprises individuals of *P. bicanaliculata* from Canada. The intraspecific distance within Clade E was 0.1% (±0.001) (Figure 3.2, Table 3.3).

Three major geographically defined lineages are present in Clade A; a Southern lineage comprising individuals from Algoa Bay, Cannon Rocks, Kidds Beach and Plettenberg Bay, a South-western lineage comprising individuals from Cape Agulhas, Rooi Els, Danger Point, Gansbaai, Italy and Portugal and a Western lineage comprising individuals from Lamberts Bay and Bloubergstrand (Figure 3.2). The intraspecific genetic diversity of this clade is 4.2% (±0.006, Table 3.3) suggesting weak to moderate genetic structure. Clade B exhibits two geographically defined lineages; a Southern lineage comprising populations from Cannon Rocks, Algoa Bay, Cape Agulhas, Plettenberg Bay and Mossel Bay and a Western lineage consisting of populations from Lamberts Bay, St. Helena Bay, Yzerfontein, Bloubergstrand, Hout Bay, Rooi Els and Danger Point. The intraspecific variability of Clade B was 2.5% (±0.003), also suggesting weak genetic structure (Table 3.3).

The unrooted mtCOI haplotype network also supported the presence of five divergent network clades (mutation average between clades = 49) for the *P. dumerilii* species-complex (Figure 3.3). Each network clade corresponded to the species clades recovered by the phylogenetic analysis and comprised the same individuals with no shared haplotypes among clades. Clade A included 56 individuals that formed 25 haplotypes across 12 localities (Figure 3.3). Twenty-one haplotypes are from South Africa and comprised three geographically defined haplogroups; a Southern group, a South-western group with Italy and Portugal specimens nesting among them and a Western group (Figure 3.3). The Southern haplogroup was characterised by the common haplotype 2 (n = 14) spread across three localities (Plettenberg Bay, Cannon Rocks and Kidds Beach) surrounded by

less frequent haplotypes separated by a maximum of 2 mutational steps (Figure 3.3). The South-western group comprised two divergent groups, the first with South African haplotypes from Rooi Els, Danger Point, Gansbaai and Cape Agulhas and the second group comprises haplotypes from Italy and Portugal and differ from each other by 24 mutation steps via 4 unsampled haplotypes (Figure 3.3). The West coast haplogroup contains haplotypes from Bloubergstrand and Lamberts Bay and consists of just three private haplotypes that differ from the South-west and South coast groups by 26 and 31 mutation steps via 5 unsampled haplotypes (Figure 3.3).

Clade B includes 52 individuals comprising 27 haplotypes spanning 13 localities in South Africa (Figure 3.3). Like the mtCOI phylogeny, two geographically defined haplogroups are observed for this species clade; West and South haplogroups. The West haplogroup includes populations from Lamberts Bay, St. Helena Bay, Yzerfontein, Bloubergstrand, Hout Bay, Rooi Els and Danger point (Figure 3.3). It is characterised by a star-like phylogeny and has an extensive branching pattern with a dominant central haplotype (Haplotype 13, *n* = 15 across 7 sites) with several private haplotypes connected to it that differ by one mutation step, one private haplotype that differs from it by 7 mutation steps and one low frequency haplotype that differs from it by two mutation steps (Figure 3.3). The South coast group includes populations from Cape Agulhas, Mossel Bay, Plettenberg Bay, Algoa Bay and Cannon Rocks (Figure 3.3). It is dominated by Haplotype 3 comprising 11 individuals from four populations and connects to one private haplotype from Plettenberg Bay with one mutation step (Figure 3.3). Clade C1 comprises populations from the Mediterranean and India and is characterised by a star-like phylogeny (Figure 3.3). The dominant haplotype consists of 22 individuals from France, Spain, Italy and India and is connected to several private haplotypes that differ from the central haplotype by one mutation step (Maximum mutation steps = four). Clade C2 comprises three individuals from the Stareso vent in France and differs from one another or unsampled haplotypes by a maximum of two mutation steps (Figure 3.3). Clade C3 contains individuals from the Vulcano vent in Italy with a dominant central haplotype (Haplotype 69, *n* = 5) that is connected to three private haplotypes via unasampled haplotypes by a maximum of 3 mutation steps (Figure 3.3).

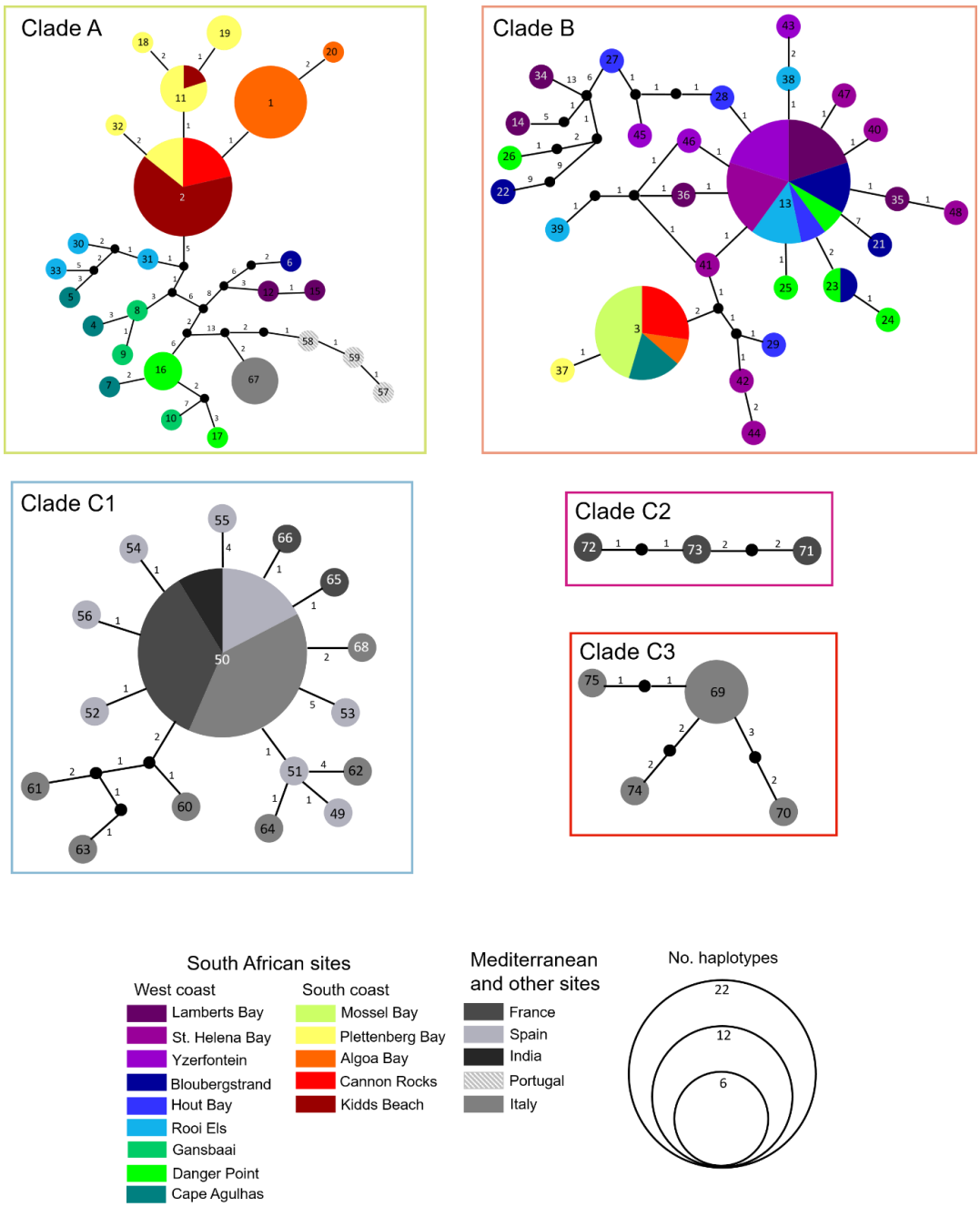

Figure 3.3: Haplotype networks for the *Platynereis dumerilii* species-complex for mtCOI data. Haplotype numbers are in each circle. Numbers on branches connecting each haplotype represent mutational steps. The size of each circle is proportional to the number of individuals present in that haplotype. Small black circles connecting two haplotypes indicate missing or unsampled haplotypes. Each site within a region is represented by a different colour. Clades that connect to one another are connected via unsampled haplotypes. Clade A – Clade B = 71 mutation steps, Clade A – Clade C2 = 61 mutation steps, Clade C2 – Clade C1 = 52 mutation steps, Clade C1 – Clade C3 = 65 mutation steps.

ITS1 Bayesian and Maximum Likelihood trees produced a well-supported (posterior probability and maximum likelihood) monophyletic group of *Platynereis* (Figure 3.4). Two sister clades are

presented, Clades A and B. Clade A consists of two sub-clades (1 – 2). Sub-clade 1 has high posterior probability and contains *P. dumerilii* from India, Italy, United Kingdom and Lab cultured specimens from France and Germany (Figure 3.4). The intraspecific variability within this clade is low, 0.4% (±0.002). Sub-clade 2 contains Species A from South Africa with *P. dumerilii* from Italy nesting with the Sub-clade. Intraspecific distances within the clade were similar to mtCOI with a 4.9% (±0.006) suggesting weak genetic structuring (Table 3.4). Unlike mtCOI, no geographic structure is observed for Species A from South Africa for ITS1. Sub-clades 1 and 2 differ from each other by 15% (±0.025) (Table 3.4). Clade B contains Species B from South Africa with a high intraspecific genetic variability of 16% (±0.013). However, like Species A no geographic structure is observed within this clade. Clade B differs from sub-clade A1 that contains *P. dumerilii* by 38% (±0.042) and differs from sub-clade 2 containing Species A by 35% (±0.039) indicating that all three clades represent independent species (Figure 3.4).

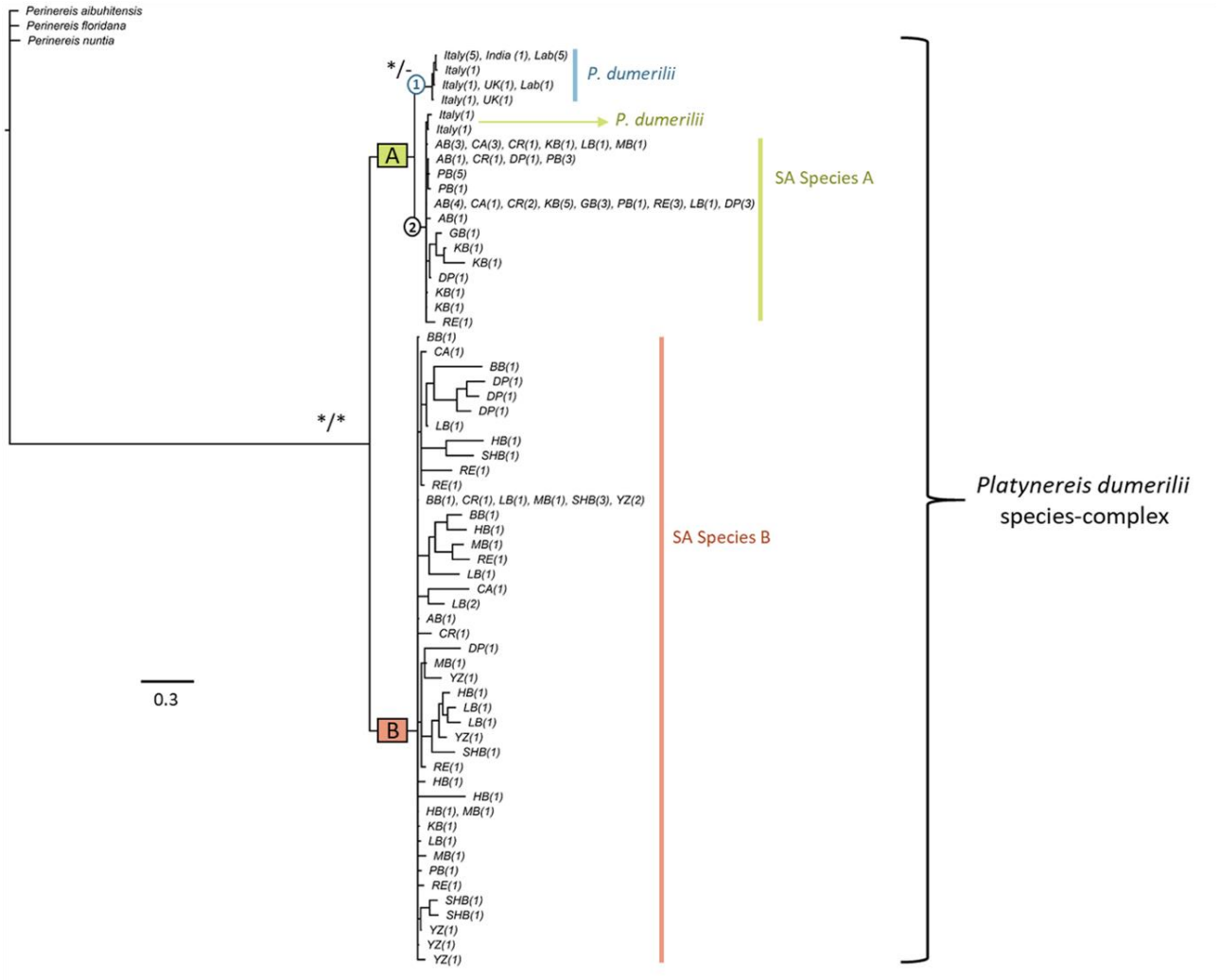

Figure 3.4: Internal Transcribed Spacer Region 1 (ITS1) tree based on Bayesian Inference and Maximum Likelihood analyses. Asterisks at nodes Asterisks at nodes indicates clades supported significantly by Bayesian posterior probability (>0.95), right side of slash and Maximum Likelihood support (>90%) on left side of slash. Clade A1 contains *Platynereis dumerilii* from elsewhere in the world (Table 3.2 – locality and reference data). Clade A2 contains *P. dumerilii* from Italy and Species A from South Africa and Clade B contains Species B from South Africa (Table 3.1 locality data).

The unrooted ITS1 network recovered three divergent network clades (mutation average between clades = 41) for the *Platynereis dumerilii* species-complex (Figure 3.5) as observed for the ITS1 phylogenetic tree (Figure 3.4). Each network clade comprised the same individuals that were recovered for species clades in the ITS1 phylogeny with no sharing of haplotypes among clades. Clade A1 contains 16 individuals across four localities (India, Italy, Lab cultures and UK) that group into five haplotypes. It is characterised by a dominant haplotype (haplotype 1, *n* = 10) shared by Italy and Lab cultured populations and is surrounded by two low frequency haplotypes and one private haplotype that differ from the central haplotype by a maximum of two mutation steps (Figure 3.5). Clade A2 comprises 24 haplotypes that were sampled from 55 individuals across 10 localities in South Africa and one locality from the Mediterranean (Figure 3.5). Clade A2 contains two dominant haplotypes (Haplotype 10, *n* = 22 and Haplotype 8, *n* = 10) that are shared by nine and six localities (Figure 3.5), respectively, exhibiting a well-mixed population, in contrast to that obtained by mtCOI network (Figure 3.3). Two of the 24 haplotypes are from the Mediterranean and are separated from the central haplotype by one mutation step (Haplotype 7) whereas Haplotype six is separated from a Rooi Els (South-western lineage/haplogroup) haplotype via three unsampled haplotypes and a total of 11 mutation steps (Figure 3.5). Clade B also contrasts with its mtCOI network by exhibiting a well-mixed population with no geographic structure. It contains 54 individuals sampled across 13 localities in South Africa forming 48 haplotypes. The network clade is characterized by an extensively branched star-like network that has a central dominant haplotype (Haplotype 15, *n* = 7 across 5 sites) surrounded by several private and two low frequency haplotypes connected to it that differ by one mutation step (Figure 3.5).

Morphology and morphometric results

The PCA reduced the eight characters into two principal components with a minimum eigenvalue of 1 that together accounted for 67% of the variation (Table 3.5). The number of segments, the length of the body and the number of paragnaths on all areas of the pharynx were the main contributors to the first component whereas longest tentacular cirri and the presence/absence of the notopodial falciger were the main contributors to the second component (Table 3.5). However, the principal component scores revealed no separation in morphological characters that distinguish between the two species (Figure 3.6).

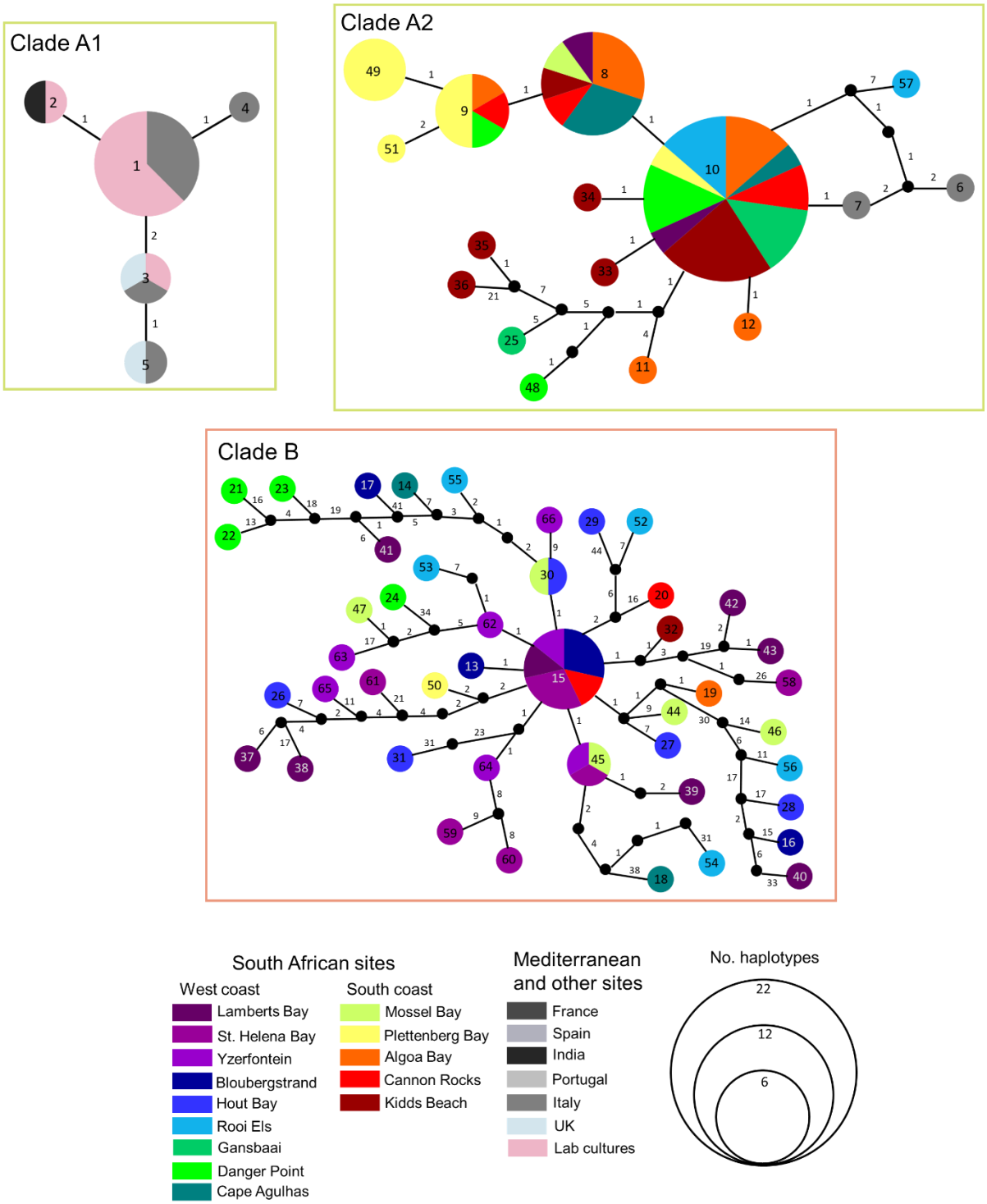

Figure 3.5: Haplotype networks for the *Platynereis dumerilii* species-complex for ITS1 data. Haplotype numbers are in each circle. Numbers on branches connecting each haplotype represent mutational steps. The size of each circle is proportional to the number of individuals present in that haplotype. Small black circles connecting two haplotypes indicate missing or unsampled haplotypes. Each site within a region is represented by a different colour. Clades that connect to one another are connected via unsampled haplotypes. Clade A1 – Clade A2 = 26 mutation steps, Clade A1 – Clade B = 46 mutation steps and Clade A2 – Clade B = 52 mutation steps.

Table 3.3: Interspecific and intraspecific distances of the mtCOI gene between the eight species clades recovered by phylogenetic analysis (in Figure 1). Clade A – Species A, *P. dumerilii* and *P. massiliensis s.l.* from South Africa, Portugal and Italy, Clade B – Species B from South Africa, Clade C1 – *P. dumerilii* from Europe and Asia, Clade C2 – *Platynereis* sp. from France, Clade C3 – *Platynereis* sp. from Italy, Clade D1 – *P. calodonta* from South Africa, Clade D2 – *P. australis* from New Zealand and Clade E *P. bicanalicluata* from Canada. Kimura 2 Parameter distances are below the diagonal with standard error estimates above the diagonal. Intraspecific distances are along the diagonal in bold for each clade with the standard error estimates after parentheses.

	A	**B**	**C1**	**C2**	**C3**	**D1**	**D2**	**E**
A	**0.042±0.006**	0.030	0.026	0.027	0.027	0.027	0.028	0.026
B	0.271	**0.025±0.003**	0.029	0.030	0.029	0.029	0.030	0.030
C1	0.261	0.273	**0.005±0.001**	0.024	0.027	0.030	0.028	0.026
C2	0.225	0.279	0.173	**0.011±0.004**	0.034	0.030	0.026	0.029
C3	0.231	0.267	0.211	0.280	**0.007±0.002**	0.029	0.035	0.030
D1	0.257	0.280	0.266	0.287	0.270	-	0.027	0.031
D2	0.265	0.292	0.253	0.229	0.311	0.233	**0.000±0.000**	0.027
E	0.228	0.291	0.211	0.259	0.280	0.268	0.257	**0.001±0.001**

Table 3.4: Interspecific and intraspecific distance of the ITS1 gene between the three species clades recovered by phylogenetic analysis (In Figure 2). Clade A1 – *P. dumerilii* from Italy, UK, India and Lab culures, Clade A2 – *P. dumerilii* from Italy and Species A from South Africa and Clade B – Species B from South Africa.

	A1	**A2**	**B**
A1	**0.004±0.002**	0.025	0.042
A2	0.150	**0.049±0.006**	0.039
B	0.387	0.354	**0.162±0.013**

Table 3.5: Eigenvalues, component loadings and the proportion of total variation contributed by the two principal components as identified by the principal component analysis on a covariance matrix of the eight morphological characters. HF – homogomph falciger, III, IV, VI, VII-VIII – paragnaths in those areas on pharynx, Longest T cirri- longest tentacular cirri.

Parameter	Component	
	1	2
Eigenvalue	4.421	1.002
Component loading		
Number of segments	0.842	-0.069
Length	0.830	-0.009
IV	0.782	-0.295
VII-VIII	0.778	-0.358
VI	0.764	-0.350
III	0.742	0.026
HF	-0.537	0.460
Longest T cirri	-0.009	0.911
% total of variance	55.0478	17.304

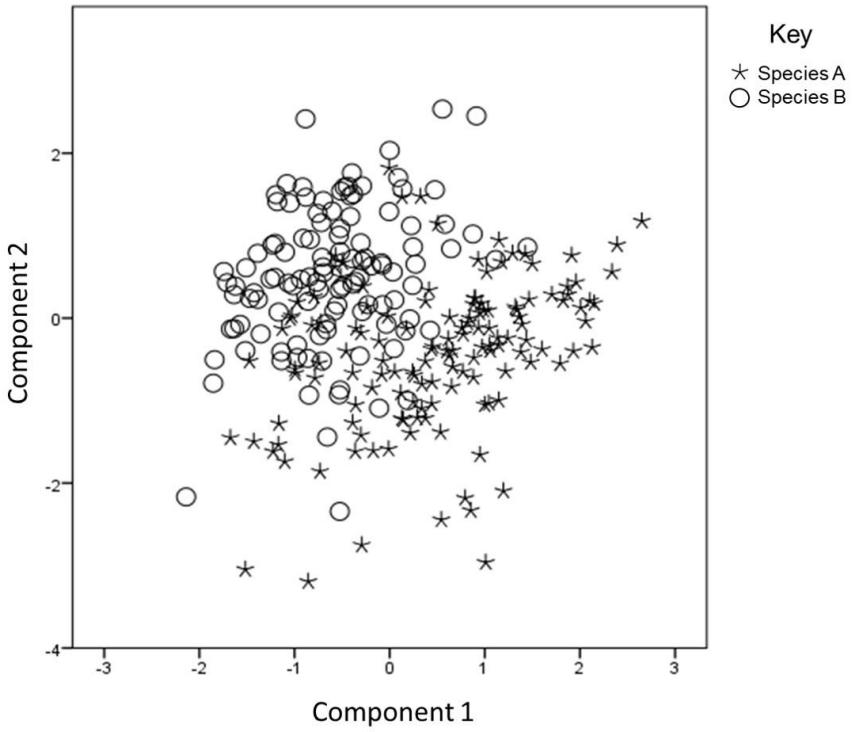

Figure 3.6: Scatterplot of principal component scores of eight morphological characters of two *Platynereis* spp from South Africa. Species A is represented by stars and Species B is represented by circles.

Discussion

This is the first study to investigate the taxonomic status of the apparently cosmopolitan *Platynereis dumerilii* and *Platynereis australis* in South Africa. Morphological examination and molecular analyses revealed several interesting findings. Species A, morphologically identified as *P. australis* and Species B, morphologically identified as *P. dumerilii* from South Africa are genetically different to their congeners from New Zealand and France, respectively. Not only had Species A and B been misidentified, but they show such high overlap in morphological characters that it was impossible to distinguish between them accurately. Species B represents an undescribed new local species in South Africa and will be therefore referred to as *Platynereis* B sp. nov. On the other hand, Species A most likely represents *Platynereis massiliensis* with genetic data also suggesting that this species originated from South Africa and therefore should be considered alien in Europe. However, the identity of Species A in South Africa cannot be determined with accuracy as *P. massiliensis* consists

of a species-complex in the Mediterranean comprising two cryptic species and it is not known which of those clades represent the real *P. massiliensis* (Wäge et al. 2017).

Platynereis massiliensis was first described in the 19th century (Moquin-Tandon 1869) from specimens found in the Marseille region of the Mediterranean Sea. This was 300 years after the commencement of transoceanic travel between Europe and Africa (Carlton 2003, 2009; Rius et al. 2015). Therefore, it is plausible that this species had unknowingly been transported from Africa to Europe where it was mistakenly described as native in the Mediterranean when it actually was an introduced species. Such incidents have been documented for several other polychaete species that were described as “new” local species in the invading environment when they actually represent introduced species (see Carlton 2009 for details; Sato-Okoshi et al. 2017; Sun et al. 2017b). For example, *Hydroides dianthus* (Verrill, 1873), a very common biofouling organism, was thought to be native to the east coast of the United States and alien in the Mediterranean Sea, however, recent molecular analyses have revealed that the animal is actually native to the Mediterranean and alien along the US east coast (Sun et al. 2017a). This may have happened with *P. massiliensis*. However, sample sizes and localities of specimens from the Mediterranean are too small and limited to infer any accurate source populations or invasion patterns and therefore will be addressed more extensively as a future direction for a different study. Since *P. massiliensis* actually represents a species complex in the Mediterranean and because one of the two clades of this complex include specimens from a site close to its’ type locality (Lucey et al. 2015; Wäge et al. 2017), it is unsure which clade represents the real *P. massiliensis.* As a result, Species A will henceforth be referred to as *P. massiliensis s.l*.

mtCOI and ITS1 results indicate that specimens previously identified as *P. dumerilii* from Portugal and Italy (Lobo et al. 2016; Miralles et al. 2016) had been misidentified since these specimens are nested among *P. massiliensis s.l.* from South Africa and Italy. We can therefore conclude that all represent a single species. Such misidentifications are understandable given the fact that the *P. dumerilii* and *P. massiliensis s.l.* forms are morphologically indistinguishable. Additionally, *P. dumerilii* from India (refer to Table 3.2 for source) is found nesting within the European *P. dumerilii* clades for both markers (Clade C1, Figure 3.2, Clade A1, Figure 3.4), therefore indicating that this

species is alien in India. This demonstrates that *P. dumerilii* is an alien, in at least parts of its' global range. Nonetheless, *P dumerilii* in and around Europe should to be re-examined to confirm its taxonomic status given the existence of the cryptic species complex.

Platynereis massiliensis s.l. (identified as *P. australis*) and *Platynereis* B sp. nov. (identified as *P. dumerilii*) according to Day (1967) were difficult to separate morphologically as demonstrated by the PCA results. Thus, the main diagnostic characters for separating these two species are not reliable; i.e., the notopodial falciger with a terminal knob on the falcigerous blade and a longer expanded and enlarged notopodial lobe in posterior parapodia were inconsistently present or absent in individuals of both species.

Platynereis massiliensis s.l. and *Platynereis* B sp. nov. were both consistently found inhabiting the same algae; *Ulva* sp., *Jania ahaerens, J. verrucose, Amphiroa ephedraea* and *Corralina officinalis.* Nonetheless, even though both species seem to prefer the same habitat, they each preferred different temperature regimes. *Platynereis massiliensis s.l.* was less abundant towards the west coast, indicating a preference for warmer temperatures whereas *Platynereis* B sp. nov. was less abundant towards the south coast indicating a preference for cooler temperatures. Such a difference in preference for environmental conditions has been documented for several cryptic species including *P. dumerilii* and *P. massiliensis s.l.* in the Mediterranean where the former species preferred non-acidified environments and the latter preferred naturally acidified environments (Calosi et al. 2013; Lucey et al. 2015; Wäge et al. 2017). Furthermore, each species in the Mediterranean was found in different algal beds which contrasts with the habitat preferences of the South African species.

The structure observed for *P. massiliensis s.l.* from South Africa displayed three mitochondrial lineages separated by two genetic barriers (Cape Agulhas and Cape Point) whereas *Platynereis* B sp. nov. only exhibited two mitochondrial lineages separated by one genetic barrier (Cape Agulhas). Similar patterns of genetic structure were observed for many marine invertebrates from South Africa where species with poor dispersal capacities tend to display several fragmented populations whereas those with planktotrophic free swimming larvae tend to exhibit populations that are less structured (Teske et al. 2007a, 2011a). Additionally, in the Mediterranean, *P. massiliensis s.l.*

displayed two divergent lineages that display some degree of structure that correspond to their sampling localities with intermediate nucleotide diversities. *Platynereis dumerilii* on the other hand, exhibited two divergent lineages, one containing the largest number of individuals with no apparent structure (Clade 4), and both nucleotide diversities that are lower than that of the two *P. massiliensis s.l.* clades (Clades 1 and 2) (Wäge et al. 2017). Based on my findings I hypothesise that *P. massiliensis s.l.* from South Africa displays a similar brooding behaviour with semi-direct developing larvae, as the Mediterranean species, producing structured populations with high diversities whereas *Platynereis* B sp. nov. probably produces planktotrophic larvae that can disperse further, resulting in less structure and low diversities. Additionally, even though in the Mediterranean, *P. massiliensis s.l.* nucleotide diversities were higher than *P. dumerilii,* it was still overall lower than the intraspecific diversities of *P. massiliensis s.l.* from South Africa. Such low nucleotide diversities of *P. massiliensis s.l.* in the Mediterranean further supports the hypothesis that *P. massiliensis s.l.* is actually alien there. In contrast to the mitochondrial structuring pattern, the nuclear marker indicated that both, *P. massiliensis s.l.* and *Platynereis* B sp. nov. in South Africa are well-mixed panmictic populations. Such contrasting patterns of genetic structure can be linked to the differing intrinsic characteristics of each gene (DeBiasse et al. 2014). Since mitochondrial DNA lacks recombination, is haploid and maternally inherited, it is representative of an effective population size that is quarter of that represented by nuclear DNA which undergoes recombination, is diploid and inherited by both parental lineages (Palumbi and Baker 1994; Burg et al. 1999; DeBiasse et al. 2014). Therefore evolutionary processes such as genetic drift strongly influence mtCOI producing several genetically structured populations (Karl et al. 1992; DeBiasse et al. 2014). Additionally, due to the smaller effective population sizes of mtCOI, it is more likely to undergo complete lineage sorting which may also result in strong mitochondrial structure but weak nuclear structure (DeBiasse et al. 2014). Thus, the discordance between the mtCOI and ITS1 genes for *P. massiliensis s.l.* and *Platynereis* B sp. nov. could be a result of the smaller effective population sizes exhibited by mtCOI.

Taxonomy

Since *Platynereis* B sp. nov. represents a genetically distinct species from *Platynereis dumerilii*, it is thus described as a new local species below.

Family **NEREIDIDAE** Blainville, 1818

Subfamily **NEREIDINAE** Blainville, 1818

Genus ***Platynereis*** Kinberg, 1865

Type species: *Platynereis magalhaensis* Kinberg, 1866

***Platynereis* B,** sp. nov. (Figs 3.7 and 3.8)

?*Platynereis dumerilii* Day 1967:306, figs 14.4 d – k, (NOT Audouin & Milne Edwards, 1833), from Day (1967)

?*Platynereis australis* Day 1967:305, fig 14.4 m, (NOT Schmarda, 1861), from Day (1967)

Material examined

Holotype. South Africa, Western Cape, Rooi Els, 1 specimen, 34°29'78.46"S 18°81'47.40"E, coll. J. Kara, 0m, 03.v.2016 from under beds of *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis*.

Paratype. South Africa, Western Cape, Yzerfontein, 1 specimen, 33°36'49.48"S 18°15'97.64"E, coll. J.Kara, 0m, 19.x.2016 from under beds of *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis*. South Africa, Western Cape, Danger Point, 1 specimen, 34°62'48.01"S 19°32'13.65"E, coll. J. Kara, 0m, 22.vi.2016 from under beds of *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis.*

Non-type material. Namibia, Luderitz, Dias Point, 5 specimens in good condition (SAMC-A20134) coll. J.H.Day 1969. Namibia, Mowe Bay, 3 specimens in good condition (SAMC-A20212), coll. J.H.Day 1969. South Africa, Western Cape, Lamberts Bay, 3 specimens in good condition (SAMC-A20707) coll. J.H.Day. Angola, Mocamedes, Praia das Conchas, 3 specimens in good condition (SAMC-A20244) coll. J.H.Day. South Africa, Western Cape, Lamberts Bay, 1 specimen

32°10’19.82”S 18°30’30.05”E, coll. J. Kara, 0m, 14.x.2016 from under beds of *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis.*

Additional material. Namibia, Luderitz, 1 specimen in good condition (SAMC-A20705) coll. J.H.Day. Falkland Islands, 2 specimens in good condition (SAMC-A20704) coll. J.H.Day. Marion and Prince Edward Islands, 3 specimens in good condition (SAMC-A21355), coll J.H.Day dredge samples 1971. Marion and Prince Edward Islands, 3 specimens in good condition (SAMC-A21174) coll. M.Branch, bottom dredge. South Africa, Western Cape, Lamberts Bay, 11, 32°10’19.82”S 18°30’30.05”E, coll. J. Kara, 14.x.2016 from under beds of *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis.* South Africa, Western Cape, St. Helena Bay, 25, 32°75’34.99”S 18°02’16.60”E, coll. J. Kara, 15.xi.2016 from under beds of *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis.* South Africa, Western Cape, Yzerfontein, 30, 33°36’49.48”S 18°15’97.64”E, coll. J. Kara, 19.x.2016 from under beds of *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis.* South Africa, Western Cape, Bloubergstrand, 15, 33°79’98.38”S 18°45’68.46”E, coll. J. Kara, 02.ix.2016 from under beds of *Ulva* sp. South Africa, Western Cape, Hout Bay, 5, 34°04’98.51”S 18°36’14.8”E, coll. L. Skein, vi.2017 subtidal among mussel beds. South Africa, Western Cape, Rooi Els, 9, 34°29’78.46”S 18°81’47.40”E, coll. J. Kara, 03.v.2016 from under *Ulva* sp. beds. South Africa, Western Cape, Danger Point, 8, 34°62’48.01”S 19°32’13.65”E, coll. J. Kara, 22.vi.2016 from under *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis.* South Africa, Western Cape, Cape Agulhas, 3, 34°81’94.05”S 20°02’81.53”E, coll. J. Kara, 04.vii.2016 from under *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis.* South Africa, Western Cape, Mossel Bay, 14, 34°18’63.87”S 22°15’92.86”E, coll. J. Kara, 27.x.2015 from under *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis.* South Africa, Western Cape, Plettenberg Bay, 4, 34°06’18.02”S 23°37’97.76”E, coll. J. Kara, 28.ii.2017 from under *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis.* South Africa, Eastern Cape, Algoa Bay, 5, 33°98’25.13”S 25°66’91.64”E, coll. J. Kara, 28.iii.2017 from under *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis.* South Africa, Eastern Cape, Cannon Rocks, 2, 33°75’15.08”S 26°54’58.36”E, coll. J. Kara, 29.iii.2017 from under *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina*

afficinalis. South Africa, Eastern Cape, Kidds Beach, 10, 33°14'71.54"S 27°70'32.59"E, coll. J. Kara, 30.iii.2017 from under *Ulva* sp., *Jania verrucosa, Amphiroa ephedra, Corralina afficinalis.*

Description

Body colour and pigmentation visible when alive, disappear shortly after preservation in 100% ethanol. Body colour variable on dorsum: medium - light brown to dark red/brown matte colour (Fig. 3.7 A – B). White luminescent pigmentation between four pairs of dark brown eye spots on prostomium (Fig. 3.7 A – B). Lateral sides of anterior segments dark green, colour prominent at chaetiger boundaries (Fig. 3.7 A – B, red arrows). Circular dark-brown/red patches of brown pigment at base of dorsal cirrus from 10th chaetiger to posterior (Fig. 3.7 A). Dull yellow dorsal midline in anterior chaetigers, from 16th chaetiger to posterior colour luminescent cream/white in the characteristic chain-like pattern for *Platynereis* (Fig. 3.7 A – B).

Robust species, 5 – 65 mm long for 30 – 84 chaetigers (n = 122). Swollen anteriorly, from chaetiger 2-4, uniform in width after 7th chaetiger, tapering posteriorly (Fig. 3.7 B). Prostomium broader than long (Fig. 3.7 C). Pair of frontal antenna, slender, tapering (Fig. 3.7 C). Pair of palps, swollen base and rounded distal ends (Fig. 3.7 C - D, red arrows). Four pairs of tentacular cirri, tapering, longest cirri extend back to chaetiger 6 – 8 (Fig. 3.7 A - C). Dark brown/black jaws on pharynx, 6 teeth (Fig. 3.7 D). Paragnaths on pharynx in distinct areas (Fig. 3.7 D), rods in tight lines or rows, Area I & II: 0, III: 3 discontinuous rows, IV: 5 rows, V: 0, VI: 2-3 rows, arc shape, VII-VIII: 2-3 rows in 5-6 groups (Fig 3.7 D).

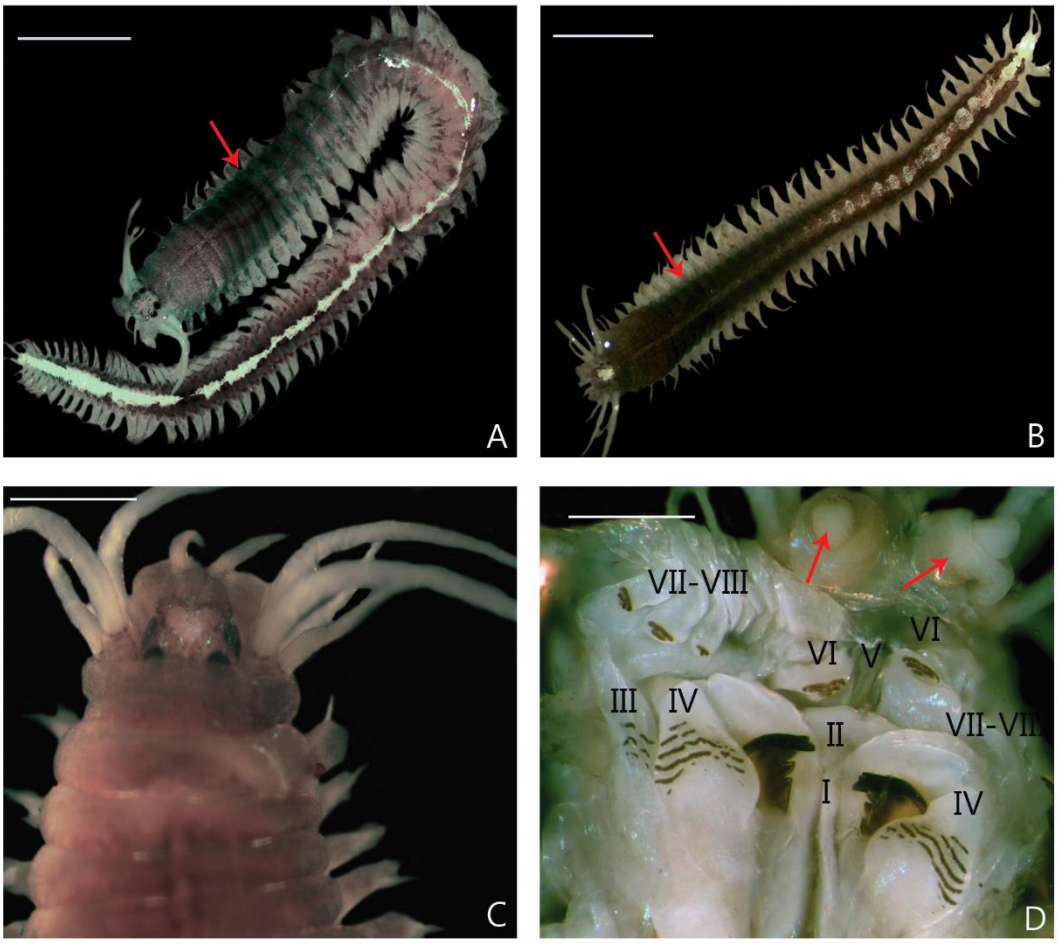

Figure 3.7: Two colour variants of *Platynereis* B sp. nov. A: Red matte with green (red arrows pointing to green pigmentation), dorsal view B: Dark brown with green (red arrows pointing to green pigmentation), dorsal view, C: Dorsal anterior view of prostomium, D: Dorsal and ventral view of pharynx with paragnaths, red arrows pointing to palps. Scale bar in A – B: 2mm, C-D: 500µm.

Parapodia of chaetigers 1 and 2 uniramous, thereafter biramous. Notopodial dorsal and ventral ligules, thick, conical, equal in length in anterior chaetigers (Fig. 3.8 A), digitiform from chaetiger 22 (Fig. 3.8 C). Notopodial postchaetal ligule present from chaetiger 12, conical (Fig. 3.8 B). Dorsal cirri simple, lack basal cirriphores, twice the length of the superior notopodial lobe (Fig. 3.8 A – E). Notopodial ligule, conical in posterior chaetigers, swollen basally forming a glandular structure (Fig. 3.8 C – E), inferior ligule, long and slender from chaetiger 32, ¾ the length of the superior ligule (Fig. 3.8 E). Dorsal neuropodial superior and inferior ligule rounded and reduced throughout (Fig. 3.8 A – E). Neuroacicular ligule conical, half the length of the ventral ligule in anterior chaetigers (Fig. 3.8 A), pointed and equal in length to ventral ligule in middle chaetigers (Fig. 3.8 A – B) and shorter than

ventral ligule in posterior chaetigers (Fig. 3.8 D – E). Neuropodial postchaetal lobe rounded anteriorly (Fig. 3.8 A), conical from chaetiger 12 to posterior (Fig. 3.8 B – E). Ventral neuropodial ligule rounded, reduced in anterior chaetigers (Fig. 3.8 A), conical in chaetiger 12 (Fig. 3.8 B), digitiform from chaetiger 22 to posterior (Fig. 3.8 C – E). Ventral cirrus equal in length to ventral ligule in anterior chaetigers (Fig. 3.8 A), half the length of the neuropodial ventral ligule from chaetiger 12 to posterior (Fig. 3.8 B – E). Notochaetae, homogomph spinigers, curved blades finely serrated (Fig. 3.8 G) and bifid homogomph falcigers from chaetiger 22 to posterior, distal tip with rounded secondary tooth, subdistal main tooth tip also rounded, connecting tendon from tip (Fig. 3.8 F). Neuropodial superior neurochaetae, thick heterogomph falcigers, terminal tendons, small concave blades (Fig. 9 H) and homogomph spinigers, curved blades finely serrated (Fig. 3.8 I). Neuropodial inferior neurochaetae, heterogomph spinigers, curved blade, finely serrated (Fig. 3.8 K), heterogomph falcigers, concave blade, finely serrated, terminal tendon (Fig. 3.8 J).

Habitat. Very common in the lower intertidal zone under *Ulva* sp., *Laurencia flexuosa* and *Jania verrucosa* beds. A soft flexible tube is formed out of mucous secretions, sand and detritus.

Distribution. From Lamberts Bay in the Western Cape to Kidds Beach in the Eastern Cape of South Africa. Day (1967) also recorded this species in Namibia and Angola.

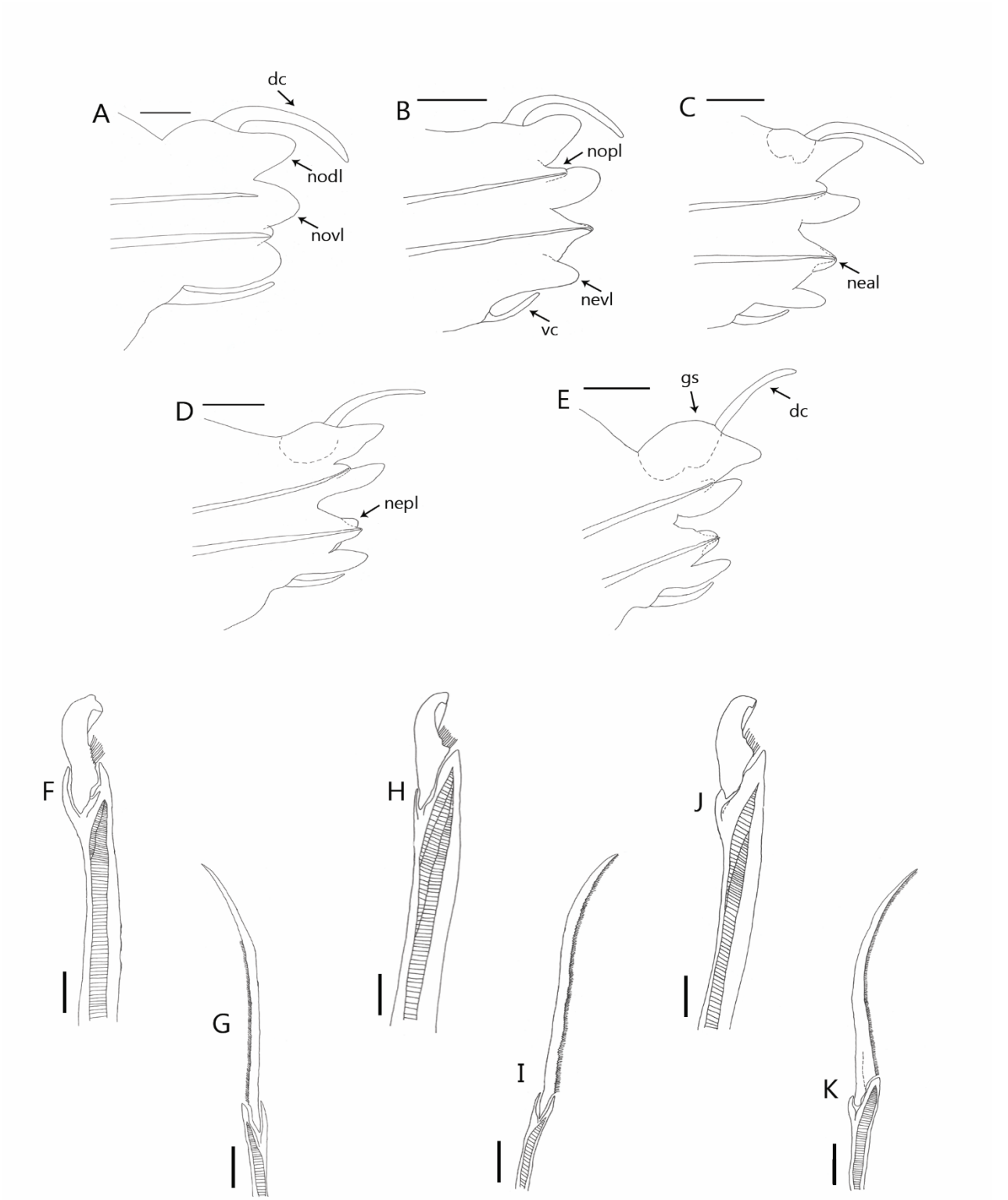

Figure 3.8: Parapodia and chaetiger type of *Platynereis* B sp. nov. A: 5th chaetiger, posterior view, B: 12th chaetiger, posterior view, C: 22nd chaetiger, posterior view, D: 32nd chaetiger, posterior view, E: 50th chaetiger, posterior view, F: notopodial homogomph bifid falciger, G: notopodial homogomph spiniger, H: neuropodial heterogomph falciger, superior fascicle, I: neuropodial homogomph spiniger, superior fascicle, J: neuropodial hetergomph falciger, inferior fascicle and K: neuropodial heterogomph spiniger, inferior fascicle. Scale bar in A: 50µm, B – E: 100µm, F – K: 12.5µm. dc: dorsal cirrus, nodl: notopodial dorsal ligule, novl: notopodial ventral ligule, nopl: notopodial

postchaetal ligule, nevl: neuropodial ventral ligule, vc: ventral cirrus, neal: neuropodial acicular ligule, nepl: neuropodial postchaetal ligule, gs: glandualr structure.

Remarks:

Mastigonereis quadridentata and *M. striata* are considered invalid names due to the absence of type material. Furthermore, it would be impossible and inappropriate to assign either of these names to the new species as it was demonstrated in this study that some *Platynereis* species are morphologically indistinguishable but represent genetically distinct species.

Freshly collected specimens of *P. massiliensis s.l.* and *Platynereis* B sp. nov. were morphologically similar to one another (Table 3.6). They differed by *Platynereis* B sp. nov. having the longest tentacular cirri that extend back to one more chaetiger (chaetiger 14). Other characters such as length, number of segments, paragnaths on areas III, IV, VI, VII-VIII all fell within the same range and notopodial falcigers were present or absent in specimens of both species (Table 3.6). Therefore, the characters used to distinguish between the two species were not strong enough to define either even though they represent genetically distinct species.

Specimens identified as *Platynereis dumerilii* by Day (collected in South Africa, Namibia and Angola) and those collected in our study were also similar with respect to their size, number of segments and arrangement of paragnaths on the pharynx (Table 3.6). The museum specimens differed by having the longest tentacular cirri that extend to chaetiger 12, which is one chaetiger less than *Platynereis* B sp. nov. and two more than *P. massiliensis s.l.* Additionally, the notopodial falciger was consistently present in all specimens whereas for the freshly collected material whose identities could be validated genetically, they were either present or absent. Thus, the specimens deposited at the museum as *P. dumerlii* could not confidently be assigned to this species as their identity could not be validated genetically. These specimens should therefore be referred to as *Platynereis* cf. B sp. nov. until such time they can be confirmed genetically. *Platynereis* B sp. nov., *Platynereis* cf. B sp. nov. and *P. massiliensis s.l.* (South Africa) closely resembled *P. dumerilii* from France. All specimens were similar with respect to the number of paragnaths on the pharynx and the length and number of segments of *P. dumerilii* from France fell within the same range as *Platynereis* B sp. nov., *Platynereis* cf. B sp. nov. and *P. massiliensis s.l.* (South Africa). The only striking differences were the presence

of the notopodial falcigers and the longest tentacular cirri in *P. dumerilii* from France that extended back to the 3rd chaetiger which is almost three times more than *Platynereis* B sp. nov., *Platynereis* cf. B sp. nov. and *P. massiliensis s.l.* (South Africa). As a result, there are not enough differences to distinguish *P. dumerilii* from France and *Platynereis* B sp. nov., *Platynereis* cf. B sp. nov. and *P. massiliensis s.l.* (South Africa) even though they are genetically different species further indicating that they all are part of a true cryptic species complex.

The presence of *P. australis sensu* Day (1967) in South Africa could not be confirmed as freshly collected specimens that conformed to this description actually represent *P. massiliensis s.l.,* as per the phylogenetic results obtained in this study (Figures 3.2 and 3.4). Museum specimens of *P. australis* were examined, but they were from sub-Antarctic islands (Marion, Prince Edward and Falkland Islands). Although they resembled *Platynereis* B sp. nov., *P. massiliensis s.l.* (South Africa) and *Platynereis* cf. B sp. nov. superficially, they are actually strikingly different. The minimum length of *P. australis* was five times that of *Platynereis* B sp. nov. and *P. massiliensis s.l.* (South Africa) and twice that of *Platynereis* cf. B sp. nov. while the minimum number of segments was almost twice that of the other three species. The length of the longest tentacular cirri extended back to chaetiger 10 in *P. australis* (Day 1967), whereas it extended to chaetiger 13 in most specimens of the other three species. Additionally, *P. australis* (Day 1967) had two more rows of rod-like paragnaths in area III in comparison to *Platynereis* B sp. nov., *Platynereis* cf. B sp. nov. and *P. massiliensis s.l.* (South Africa), which all had 3 rows. *Platynereis australis* (Day 1967) also differed from the other species in terms of the consistent absence of the notopodial falciger which was always present in *Platynereis* cf. B sp. nov. but only present sometimes in *Platynereis* B sp. nov. and *P. massiliensis s.l.* (South Africa). The only similarities between *P. australis* (Day 1967) and the other three species are that they have similar rows of rod-like paragnaths in areas IV, VI and VII-VIII which could just indicate that this is a variable character within the genus. Due to the several differences observed, it is concluded that the sub-Antarctic *P. australis* (Day 1967) is indeed morphologically different to *Platynereis* B sp.nov., *Platynereis* cf. B sp. nov. and *P. massiliensis s.l.* (South Africa).

Table 3.6: Comparison of diagnostic characters of *Platynereis* species from South Africa, France, sub-Antarctic Islands and New Zealand. (+/-, indicates present and absent, + indicates present, - indicates absent).

	***Platynereis* B. sp. nov.**	***Platynereis* cf. B (as *P. dumerilii* in Day 1967)**	***"P. massiliensis"***	***P. australis* complex**	***P. australis***	***P. dumerilli***
Reference/Museum ID	This study	Collected by Day 1969 SAMC-A20134, SAMC-A20212, SAMC-A20707	This study	Collected by Day SAMC-A20705, SAMC-A20704, SAMC-A21355, SAMC-A21174	Read 2007	Fauvel 1923
Locality	South Africa	South Africa	South Africa	Marion, Prince Edward and Falkland Island	New Zealand	France
Length (mm)	5 – 65	15 – 35	5 – 60	25 – 70	200	30
Number of segments	34 – 84	34 – 72	27 – 75	50 – 78	160	64
Longest tentacular cirri (chaetiger number)	13	12	14	10	12	3
Number of paragnaths, Areas:						
III	3 rows	3 rows	3 rows	5 rows	5 rows	1-3 rows
IV	5 rows	6 rows	3-4 rows	6 rows	10 rows	5-6 rows
VI	2-3 rows, arc	3 rows, arc	1-2 rows, arc	3 rows, arc	4 rows	2-3 rows
VII-VIII	2-3 rows, 5-6 groups	3 rows, 5 groups	3 rows, 4 groups	2 rows, 6 groups	4 rows, 5 groups	2 rows, 5 groups
Notopodial falciger	+/-	+	+/-	-	-	+

The sub-Antarctic *P. australis* (Day 1967) were morphologically similar to *P. australis* (Read 2007) from the type locality in New Zealand (Table 3.6). The most striking differences were that the New Zealand specimens were almost three times the size of the sub-Antarctic specimens and had almost twice as many segments. Additionally, the New Zealand specimens (Read 2007) had 4 more rows of rod-like paragnaths in area IV and two more rows and an additional group in area VII-VIII than the sub-Antarctic specimens (Table 3.6). Nonetheless, the two species did share some similarities in having similar numbers of paragnaths in area III and the consistent absence of the notopodial falciger. The smaller size of sub-Antarctic specimens and the striking differences in paragnaths on the pharynx suggest that these specimens are a different species to *P. australis* from New Zealand. Thus, until the identity of the subantarctic specimens can be confirmed genetically, they should at best be referred to as *P* cf. *australis*.

Species complexes

From the preceding discussion and also considering conclusions drawn by Wäge et al. (2017) it is evident that individuals in Clades A – C (Figures 3.2 – 3.5) represent a monophyletic clade comprising genetically distinct species that are morphologically indistinguishable from each other. This supports the long-standing hypothesis of a *Platynereis dumerilii* species-complex (Pfannenstiel and Grunig 1984). Species complexes are hypothesised to occur as a result of non-adaptive radiation resulting in genetically diverse lineages that do not experience adaptive phenotypic changes (Gittenberger 1991; Rundell and Price 2009) and are very common among polychaetes (eg: Manchenko and Radashevsky 1998; Westheide and Hass-Cordes 2001; Halt et al. 2009). The lack of morphological changes could be a result of strong stabilising selection that has favoured a common shared morphotype especially when an adaptive peak is reached while the rate of molecular evolution remains constant (Palumbi and Benzie 1991; Struck et al. 2018). Since *P. dumerilii* is considered a morphologically slowly evolving species with a slower rate of molecular evolution in comparison to other species (Zantke et al. 2014), it is plausible that *P. dumerilii* has retained its current morphology because of its adaptive advantage, but molecularly continues to diversify into several distinct lineages as a result of reproductive isolation resulting in a species complex. However, such a hypothesis will benefit from including additional sequences of members

from the species-complex to better understand the underlying evolutionary mechanisms of species-complexes.

Chapter Four:

Genome-wide scans of single nucleotide polymorphisms reveal high connectivity for the marine annelid, *Pseudonereis podocirra* (Schmarda, 1861) across the heterogeneous South African coast.

Introduction

Understanding the distribution of intraspecific genetic variability is essential for elucidating the processes that have influenced a species' genetic structure, diversity, distribution and demographic history (Palumbi 1994; Grosberg et al. 1998; Hellberg et al. 2002; Marko and Hart 2011). Furthermore, investigating these processes helps us understand the relative contributions that each (i.e. genetic drift, gene flow, natural selection and mutations) makes to shaping the evolution of the species in question (Nielsen 2005; Pool et al. 2010), something which is currently poorly understood in the marine environment (Reitzel et al. 2013). Not only is such information important to understanding poorly understood marine animals (Hart and Marko 2010; Marko and Hart 2011), but it is essential for conserving and protecting them (Reitzel et al. 2013) and the processes that maintain their biological diversity and distribution (Teske et al. 2011a; Lexer et al. 2013).

The four inshore ecoregions (i.e. the Southern Benguela, Agulhas, Natal and Delagoa) of the South African coast are controlled by strong temperature and productivity gradients (Table 1.2) (Griffiths et al. 2010; Teske et al. 2011a) and present a unique opportunity to investigate and identify the potential drivers of marine species diversity, distribution and genetic structure. Many marine invertebrate species tend to display regionally structured lineages that correspond to one or more of these ecoregions, indicating that the boundaries of these ecoregions may represent strong phylogeographic barriers to gene flow (Teske et al. 2011a). Nonetheless, the boundaries as potential barriers are not absolute as several species are known to have a continuous distribution across them (Teske et al. 2011a). The structuring patterns of many marine species are largely influenced by the interplay between larval development mode and oceanographic currents, in addition to phylogeographic barriers (Teske et al. 2011a). For example, species that have direct developing larvae tend to show more regionally structured lineages that are strongly influenced by more than one phylogeographic break as has been observed for *Exosphaeroma hylecotes* Barnard, 1940 and *Iphinoe truncate* Hale, 1953 (Teske et al. 2007a). In contrast, species that have a pelagic larval stage tend to display less regional structure, such as that observed for *Boccardia polybranchia* (Haswell, 1885) (Williams et al. 2016), *Upogebia africana* (Ortmann, 1894) and *Perna perna* (Linnaeus, 1758) (Teske et al. 2007a; Zardi et al. 2007). In some cases, for example the clinid fish, *Clinus cottoides*

Valenciennes, 1836, regionally structured populations were found to be influenced by the local inshore current and eddie systems (von der Heyden et al. 2008). In an instance where high connectivity between populations regardless of larval development mode have been demonstrated for a species, such a pattern was attributed to pre-and post-glacial expansions leaving the impression of high gene flow such as that observed for *Tetraclita serrata* Darwin, 1954 (Reynolds et al. 2014) and *Bullia rhodostoma* Reeve, 1847 (Muteveri et al. 2015). On the other hand *Polydora hoplura* Claparède, 1868 and *Boccardia proboscidea* Hartman, 1940, high connectivity reflected the unnatural movement of larvae and adults with their host aquaculture species (Simon et al. 2009; Williams et al. 2016). Finally, major current systems have been demonstrated to play a role in the dispersal of larvae of endemic species, as is the case for influence of the east coast's Agulhas current on the panmictic *Caffrogobius caffer* (Gűnther, 1874) (Neethling et al. 2008). Even though one or more of these species share similar habitats, i.e. the rocky shores, sandy beaches or estuaries, each shows different patterns of structure and connectivity that are influenced by different factors. Similarly, different phylogeographic patterns were observed for eleven sympatric limpet species that share similar life history traits (Mmonwa et al. 2015). Since a general hypothesis about the historical and contemporary structuring patterns cannot be applied to all species occurring along the coast, it is thus important to determine the species-specific evolutionary factors that drive biological diversity, structure and the distribution of a species along the South African coast.

Pseudonereis podocirra (Schmarda, 1861) represents an ideal subject for further investigation as it is known to occur from Lamberts Bay on the west coast to Kidds Beach on the south-east coast, spanning at least two of South Africa's ecoregions (Chapter 2). Furthermore, *P. podocirra* is known to live in sympatry along the coast with *Platynereis massiliensis s.l.* (Moquin-Tandon, 1869) and *Platynereis* B sp. nov. Kara, Macdonald, Simon, 2018 and is presumed to have a larval strategy similar to that of the latter species, which is a long pelagic larval dispersal phase (Chapters 2 and 3). *Platynereis* B sp. nov. displayed two well-mixed lineages that were separated by the Cape Agulhas phylogeographic barrier whereas *P. massiliensis s.l.* displayed three well-structured lineages that were separated by the Cape Agulhas and Cape Point phylogeographic barriers (Chapter 3). Given the presumed similarity in larval development mode to *Platynereis* B sp. nov. and their overlap in

distribution, contrary to expectations, *P. podocirra* showed a well-connected meta-population that spans these phylogeographic breaks (Chapter 2). Although single mitochondrial markers have proven useful in elucidating the genetic structure and connectivity patterns of species (e.g. Teske et al. 2006; Zardi et al. 2007; Neethling et al. 2008; von der Heyden et al. 2010), the patterns produced are biased toward historical events that are maternally inherited thus representing only one quarter of the effective population size (Palumbi and Baker 1994; Burg et al. 1999; DeBiasse et al. 2014). It is therefore important to use additional markers that are able to display patterns inherited by both parental lineages that will elucidate more recently evolved genetic patterns (Teske et al. 2011a). For example, when using a nuclear intron Teske et al. (2014) found regionally structured populations in the crab, *Hymenosoma orbiculare* Desmarest, 1823 which contrasted with the single non-structured lineage observed for the same species using only a mitochondrial marker. Thus, *P. podocirra* needs to be re-examined, preferentially using a multi-locus dataset to determine whether the lack of genetic structure could be an artefact of using a single mitochondrial DNA marker. Multi-locus nuclear datasets such as single nucleotide polymorphisms (SNPs) serve as invaluable markers as they represent point mutations that result in single base pair differences, are found in abundance throughout the genome, are biallelic and slow evolving, thus providing a suitable method of assessing genomic patterns within a species (Brumfield et al. 2003; Kumar et al. 2012; Mesak et al. 2014). Furthermore, SNPs are known to detect fine-scale differentiation of very recently evolved genetic patterns, not necessarily detected by other multi-locus markers (Vendrami et al. 2017). This has been demonstrated by Vendrami et al. (2017) where SNP markers proved more useful at detecting recently evolved fine-scale structure of the king scallop *Pecten maximus* (Linnaeus, 1758) as opposed to microsatellite markers which revealed no structure.

Considering that *P. podocirra* has a distribution spanning three of South Africa's ecoregions and is presumed to have a long pelagic larval stage, three hypotheses with different structuring patterns are proposed (Table 4.1): (i) *P. podocirra* has two well-mixed regionally structured lineages, west and south, that are strongly influenced by the Cape Agulhas phylogeographic barrier, (ii) *P. podocirra* has four regionally structured lineages, west, south-west, south and south-east, that are influenced by the Cape Point, Cape Agulhas and Algoa Bay phylogeographic breaks and the respective

temperature regimes of the biogeographic provinces and lastly (iii) *P. podocirra* has two regionally structured lineages, west and south, that are strongly influenced by the Cape Point barrier.

Table 4.1: Three scenarios used to test population structure of eleven populations of *Pseudonereis podocirra.*

	West coast	**South-west coast**	**South coast**	**South-east coast**
Scenario 1	LB, SHB, YZ, ST, RE	-	MB, PB, SFB, AB, CR, KB	-
Scenario 2	SHB, LB, YZ	ST, RE	MB, PB, SFB	AB, CR, KB
Scenario 3	SHB, LB, YZ	-	ST, RE, MB, PB, SFB, AB, CR, KB	-
LB- Lamberts Bay, SHB- St. Helena Bay, YZ- Yzerfontein, ST- Strand, RE- Rooi Els, MB-Mossel Bay, PB-Plettenberg Bay, SFB- St. Francis Bay, AB- Algoa Bay, CR- Cannon Rocks, KB- Kidds Beach				

These three hypotheses will be tested using a polymorphic SNP dataset to determine whether there is any recently evolved fine-scale differentiation patterns which could not be detected by the mitochondrial marker. The polymorphic SNPs will be isolated using a high-throughput sequencing approach coupled with ezRAD which provides an invaluable, cost-effective method that provides a reduced representation of the entire genome to identify patterns of fine-scale genomic variation (Davey and Blaxter 2010; Hohenlohe et al. 2010; Davey et al. 2011; Reitzel et al. 2013; Toonen et al. 2013). Thus, providing high coverage of sequencing even for non-model organisms that do not have a fully sequenced or annotated genome (Davey and Blaxter 2010; Davey et al. 2011; Toonen et al. 2013), such as *P. podocirra.*

Materials and Methods

Sample Collection

A total of 253 specimens were collected from eleven sites along the South African coast from November 2015 to March 2017 (Figure 4.1, Table 4.2). Specimens were collected from the rocky intertidal zones at low tide at each site. Specimens were found among *Perna perna* (Linnaeus, 1758) and *Mytillus galloprovincialis* Lamarck, 1819 beds and in sand tubes of *Gunnarea gaimardi* (Quatrefages, 1848) and stored in bags of seawater for processing in the laboratory. Specimens

were anesthetised with 7% $MgCl_2$ diluted in distilled water, photographed and thereafter preserved in 100% ethanol and stored at room temperature for molecular analysis.

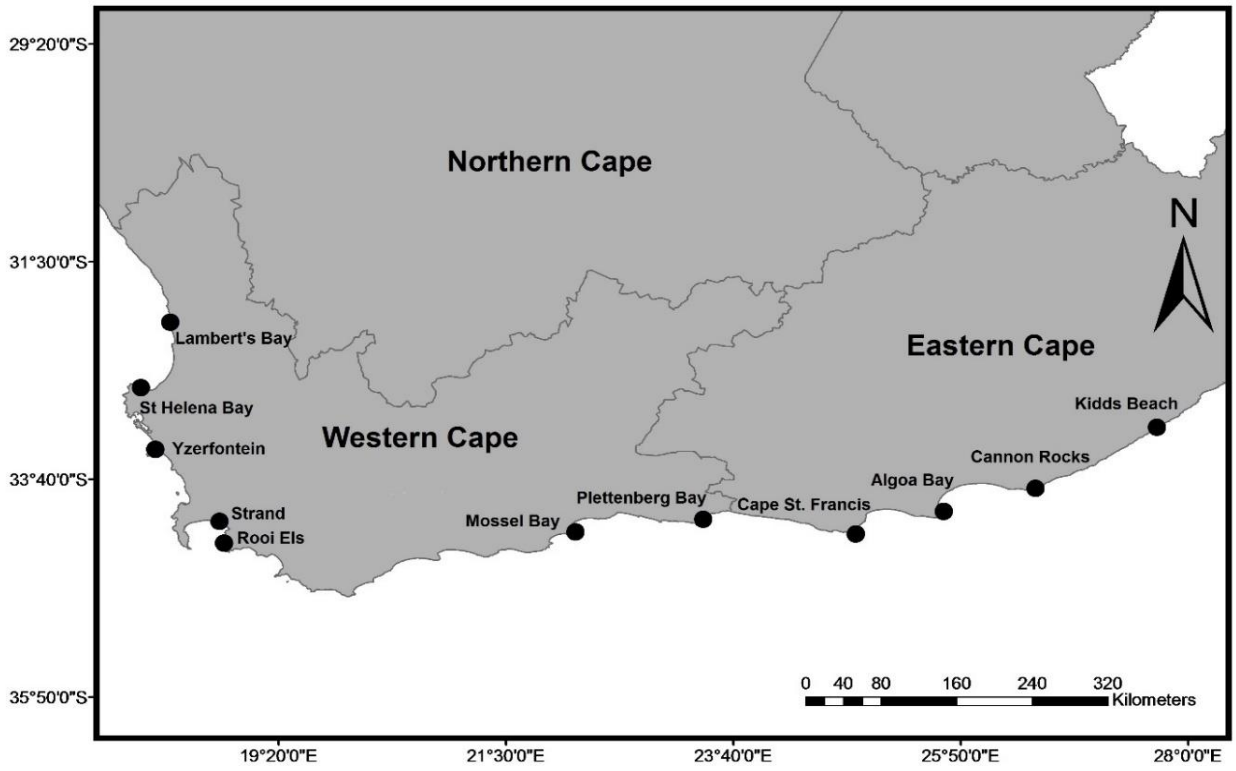

Figure 4.1: Eleven sampling sites of *Pseudonereis podocirra* along the South African coast.

DNA extraction, ezRAD library preparation and sequencing

Genomic DNA was extracted from 25 mg of tissue from each sample following the instructions of the ZR Genomic Tissue MiniPrep Kit with one minor modification: the DNA was eluted in molecular grade water instead of the elution buffer provided with the kit. The quality of each DNA sample was determined by Agarose gel electrophoresis (1%) and the quantity determined by Qubit Assays (Qubit Quant iT dsDNA HS Assay system available at the Central Analytical Facility at Stellenbosch University) to ensure that all samples used for downstream library preparation were of high quality and quantity. Thereafter, equal quantities of DNA from each individual within a population were pooled (see Table 4.2 for number of individuals pooled per population) into one library to a total of 300 ng/µl of DNA, resulting in 11 pooled samples. Each pooled sample was then frozen in liquid nitrogen and sent to the Hawaii Institute of Marine Biology (HIMB) core facility for library preparation and MiSeq Illumina sequencing. DNA was digested following the protocol by Toonen et al. (2013)

using the high frequency cutter isoschizomer enzyme DpnII (New England Biolabs®). An SPRI bead size selection step was performed and thereafter validated using the Agilent 2100 BioAnalyzer, quantified by qPCR and paired-end sequenced using the Illumina MiSeq platform.

Table 4.2: Sampling localities of *Pseudonereis podocirra* from eleven sites along the South African coast with date of collection, geographic co-ordinates of sites and number of individuals sampled per population.

Sample Site	Date collected	Co-ordinates	Number of individuals
Mossel Bay	27 – 10 – 2015	34º18'63.87"S 22º15'92.86"E	32
Rooi Els	03 – 05 – 2016	34º29'78.46"S 18º81'47.40"E	10
Lamberts Bay	14 – 10 – 2016	32º10'19.82"S 18º30'30.05"E	34
Strand	17 – 10 – 2016	34º11'88.18"S 18º82'49.51"E	18
Yzerfontein	19 – 10 – 2016	33º36'49.48"S 18º15'97.64"E	33
St. Helena Bay	15 – 11 – 2016	32º75'34.99"S 18º02'16.60"E	29
Cape St. Francis Bay	27 – 02 – 2017	34º20'65.48"S 24º83'44.86"E	9
Plettenberg Bay	28 – 02 – 2017	34º06'18.02"S 23º37'97.76"E	7
Algoa Bay	28 – 03 – 2017	33º98'25.13"S 25º66'91.64"E	20
Cannon Rocks	29 – 03 – 2017	33º75'15.08"S 26º54'58.36"E	33
Kidds Beach	30 – 03 – 2017	33º14'71.54"S 27º70'32.59"E	28

Bioinformatics workflow

A standard quality control filter was run by the HIMB core facility and the raw Illumina reads were parsed into FASTQ files. The raw reads were analysed using FastQC (Andrews 2010) and thereafter quality control steps such as trimming the adapter sequences, removing overrepresented sequences and reads with a Phred quality score less than 25, were performed using TrimGalore! on a Galaxy instance (Afgan et al. 2018). Since no reference genome was available for *P. podocirra,* high quality reads were assembled *de novo* using SPAdes (Bankevich et al. 2012) with a kmer=71 to produce a

final assembly of reference contigs. Each population's high quality reads were then mapped back to the reference contigs using the BWA MEM algorithm (Li 2013) with the number of threads set to 16 and a mapping score of 20. Mapping success (number of mapped versus unmapped reads) was determined using the stats module in SAMtools (Li et al. 2009) with mapping quality set to exclude values below 20. SAM files were converted to BAM files using SAMtools with a mapping quality above 20. BAM files were then sorted according to the alignment position using SAMtools. The sorted BAM files were then used to call variants using the mpileup module in SAMtools on the Galaxy instance with a minimum quality score of 20 and a maximum read depth of 1000 reads per locus. The above step was done twice, once to create a combined mpileup file that included all populations for export to Genepop and a second to create pileup files for each population for further population genetic analyses with PoPoolation (Kofler et al. 2011a) and PoPoolation2 (Kofler et al. 2011b).

Genomic diversity and structure

The individual pileup files were subsampled to ensure uniform coverage across all populations using the subsample-pileup.pl module in PoPoolation to account for sequencing bias with the minimum coverage set to 10, maximum coverage set to 500, the minimum allele count was set to 2 and the quality score set to 10. Thereafter, the number of SNPs, Tajima's π, Watterson's Θ_W and Tajima's D were calculated using the variance-sliding.pl module in PoPoolation in order to characterise the genetic diversity and to detect any demographic events in each population, using a sliding window approach with a minimum count of 2, minimum coverage of 10, maximum coverage of 500, minimum quality of 10, window size of 100 and step size of 100. The combined mpileup file that included all populations was converted to a sync file using the mpileup2sync.pl module in PoPoolation2 with a minimum quality of 20. The sync file was then subsampled to uniform coverage and converted to a Genepop file using the subsample_sync2genepop.pl module in PoPoolation2 with a minimum coverage of 2, target coverage of 10 and maximum coverage of 500. A Perl script was used for further editing of the Genepop files by merging all the contigs and to identify locus positions. Pairwise F_{ST} comparisons with its associated 95% confidence intervals were calculated using the diveRsity package in the R environment using the edited Genepop file as input. Formatomatic (Manoukis 2007) was used to convert the Genepop file to an Arlequin (Excoffier and Lischer 2010) file which was

subsequently used to calculate the gene diversity in each population, to calculate AMOVAs to detect any population structure and to conduct a Mantel's Test to determine whether there is isolation by distance. The AMOVA and IBD analyses were run for 2000 simulations and populations were structured according to three scenarios (Table 4.1).

Results

Genome sequencing and assembly

Sequencing of the ezRAD libraries generated a total of ~48.6 million reads of 300bp in length. After trimming reads to ~250bp and quality filtering, on average 43.2 million reads were retained (Table 4.3). Since no reference genome was available, the *de novo* assembly resulted in 705,240 reference contigs that ranged from 35bp – 16,480bp in length. These contigs were then combined to create a reference contig for further downstream analyses. A total of ~33 million reads were mapped onto the *de novo* assembled reference contig, with the number of mapped reads ranging from 2.3 – 3.5 million reads for each population (Table 4.3).

Table 4.3: The number of sequenced reads, reads after quality control, mapped reads and insert size across eleven populations along the South African coast.

Samples Site	Number of reads sequenced	Number of reads after QC and trimming	Number of mapped reads after quality filtering	Average insert size (bp)*
Lamberts Bay	5 024 852	4 929 910	3 532 033	193
St. Helena Bay	4 798 018	4 705 088	3 324 891	200
Yzerfontein	4 338 640	4 285 994	3 058 395	199
Rooi Els	4 070 514	3 676 638	2 354 877	188
Strand	4 088 560	3 994 594	2 786 445	190
Mossel Bay	3 965 152	3 851 032	2 733 310	233
Plettenberg Bay	4 471 252	4 357 214	3 165 545	237
St. Francis Bay	4 531 800	4 498 174	3 268 347	222
Algoa Bay	4 509 980	4 461 784	3 103 889	227
Cannon Rocks	4 815 956	4 756 002	3 468 147	242
Kidds Beach	4 068 366	4 032 240	2 905 354	251
**Average Insert size: sequenced length of DNA between a paired read (read 1 and read 2)*				

Genomic diversity and population structure

A total of 235,053 SNP's were identified across all eleven populations of *Pseudonereis podocirra* with the highest number of SNPs found in St. Helena Bay and the lowest in Mossel Bay (Table 4.4). Nucleotide diversity for all eleven populations ranged between 0.015 – 0.021 with the lowest recorded for a south-west population Rooi Els (π=0.015). Similarly, the population mutation rate ranged between 0.015 – 0.022, with the highest recorded for Yzerfontein (Θ_W=0.022) (Table 4.4). Nucleotide diversities and population mutation rate (Θ_W) were low for all populations and similar to one another (Table 4.4). The average genomic diversity for all populations was (0.094± 0.05), with the most diverse population being from the West-coast, St. Helena Bay (0.124±0.05) and the least diverse being one west-coast population, Lamberts Bay and two south-east coast populations, Cannon Rocks and Kidds Beach (0.011±0.05) (Table 4.4). The number of private SNPs varied between populations, with Lamberts Bay, Strand, Algoa Bay and Kidds Beach having zero private SNPs and St. Helena Bay having the largest number of private SNPs (1819) (Table 4.4).

The mean pairwise F_{ST} values across all eleven populations were low and none significantly different frome each other (i.e., 95% CI did not contain the value of the null hypothesis=0), with the exception of four comparisons (Table 4.5). Significant fine scale differentiation was found between Mossel Bay and Yzerfontein (0.044), Mossel Bay and St. Francis Bay (0.047), Cannon Rocks and Lamberts Bay (0.051) and lastly Lamberts Bay and St. Francis Bay (0.037) (Table 4.5). The lowest pairwise genetic distance was recorded for two South-coast populations, Plettenberg Bay and Algoa Bay (0.026) and the highest was recorded between a West-coast and South-coast population, St. Helena Bay and Mossel Bay (0.058), respectively (Table 4.5).

Table 4.4: The number of SNPs, Number of private SNPs per population, Tajima's π, Watterson's Θ_W, Gene diversity and Tajima's D for eleven populations of *Pseudonereis podocirra*. Gene diversity with standard error estimates in parenthesis.

Sample Site	Number of SNPs	Tajima's π	Watterson's Θ_W	Genomic diversity	Tajima's D	Number of private SNPs/pop
Lamberts Bay	21,401	0.018	0.020	0.011(±0.05)	-0.113	0

St. Helena Bay	23,637	0.018	0.019	0.124(±0.06)	-0.125	1819
Yzerfontein	22,558	0.018	0.022	0.118(±0.05)	-0.118	1060
Rooi Els	21,062	0.015	0.018	0.111(±0.05)	-0.098	731
Strand	21,271	0.016	0.017	0.114(±0.05)	-0.128	0
Mossel Bay	20,011	0.017	0.019	0.108(±0.05)	-0.103	745
Plettenberg Bay	20,739	0.019	0.015	0.110(±0.05)	-0.091	695
St. Francis Bay	20,808	0.019	0.019	0.108(±0.05)	-0.139	823
Algoa Bay	21,271	0.020	0.018	0.112(±0.05)	-0.140	0
Cannon Rocks	21,051	0.019	0.016	0.011(±0.05)	-0.114	794
Kidds Beach	21,244	0.021	0.018	0.011(±0.05)	-0.136	0

The AMOVAs computed for all three hypotheses yielded similar results where significant values were obtained for all three fixation indices (p<0.05) with the exception of scenario 2 where the “among groups” index was non-significant (p>0.05) (Table 4.6). In all scenarios 91% of the variation can be explained by differences within each population instead rather than the structure designated by each hypothesis (Table 4.6). Furthermore, the isolation by distance results for each hypothesis indicates that there is no significant correlation between the genetic and geographic distance between populations (r = 0.00, p=1.00) (Table 4.6). All eleven populations displayed similar negative values for Tajima’s D (Table 4.4). One South-coast population, Plettenberg Bay and one South-west population, Rooi Els, displayed the lowest Tajima’s D values (-0.091 and 0.098, respectively), whereas the remaining populations had values ranging between -0.113 to -0.140 (Table 4.4).

Table 4.5: Population pairwise F_{ST} estimated for eleven populations of *P. podocirra* in South Africa. F_{ST} values below diagonal with 95% lower and upper confidence intervals above diagonal. Significant values in bold. Abbreviations for population names are found in Table 4.2.

	LB	**SHB**	**YZ**	**RE**	**ST**	**MB**	**PB**	**SFB**	**AB**	**CR**	**KB**
LB	-	-0.011; 0.124	-0.013;0.018	-0.002;0.097	-0.022;0.066	-0.021;0.205	-0.016;0.082	**0.003;0.079**	-0.008;0.087	**0.005;0.063**	-0.003;0.078
SHB	0.046	-	-0.035;0.109	-0.008;0.116	-0.035;0.104	-0.004;0.131	-0.010;0.013	-0.011;0.103	-0.029;0.098	-0.022;0.112	-0.002;0.120
YZ	0.032	0.033	-	-0.041;0.120	-0.001;0.106	**0.008;0.128**	-0.041;0.120	-0.005;0.107	-0.021;0.108	-0.026;0.103	-0.017;0.160
RE	0.034	0.045	0.035	-	-0.010;0.083	-0.004;0.090	-0.034;0.088	-0.044;0.140	-0.012;0.066	-0.009;0.092	-0.121;0.113
ST	0.028	0.036	0.027	0.029	-	-0.025;0.132	-0.021;0.067	-0.013;0.147	-0.014;0.079	-0.005;0.074	-0.016;0.070
MB	0.041	0.058	**0.044**	0.036	0.040	-	-0.032;0.136	**0.011;0.126**	-0.001;0.108	-0.026;0.053	-0.019;0.167
PB	0.032	0.046	0.032	0.028	0.027	0.038	-	-0.025,0.122	-0.031;0.090	-0.039;0.138	-0.041;0.171
SFB	**0.037**	0.052	0.039	0.038	0.035	**0.047**	0.036	-	-0.024;0.136	-0.016;0.120	-0.014;0.122
AB	0.032	0.046	0.033	0.030	0.030	0.039	0.026	0.034	-	-0.044;0.113	-0.037;0.165
CR	**0.035**	0.048	0.038	0.034	0.031	0.042	0.034	0.034	0.030	-	-0.018;0.090
KB	0.030	0.044	0.032	0.031	0.027	0.039	0.028	0.028	0.026	0.029	-

Table 4.6: AMOVA and Isolation by distance results for Scenarios 1-3 for eleven populations of *Pseudonereis podocirra*. Bold values indicate significant values.

	SOV	df	SS	VC	% variation	FI	p-values	IBD
Scenario 1	**Among pops**	1	10506.74	9.603	0.27	0.002	**0.007**	
	Among pops within groups	9	85131.84	308.45	8.55	0.085	**0.019**	
	Within pops	209	687589.00	3289.89	91.18	0.088	**0.011**	
	Total	219	783227.58	3607.96				
	***r* (p-value)**							0.00 (1.00)
Scenario 2	**Among pops**	3	29484.04	6.91	0.19	0.001	0.059	
	Among pops within groups	7	66154.53	308.03	8.55	0.085	**0.037**	
	Within pops	209	687589.00	3289.89	91.26	0.087	**0.010**	
	Total	219	783227.58	3604.85				
	***r* (p-value)**							0.00 (1.00)
Scenario 3	**Among pops**	1	10958.61	17.75	0.49	0.004	**0.011**	
	Among pops within groups	9	84679.96	305.94	8.47	0.085	**0.019**	
	Within pops	209	687589.58	3289.89	91.04	0.089	**0.010**	
	total	219	783227.58	3613.60				
	***r* (p-value)**							0.00 (1.00)

SOV- Source of variation, df- degrees of freedom, SS- sum of squares, VC- variance component, FI-fixation indices, IBD- isolation by distance

Discussion

With the use of a high-throughput pooled RAD-seq method this study represents the first high-density SNP-based genome scan of genomic diversity and differentiation in a South African marine annelid, namely *Pseudonereis podocirra.* Analyses of population structure and isolation by distance indicate that *Pseudonereis podocirra* sampled from eleven sites along the South African coast show a well-mixed panmictic population which is contrary to all three structuring patterns hypothesised for this species.

Patterns of connectivity and weak differentiation

The high levels of connectivity obtained in the present study are concordant with those obtained in Chapter 2 for *P. podocirra,* wherein mitochondrial network analyses recovered no geographic structure between the same eleven populations. The observed patterns of concordance obtained for both markers are surprising since SNP markers are known to detect even the most recently diverged lineages (Brumfield et al. 2003; Morin et al. 2004; Vendrami et al. 2017). Thus, it was expected that the strong temperature regime coupled with oceanic frontal systems and upwelling cells would have acted as barriers to gene flow resulting in regionally structured lineages of *P. podocirra* that was not recovered with mtDNA. The AMOVA and IBD analyses demonstrate no well-defined geographic structure supporting any of the three hypotheses proposed and attributed a large portion of the variation to within population variation, thus affirming that neither variable temperatures nor isolation by distance are limiting factors in the dispersal of *P. podocirra* larvae. The number of private SNPs obtained for all populations do not show any geographical partitioning and the lack of private SNPs obtained for geographically distant populations in the west, south-west coast (Lamberts Bay and Strand) and the south-east coast (Algoa Bay and Kidds Beach), coupled with the low F_{ST} values for all populations suggest that there is extensive contemporary gene flow among populations. However, F_{ST} values did to some extent increase with an increase in distance between populations, for example the differentiation between St. Helena Bay on the west coast and Mossel Bay on the south coast was higher (F_{ST}=0.058, distance=~537 km) compared to St. Helena Bay and Yzerfontein on the west coast (F_{ST}=0.033, distance=~86.5 km), thus indicating that fewer individuals are dispersed over larger distances as opposed to sites located close to one another. The pattern of high

connectivity observed for *P. podocirra* is suggestive of planktonic larvae with a long pelagic stage thus enabling dispersal over long distances. Such larvae are commonly produced by *Pseudonereis* Kinberg, 1865 species including the closely related *Pseudonereis anomala* Gravier, 1899 (Çinar and Altun 2007; Hamdy et al. 2014). This further supports the conclusion that *P. podocirra* probably also reproduces in this manner and with the aid of the oceanic currents, larvae are transported over vast distances producing a panmictic meta-population. Furthermore, it is interesting that dispersal is taking place across the Cape Agulhas, Cape Point and Algoa Bay phylogeographic barriers, which have in previous studies been identified as strong barriers to gene flow for several other marine species (e.g. Evans et al. 2004; Teske et al. 2007a, b; von der Heyden et al. 2008), including the polychaetes *Boccardia polybranchia* (Williams et al. 2016)*, Arenicola loveni* Kinberg, 1866 (Naidoo 2017), *Platynereis massiliensis s.l.* and *Platynereis* B sp. nov. (Chapter 3). For many of these species, it was hypothesised that dispersal across these phylogeographic barriers are most likely limited by the inability of larvae to survive the sudden changes in temperatures (Teske et al. 2011a; Muller et al. 2012), which are much lower on the west coast on the south and east coasts (Griffiths et al. 2010; Smit et al. 2013). Additionally, the formation of warm and cold core eddies around Cape Point (Lutjeharms and van Ballegooyen 1988) and upwelling of cold water cells along the Cape coast have been identified as strong barriers to dispersal for many other species (von der Heyden et al. 2008; Teske et al. 2011a). Thus, on the basis of the observed connectivity patterns, it may be postulated that larvae of *P. podocirra* are able to withstand the varying temperatures along the coast due to the facilitation of transport by oceanic currents. These patterns of connectivity are concordant with those obtained for *Caffrogobius caffer, Palaemon peringueyi* (Stebbing, 1915) and *Palaemon capensis* (de Man in Weber, 1897) where high gene flow between populations was maintained by the dispersal capability of the larvae assisted by oceanic currents (Neethling et al. 2008; Wood et al. 2017).

Nonetheless, weak but significant differentiation was found between Lamberts Bay and Cannon Rocks, Lamberts Bay and Cape St. Francis Bay, Yzerfontein and Mossel Bay and Mossel Bay and Cape St. Francis Bay. As genetic differentiation is dictated by the amount of gene flow between local populations (Slatkin 1985), this would lead to the assumption that these populations in particular

have recently experienced reduced or interrupted gene flow. Such differentiation patterns could be a result of the different selective forces acting on individuals at each locality (Nielsen et al. 2018), which are indeed represented by the low and negative Tajima's D values. Lamberts Bay, Yzerfontein, Cape St. Francis Bay and Mossel Bay represent semi-enclosed bays and thus could be playing a role in the local retention of larvae (Nicastro et al. 2008; Von Der Meden et al. 2008). This has been documented for the native mussel, *Perna perna* where populations located in bays showed a higher genetic divergence than populations along the open coast indicating that a large fraction of larvae are retained due to the reduced wave action and semi-enclosed characteristic of the bay (Nicastro et al. 2008).

The number of private SNPs are those that are unique to a single population among a broader range of populations and thus are primarily influenced by gene flow and genetic drift (Slatkin 1985; Slatkin and Takahata 1985). Thus, populations that exhibit a larger number of private SNPs are assumed to be demographically isolated to some extent (Slatkin 1985). Seven populations, St. Helena Bay, Yzerfontein, Rooi Els, Mossel Bay, Plettenberg Bay, Cape St. Francis Bay and Cannon Rocks all had high numbers of private SNPs and are thus considered to be unique. Such differentiation could be attributed to different selective forces acting at these sites which are located in different ocean basins and thus affected by different environmental conditions (Nielsen 2005). Such a scenario is plausible as the differentiated populations could be linked to the distribution in habitat of *P. podocirra* which are predominantly mussel beds attached to the rocky shores. The South African coast is characterised by a patchy distribution of rocky shores interspersed by sandy beaches (Bally 1987; Griffiths et al. 2010). The sandy beaches found scattered between these sites could result in reduced gene flow creating such isolated populations. Nonetheless, such a scenario does not explain the genomic similarity between, for example, the northwestern-most population, Lamberts Bay and the southeastern-most population, Kidds Beach. Alternatively, these genetically unique sites could represent post-glacial secondary contact zones where taxa from previously isolated populations expanded their ranges and admixed resulting in unique allele frequencies (Avise et al. 1987; Taberlet et al. 1998). The largest numbers of private SNPs obtained for the two west coast populations, St. Helena Bay and Yzerfontein are consistent with results obtained in Nielsen et al. (2018) where two

rocky shore species, *Scutellastra granularis* (Linnaeus, 1758) and *Parechinus angulosus* (Leske, 1778) displayed the largest numbers of private SNPs in the two northern-most west coast populations and it was suggested that such a pattern could most likely be the result of a post-glacial southward expansion of these west coast populations (Nielsen et al. 2018) thus, leading to the assumption that historically *P. podocirra* could have expanded their distribution range in a southward direction.

Such a scenario of post-glacial expansion is plausible as Tajima's D for all populations were low and negative, indicating that populations have accumulated an abundance of low frequency polymorphisms which is either the result of a selective sweep where natural selection removed all variation within populations (Nielsen 2005) or that populations experienced sudden expansions (Stajich and Hahn 2005; Biswas and Akey 2006; Korneliussen et al. 2013). Additionally, the low nucleotide and genomic diversity estimates that did not vary between all populations is usually indicative of populations that have been through a bottleneck or of populations that have recently colonized a new habitat after glacial periods where the signal of a founding event still persists (Lowe et al. 2009; Hart and Marko 2010; Lowe and Allendorf 2010; Garcia-Cisneros et al. 2016). Patterns of post-LGM expansion have been documented for several marine invertebrate species along the South African coast (e.g. von der Heyden et al. 2010; Muller et al. 2012; Reynolds et al. 2014; Toms et al. 2014; Mmonwa et al. 2015; Wood et al. 2017), thus making it possible that *P. podocirra* could have experienced similar conditions historically. However, these are hypotheses that will benefit from further analysis using an increased sample size to reduce the possibility of ascertainment bias of the current SNP dataset to help us clearly separate demographic events from natural selection. Alternatively, such high connectivity could be due to the artificial movement of bait worm species between localities as it is common practice for recreational fisherman to discard unused bait back into the water (Arias et al. 2013). In South Africa, *P. podocirra* is commonly used as a bait species (van Herwerden 1989) and it is found that many fisherman do not necessarily fish in the same place they had collected the bait from (pers. comms. SANParks), thus making it possible that the lack of genetic structure could be a result of such baiting and fishing practices.

Limitations of the study

The most important limitation of SNP datasets is the influence of ascertainment bias introduced into an analysis as a result of the method in which the SNPs are identified or "called" (Brumfield et al. 2003; Morin et al. 2004). The RAD-seq approach used in this study has been demonstrated to have unbiased population statistic parameters as a larger number of genomic regions are sequenced compared to other methods (Reitzel et al. 2013). Additionally, the use of stringent criteria implemented in this study, such as using a meaningful "cut-off" value during the SNP filtering process, could have further contributed to the reduction in possible biases associated with SNP identification (Brumfield et al. 2003). A potential source of bias could have resulted from the numbers of individuals (7-33 individuals per population, refer to Table 4.1) used in the populations under study which are lower than the recommended 40-100 individuals per pool (Brumfield et al. 2003; Morin et al. 2004; Gautier et al. 2013; Schlötterer et al. 2014). Nonetheless, Emerson et al. (2010) successfully elucidated the phylogeographic structure of a pitcher plant mosquito, *Wyeomyia smithii* Coquillett, 1901, using pools of six individuals per population and Mimee et al. (2015) pooled 15 cysts per population in their investigation into the population genetics of cyst nematodes, which is approximately half that of the individuals used than our largest pooled sample (33, Table 4.1). The patterns of genomic diversity in this study are thus deemed to be an accurate reflection of contemporary distribution, albeit that historical population dynamics have undoubtedly also played a role.

Chapter Five:

Understanding evolutionary processes and historical phylogeography of three (pseudo)cryptic nereidid polychaetes.

Introduction

Complexes of local cryptic species are becoming more prevalent in the literature as integrative taxonomic methods are popularly used when challenging the “cosmopolitan” distributions of several marine invertebrate species (Klautau et al. 1999; Gómez et al. 2002; Barroso et al. 2009; Pérez-Portela et al. 2013; Dijoux et al. 2014; Simon et al. 2017). Since cryptic species represent genetically distinct species that have acquired very little morphological divergence over time (Avise et al. 1987), speciation in such cases are hypothesised to have diversified without a change in gross morphology (Gittenberger 1991). When this happens, sexual selection, genetic drift or a combination of the two act to fix genes for reproductive incompatibility throughout the population, which does not necessarily require an accompanying change in gross morphology (Bickford et al. 2007).

Four hypotheses have been proposed to explain cryptic speciation: recent divergence, parallelism, stasis and morphological convergence (Fišer et al. 2018; Struck et al. 2018). In the first two cases, evolutionary timescales are more recent (~ thousands of years ago) whereas in the latter two, they are more historical (~ millions of years ago) (Struck et al. 2018). Recent divergence is when cryptic species are closely related and divergence has occurred only recently leaving very little time for substantial morphological differences to accumulate whereas in parallelism cryptic species are not closely related, but have evolved independently from morphologically similar ancestors (Fišer et al. 2018; Struck et al. 2018). Morphological stasis occurs when species are closely related or are part of a species complex but have diverged from each other over millions of years ago (Fišer et al. 2018; Struck et al. 2018). The high morphological similarity shared between these species is suggested to be a result of either low standing genetic variation and or strong stabilising selection which has to retain the most common morphology as traits do not change drastically over time (Bickford et al. 2007; Fišer et al. 2018; Struck et al. 2018). On the other hand, morphological convergence occurs when distantly related taxa have a high degree of morphological similarity resulting from similar selection pressures imposed by extrinsic factors (Fišer et al. 2018; Struck et al. 2018).

Due to the intricate processes that shape cryptic species, information from several disciplines such as historical biogeography, palaeontology, geology, population genetics, phylogenetic systematics and ecology (i.e. commonly known as phylogeography) are vital when investigating cryptic speciation hypotheses (Avise 2000; Beheregaray and Caccone 2007). Phylogeography therefore has the potential to uncover the chronology of demographic variation, reproductive isolation and speciation events that occurred throughout a species' history (Avise 2000; Beheregaray and Caccone 2007). It has proven useful in teasing apart cryptic species and the factors responsible for the distribution, structure and connectivity of several marine species (e.g. Uthicke and Benzie 2003; Ciofi et al. 2006; Schön 2007; Marko et al. 2010; Hu et al. 2011; Teske et al. 2013; von der Heyden et al. 2013; Zhang et al. 2014; Struck et al. 2017). However, the four hypotheses (mentioned above), that were proposed to explain cryptic speciation are less studied and thus poorly understood (Fišer et al. 2018).

The South African coastline is of particular interest in phylogeographic and cryptic species research as the differences between the two major current systems, the warm Agulhas on the east and cold Benguela on the west, give rise to a variation in temperature, nutrients and productivity, resulting in the classification of four inshore marine ecoregions (Figure 1.2) (Griffiths et al. 2010; Sink et al. 2012). Since each of these ecoregions are known to support their own diverse assemblages of flora and fauna (von der Heyden 2009; Griffiths et al. 2010; Sink et al. 2012), this provides the unique opportunity for researchers to investigate the importance of ecological gradients that contribute to population structure, distribution, connectivity (Teske et al. 2011a) and speciation events (Teske et al. 2018b).

The plethora of phylogeographic research on marine species within South Africa revealed that many species with distributions that span most of the coastline have regionally structured lineages that are primarily dictated by the interplay between intrinsic characteristics of larval developmental patterns, oceanographic currents and frontal systems and the ecoregions (Teske et al. 2011a, 2013, 2018b). By contrast, some species tend to display genetically identical populations suggesting that oceanic currents and frontal systems assisted in the dispersal and thus connectivity of populations (e.g. Teske et al. 2007; Neethling et al. 2008; von der Heyden et al. 2010, 2013) and that their larvae were hardy enough to survive these drastic changes in temperatures. In some cases historical

biogeography was found to have a profound influence on the contemporary genetic patterns of species (e.g. Reynolds et al. 2014; Muteveri et al. 2015). A common trend found was that the Pleistocene served as an important historical climatic event that influenced population expansions (e.g. Muller et al. 2012; von der Heyden et al. 2013; Reynolds et al. 2014; Mmonwa et al. 2015; Muteveri et al. 2015) whilst others found substantial evidence for post-LGM (Last Glacial Maximum, ~26,000 – 18,000 years ago, Clark et al. (2009)) expansions due to warming conditions (e.g. Gopal et al. 2006; Matthee et al. 2006; Neethling et al. 2008; von der Heyden et al. 2010).

In view of the lack of understanding of the distribution, cryptic diversity and population genetic patterns of polychaete worms in South Africa, Chapters 3 and 4 investigated the genetic structure of three historically misidentified nereidid polychaete species, *Pseudonereis podocirra* (Schmarda, 1861), *Platynereis massiliensis s.l.* (Moquin-Tandon, 1869) and *Platynereis* B sp. nov. Kara, Macdonald, Simon, 2018, with overlapping distributions and a slight difference in specific habitat preferences (Chapter 2). *Platynereis massiliensis s.l.* displayed three structured lineages that corresponded with the Cape Point and Cape Agulhas genetic breaks whereas *Platynereis* B sp. nov. displayed two well-mixed lineages separated by the Cape Agulhas genetic break (Chapter 3). In contrast, high-throughput SNP (Chapter 4) and mitochondrial (Chapter 2) datasets for *P. podocirra* revealed high connectivity between populations across the phylogeographic breaks identified for *P. massiliensis s.l.* and *Platynereis* B sp. nov. Furthermore, *P. massiliensis s.l.* and *Platynereis* B sp. nov. were found to be part of a true cryptic species complex due to their identical morphologies (Chapter 3) whereas *P. podocirra* and *Pseudonereis variegata* (Grube, 1857) are better described as pseudocryptic (Chapter 2).

While the patterns of genetic structure observed for *P. massiliensis s.l.* and *Platynereis* B sp. nov. can be explained by biogeography in addition to larval development mode (inferred from Wäge et al. (2017)), the patterns of connectivity observed for *P. podocirra* indicate that this species' distribution is most likely affected by older factors such as historical climatic changes (Chapter 4), as observed for other marine invertebrates with similar connectivity patterns (e.g. Reynolds et al. 2014; Muteveri et al. 2015). Therefore, the first aim is to investigate the influence of historical climatic conditions on

shaping the contrasting patterns of phylogeography observed for *P. massiliensis s.l., Platynereis* B sp. nov. and *P. podocirra*.

Platynereis massiliensis s.l. and *Platynereis* B sp.nov. are morphologically identical sympatric species (Chapter 3), whereas *P. podocirra* and *P. variegata* represent allopatric species that have acquired only minimal morphological differences. Therefore, the second aim was to investigate the mechanisms responsible for their cryptic speciation patterns over evolutionary timescales in order to better understand why the polychaete group is riddled with cryptic species complexes. Both of these aims will be addressed by generating a date chronogram using mtCOI data generated in Chapters 2 and 3.

Materials and Methods

A dated chronogram was generated using BEAST v2.4.8 (Bouckaert et al. 2014) based on mtCOI data generated for *Pseudonereis podocirra* (Chapter 2) and *Platynereis massiliensis s.l., Platynereis* B sp. nov. (Chapter 3) together with additional sequences of *Pseudonereis variegata* from Chile and *Pseudonereis* sp. from South Korea and China (Accession numbers and reference data: Chapter 2) and *Platynereis massiliensis, Platynereis dumerilii* (Adouin & Milne Edwards, 1833), *Platynereis* sp. (Accession numbers and reference data: Chapter 3). The outgroup used to root the tree was *Namanereis* sp. (JX420279). The HKY model of evolution was used with a strict clock and fixed mutation rate (2.2%) to calibrate the tree for divergence time estimations. The same mutation rate has been successfully used for other nereidid polychaetes (Chevaldonné et al. 2002; Jolly et al. 2006; Glasby et al. 2013). The analysis was run for 10 000 000 generations and the resulting log files were checked in Tracer v1.5 (Rambaut et al. 2018) to ensure that parameters converged. The sampled trees were combined into a single tree using TreeAnnotator (Bouckaert et al. 2014) with the first 25% of trees discarded as burn-in and the maximum clade credibility tree was selected as the target tree. The final tree was viewed using FigTree v1.4.3 (Rambaut 2016) and edited in Photoshop CC 2014. The resulting divergence times should be interpreted as an estimate as the

date chronogram was estimated using a single marker thus reflecting gene divergence times and not species divergence times.

Results and Discussion

Contemporary and historical patterns of genetic structure and connectivity

In contrast to the geographic and genetic structure found for *Platynereis massiliensis s.l.* and *Platynereis* B sp. nov., *Pseudonereis podocirra* exhibited patterns of a well-mixed meta-population (Chapters 2 and 3). In addition to the star-like topology for the mitochondrial network analysis, genomic diversity estimates were low and Tajima's D values were low and negative (Chapter 4). As a result, it was hypothesised that populations of *P. podocirra* could have experienced historical expansion events (Chapter 4). Furthermore, the west coast was flagged as an area of genetic uniqueness due to the large numbers of private SNPs found at two west coast populations (Chapter 4). It was therefore further hypothesised that the west coast populations of *P. podocirra* underwent a postglacial southward expansion (Chapter 4).

The above-mentioned hypotheses are supported by the patterns illustrated in the date chronogram (Figure 5.1) that demonstrates the existence of two evolutionarily old lineages of *P. podocirra,* one on the west and south coasts, respectively, and a fourth more recently derived lineage on the south-east coast (Blue clade, Figure 5.1). Lineage one (L1, ~3 million years ago (MYA) – 2 MYA) exclusively contains individuals from St. Francis Bay and Plettenberg Bay, lineage two (L2, 3 MYA – 0.7 MYA) contains a mix of individuals from Lamberts Bay and Yzerfontein from the west coast, Strand and Rooi Els from the south-west coast and Mossel Bay from the south coast (Figure 5.1) and lineage three contains more recently derived individuals (L3, 0.06 MYA – present) which predominantly consists of those from south and south-east coast populations (Figure 5.1). The observation that L1 exclusively contains individuals from Cape St. Francis and Plettenberg Bay indicates that these two populations were isolated from the rest of the south and west coast populations for at least 40 000 years which explains the differentiation observed with mtCOI (Chapter 2) and SNP (Chapter 4) datasets between these populations (Figure 5.1). These results contrast

with those obtained for the rocky shore fish, *Clinus cottoides* Valenciennes, 1836, which showed a divergence between west and south coast lineages ~54 000 years ago coinciding with the southward expansion of the Southern Coastal Plain (SCP) exposing vast sandy beaches separating the two rocky shore lineages (Toms et al. 2014). However, the split between L1 and L2 of *P. podocirra* (~6.4 MYA, CI: 5.1-7.4 MYA) (Figure 5.1) predates the expansion of the SCP (75 000 - 14 000 years ago, Compton 2011) and could instead be attributed to conditions during the Miocene (Rovere et al. 2014).

It is believed that taxa that have persisted for long periods, surviving a wide range of environmental fluctuations are more resilient than more recently evolved, vulnerable taxa (Cattin et al. 2016). Since *P. podocirra* is evolutionarily older than the two *Platynereis* species (~6.4 MYA and ~4.7 – 2.5 MYA, respectively) (Figure 5.1), age might have contributed to producing the contrasting patterns of structure observed (Chapters 2, 3 and 4). As a result, the two old *P. podocirra* lineages, L1 and L2 would have stood the test of time, surviving several glacial and interglacial periods from the late Miocene to Pliocene periods, maintaining gene flow within each lineage. Thus, with the formation of the present-day ecoregions, this resilient species would have had a higher genetic fitness allowing gene flow to still persist beyond the present-day thermal and oceanographic barriers to gene flow. However, such a scenario has never been investigated before and thus would benefit from future exploration.

Patterns of evolutionarily old lineages occurring on the west and south coasts observed for the two *Platynereis* species are similar to those observed for *Pseudonereis. Platynereis* B sp. nov. is represented by one evolutionarily old lineage (L1, 1.5-0.7 MYA) that is present along the west coast whereas the two more recently derived lineages, consist of individuals from west, south and southeast coasts (L2, 0.08 – present) and from the west and southwest coasts only (L3, 0.05 – present) (Figure 5.1). *Platynereis massiliensis s.l.* on the other hand displayed three evolutionarily old lineages with one recent derived lineage. Lineages 1, 2 and 3 (1.3-0.2 MYA) consisted of individuals from the west and southwest coasts only, whereas lineage 4 (0.08 MYA – present) consisted of individuals from a mixture of locations with the south and southeast coast individuals predominating (Figure 5.1).

The more recently derived individuals of all three species are found along the south and southeast coasts, providing strong support for south-eastern expansions of their populations, while recently derived individuals of *Platynereis* B sp. nov. and *P. podocirra* were also found along the west coast. The timing of the evolution of the more recently derived lineages for all three species coincides with the middle to late Pleistocene (Ramsay and Cooper 2002), when a gradual rise in sea level occurred creating most of the present-day shoreline (Davies 1972). Therefore, it is very likely that when sea level increased, additional suitable rocky habitats became available (Davies 1973) which marked the expansion of the west and southwest coast lineages in a southeast and western direction. Additionally, the observation of recently derived lineages along the west coast in addition to south and southeast coasts for *Platynereis* B sp. nov. and *P. podoccira* suggests that these populations radiated up the west coast in response to increases in sea surface temperatures during this period (Compton 2011). These results are consistent with other studies (e.g. Matthee et al. 2007; von der Heyden et al. 2010, 2013; Muller et al. 2012; Reynolds et al. 2014; Mmonwa et al. 2015; Muteveri et al. 2015) and indicate that the Pleistocene glacial cycles impacted many South African marine invertebrate species, as well as other marine species around the world (e.g. Schön 2007; He et al. 2010; Marko et al. 2010; Leyton et al. 2015; Derycke et al. 2016). Furthermore, the west, southwest and south coasts of South Africa serve as important sites for evolutionarily old and diverse lineages as all three study species exhibited diverse ancestral lineages that originated from here. This is similar to the plough shell, *Bullia rhodostoma,* where it was found that a range expansion was more important among the western-most localities (Muteveri et al. 2015). Finally, in accordance with Matthee et al. (2007); Reynolds et al. (2014) and Nielsen et al. (2018) it was found that the west coast populations tend to harbour more genetically diverse (Chapters 2, 3 and 4) populations as opposed to the south coast.

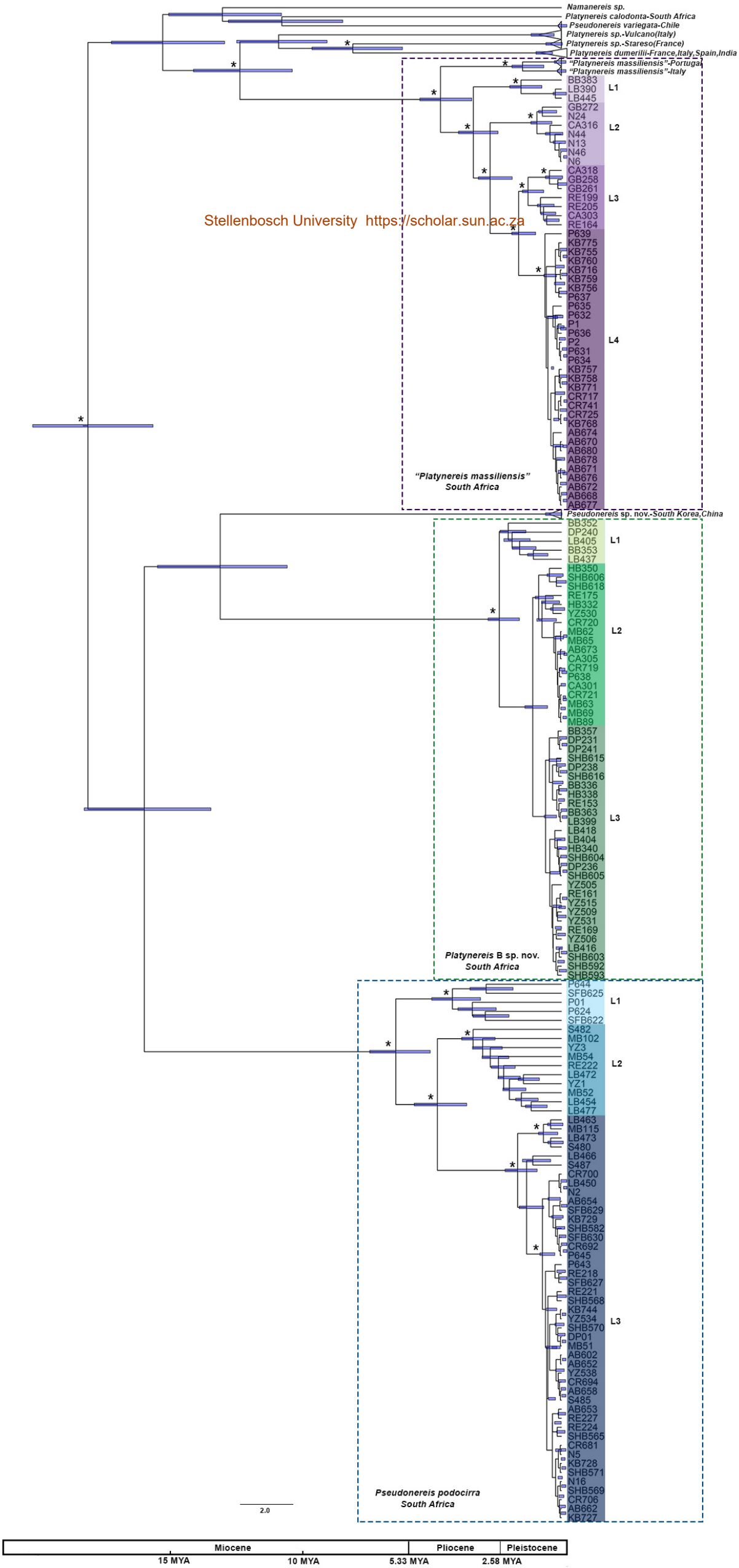

Figure 5.1: Date chronogram of BEAST consensus tree depicting divergence times of *Platynereis massiliensis s.l.* (South Africa, Italy, Portugal, Purple clade), *Platynereis* B sp. nov. (South Africa, Green clade), *Platynereis dumerilii* (Italy, Spain, France, India), *Platynereis* sp. (Stareso (France) and Vulcano (Italy) vents), *Pseudonereis podocirra* (South Africa, Blue clade) and *Pseudonereis variegata* (Chile). Node Bars represent the 95% CI for the divergence estimates. Asterisk at tree nodes represent clades with Bayesian posterior probability of >90%. Abbreviations: L1, L2 etc represent Lineage 1, Lineage 2, etc within each of the South African clades.

In South Africa, *Platynereis massiliensis s.l.* and *Platynereis* B sp. nov. inhabit the same habitat but display contrasting patterns of genetic structure indicative of different modes of reproduction (Chapter 3), suggesting that speciation occurred sympatrically and is maintained through reproductive isolation. Furthermore, results from the calibrated chronogram reveal that divergences between these two species are ancient because they last shared a common ancestor ~18.1 million years ago (MYA) (CI: 20-15 MYA) (Figure 5.1). Since the two species have been evolutionarily independent for millions of years without accumulating any morphological differences, they are experiencing morphological stasis (cf. Struck et al. (2018)). Morphological stasis, the lack of morphological differentiation accompanied by genetic diversification, could result from low standing genetic variation and or strong stabilising selection acting to retain a common morphology as most traits do not change drastically over time (Bickford et al. 2007; Fišer et al. 2018; Struck et al. 2018). The latter scenario is plausible as even though distributional ranges of *P. massiliensis s.l.* and *Platynereis* B sp. nov. were limited by temperature, they were present in the same habitat (i.e. the same algae on the fringing intertidal zone) along the South African coast (Chapter 3) suggesting that strong stabilising selection has acted to retain a common shared morphology as a result of a common shared ecology (Bickford et al. 2007; Struck et al. 2018).

Since both species' ranges are limited by different temperatures, thermal adaptation has likely played a role in limiting gene flow between ecoregions by reducing migrant fitness and subjecting them to competitive exclusion (Teske et al. 2011a). The latter is further demonstrated by the fewer individuals of *Platynereis* B sp. nov. found along the warmer coasts and of *P. massiliensis s.l.* along the colder coasts (Table 3.1). These results are consistent with that of Teske et al. (2018b) who found evidence for incipient speciation of thermally adapted lineages of the sand goby, *Psammogobius knysnaensis* Smith, 1935 across the Atlantic/Indian Ocean boundary. These results further demonstrate that even though both species, *Platynereis massiliensis s.l.* and *Platynereis* B sp. nov. most likely developed physiological tolerances to different temperatures (Bickford et al. 2007), their morphology was nonetheless still constrained.

Despite the ancient divergence between *Platynereis massiliensis s.l.* and *P. dumerilii* (~10.9 MYA, CI: 9 – 12.1 MYA) (Figure 5.1) both species are morphologically identical suggesting that these two species are also undergoing morphological stasis. Whilst Wäge et al. (2017) did not directly relate the speciation of *P. massiliensis s.l.* and *P. dumerilii* to differences in ecological conditions, they did conclude that the prevalence of the brooding habit in acidified sites suggested that this reproductive habit enabled the former species to survive in stressful conditions. Since South Africa was suggested as the native range of *P. massiliensis s.l.* (Chapter 3) it is probable that this species evolved to have a brooding reproductive strategy in response to an unknown pressure here. This reproductive strategy then facilitated its' establishment in acidified environments in its introduced range (i.e. Mediterranean). This is further supported by the similar water temperatures at the shallow hydrothermal vent systems at Castello Aragonese d'Ischia (temperature range: 18 – 19 °C (Martin et al. 2008)) and in the southwest coast region of South Africa (temperature range: 17 – 21 °C (Smit et al. 2013)), that was suggested as the source of the introduction (Figure 3.2). Since *P. massiliensis s.l.* is found in the Atlantic and to some extent in the western extremeties of the Indian Ocean and *P. dumerilii* in the Mediterranean, the initial seperation of these two species could have occurred allopatrically due to large scale oceanic currents, and inability of larvae to survive in the open ocean (Knowlton 1993; Avise 2000). Furthermore, their identical morphologies coupled with ancient divergences suggests that they too are probably experiencing morphological stasis. Alternatively, convergence could also result in high morphological similarity of species (Fišer et al. 2018; Struck et al. 2018). However, it may not be a plausible explanation as *P. dumerilii* and *P. massiliensis s.l.* are sister taxa, although this could be an artefact of a "limited phylogeny" where only available taxa were used to construct the phylogeny (Figure 5.1). Furthermore, convergence requires that distantly related species are exposed to the same ecological pressures, hence live in the same habitat (Fišer et al. 2018; Struck et al. 2018), but *P. dumerilii* is found subtidally in the Mediterranean (Wäge et al. 2017) whereas *P. massiliensis s.l.* inhabits the fringing intertidal zones in South Africa (Chapter 3). Thus, allopatric speciation coupled with morphological stasis seems a more likely mode and mechanism of speciation, respectively.

The three *Platynereis* species from the Mediterranean, a brooding *Platynereis* sp. from the Vulcano vent in Italy, the *Platynereis* sp. clade from the non-acidified Stareso sites in France and the *P. dumerilii* clade from France, Spain, Italy and India (Wäge et al. 2017) shared a common ancestor ~10.9 MYA (9-14 MYA). The mechanisms of speciation within this region are complex, as Wäge et al. (2017) suggested that St. Caterina and sites at Castello (Italy) could most likely represent areas of sympatry as individuals of *P. massiliensis s.l.* and *P. dumerilii* coexisted there. However, this conclusion was made under the assumption that the former species was actually native to the Mediterranean and since it is now demonstrated to be alien there (Chapter 3), this conclusion is no longer plausible. In the case of *Platynereis* sp. (Vulcano acidified vents), speciation from *Platynereis* sp. (Stareso) and *P. dumerilii* probably occurred as a result of reproductive isolation and different physiological pressures imposed by the habitat as the latter two species are presumed to be broadcast spawners that live in non-acidified sites (Wäge et al. 2017). By contrast, the factors contributing to the speciation of *P. dumerilii* and *Platynereis* sp. (Stareso) are unknown and should thus be investigated further. Additionally, these three species in the Mediterranean have been independent for millions of years (Figure 5.1) without accumulating any morphological differences irrespective of differences in habitat preferences and reproductive strategies suggesting that they are also undergoing morphological stasis (Struck et al. 2018).

Pseudonereis podocirra from South Africa and *Pseudonereis* sp. from Korea were historically misidentified as *Pseudonereis variegata* from Chile due to significant overlap in morphological characters (Chapter 2; Park 2018). However, the former two species can be distinguished from *P. variegata* by small but discernible morphological differences (Table 2.3 and Park 2018) and large genetic distances (Table 2.4 and Park 2018). The three species last shared a common ancestor ~18.5 MYA (CI: 15-20 MYA) (Figure 5.1) indicating that even though they have been evolutionarily independent for millions of years, they have only accumulated slight morphological differences in the intervening time. *Pseudonereis podocirra* occurs in the Atlantic and Indian oceans and *P. variegata* and *Pseudonereis* sp. in southeast and northwest, respectively of the Pacific Ocean. The three species probably speciated allopatrically, and gene flow between them would have been inhibited shortly after seperation due to large-scale oceanic currents, unsuitable habitats between populations

(i.e. open ocean as these are intertidal rocky shore species), extreme temperature and salinity gradients (Knowlton 1993; Palumbi 1994). An alternative explanation for a shared morphology between the three geographically distant species is that each species had separate ancestors with a shared morphology that somewhat resembled the present day *Pseudonereis* species which is further supported by the polyphyly observed (Figure 5.1). Due to their specialised, common habitats, (i.e. mussel beds) (Sampertegui et al. 2013; Park 2018; Chapter 2), strong stabilising selection would have acted to preserve this morphology type making it relatively constant through time with only minute changes (Struck et al. 2018). If all three species indeed had separate ancestors that morphologically resembled one another and that inhabited mussel beds, this would also bring convergent evolution into question as an alternative mechanism of speciation (Fišer et al. 2018; Struck et al. 2018). Convergence requires species to be distantly related and thus not sister taxa as demonstrated in the phylogeny in Figure 2.5. Such a close relationship between species could be a result of the limited taxa that were available for inclusion in the phylogeny, leaving convergent evolution a plausible mechanism of speciation. Evidence of morphological stasis on common ancestors through time for the three *Pseudonereis* species is further supported by the large morphological overlap found for 10 additional species of *Pseudonereis* that only differed in the number and arrangement of paragnaths present on the pharynx and minute differences in parapodial morphologies (Bakken 2007). Thus, without finer examination, it is hypothesised that if all species were placed side by side, they would indeed be mistaken for one species. Nonetheless, these hypotheses will benefit from further examination of the last shared common ancestor of all species belonging to the genus.

It is therefore evident from the current research that *P. podocirra, P. variegata* and *Pseudonereis* sp. are examples of species that are experiencing either convergence or morphological stasis. *Platynereis massiliensis s.l.* and *Platynereis* B sp. nov. displayed evidence of speciation as a result of reproductive isolation and preferences for different temperatures, *Platynereis* sp. (Vulcano) was suggested to have speciated from *Platynereis* sp. (Stareso) and *P. dumerilii* as a result of reproductive isolation coupled with preferences for different ecological conditions, whilst the speciation between *Platynereis* sp. (Stareso) and *P. dumerilii* are largely unknown. Furthermore, all

four species have undergone morphological stasis evidenced by their identical morphologies. These results contrast with that obtained for three geographically distant species of *Stygocapitella* genus where it was hypothesised that long distance dispersal occurred via the Paleo-Tethys Ocean which acted to homogenise their morphologies (Struck et al. 2017). Furthermore, their subsequent split was attributed to the formation of two Southern hemisphere species, one corresponding to the Panthalassic Ocean and the other to the Paleo-Tethys Ocean (Struck et al. 2017).

Chapter Six: Final Discussion

Summary

Cosmopolitanism in the polychaete group has increasingly been shown to be an artefact of several historical factors such as the “European taxonomic bias”, the conservative views of influential taxonomists, poor species descriptions and the lack of the type material (Hutchings and Kupriyanova 2018). This high occurrence of apparent cosmopolitan species significantly underestimated native and alien diversity and distribution of polychaete species in different regions (Nygren 2014) as further taxonomic and molecular revisions revealed the presence of several misidentified (e.g. Molina-Acevedo and Carrera-Parra 2015; Villalobos-Guerrero and Carrera-Parra 2015; Simon et al. 2017, 2018) and cryptic species (e.g. Halt et al. 2009; Glasby et al. 2013). The misrepresentation of species on alien inventories has serious implications for conservation and management as early warning of potential introductions, prevention and control measures heavily rely on accurate alien species identfications and lists (McGeoch et al. 2012). Additionally, incorrect identities of native species complicate our understanding of regional patterns and underlying processes that are responsible for population structure, connectivity, diversity and speciation mechanisms of cryptic and pseudocryptic marine invertebrate species (Bermingham and Moritz 1998; Teske et al. 2011a; Nygren 2014; Fišer et al. 2018; Struck et al. 2018), particularly within the context of the heterogeneous nature of South African coastline. Thus, it is imperative that species are identified correctly by conducting thorough taxonomic and molecular investigations before a species name is assigned to a specimen as the polychaete group is riddled with cryptic species complexes, in addition to its convoluted taxonomic history (e.g. Halt et al. 2009; Nygren and Pleijel 2011; Glasby et al. 2013; Struck et al. 2017). As a result, two broad research questions were addressed below.

Research Question 1: Have nereidid polychaetes indigenous to South Africa been misidentified as cosmopolitan, resulting in an underestimation of native diversity?

This research question was addressed by investigating the taxonomic and molecular status of three species that have type localities outside of South Africa and with wide global distributional ranges. Investigations were carried out using thorough taxonomic revisions with molecular datasets to

determine whether species names were assigned correctly and consequently whether their classification as alien and cosmopolitan were correct.

Day (1967) considered *Platynereis australis* Schmarda, 1861*, Platynereis dumerilii* Audouin & Milne Edwards, 1833 and *Pseudonereis variegata* Grube, 1857 as cosmopolitan species. Multiple junior synonyms of *P. dumerilii* and *P. variegata* were initially described as native species in various locations, including South Africa but were synonymised later due to the high morphological resemblance to their congeners from France and Chile, respectively (Augener 1913; Fauvel 1921; Day 1953; Hartman 1959). As a result, all three species were considered cosmopolitan due to their disjunct global distributions (Day 1967; Read 2018; Read and Fauchald 2018d). However, the incorrectly assigned junior synonyms of *P. variegata* have recently been reinstated in some parts of their disjunct distributions (Villalobos-Guerrero and Carrera-Parra 2015; Conde-Vela 2018; Park 2018). On the other hand, *P. dumerilii* and *P. australis* were recently found to be part of species complexes in their respective native ranges (Read 2007; Calosi et al. 2013; Lucey et al. 2015; Wäge et al. 2017). Additionally, the inconsistencies between the two major works listing alien polychaete species locally (Mead et al. 2011b) and globally (Çinar 2012) and the lack of thorough taxonomic investigation before statuses were assigned, have resulted in all three species classifications in South Africa to be considered questionable. Since all three nominal species have type localities outside of South Africa, multiple synonymised names, disjunct global distributions and were found to be incorrect identifications in parts of their distributions, they were all considered as priority for further investigation. Additionally, all three species have a widespread distribution across the coast of South Africa (Day 1967) and since many marine invertebrate species tend to display structured lineages across such a wide distribution (e.g. Zardi et al. 2007; von der Heyden et al. 2008; Teske et al. 2011a, 2018), it is thus very likely that the same is possible for the above species.

An update on the status of South African Nereididae

Thorough taxonomic and molecular revisions revealed that the three species previously considered cosmopolitan, *P. variegata* (Chapter 2) and *P. australis* and *P. dumerilii* (Chapter 3)*,* are actually indigenous species that were historically misidentified. The local *Pseudonereis podocirra* was

previously incorrectly synonymised as *P. variegata* from Chile, the local *P. massiliensis s.l.* (Moquin-Tandon, 1869) was misidentified here as *P. australis* from New Zealand and *Platynereis* B sp. nov. Kara, Macdonald, Simon, 2018 was misidentified as *P. dumerilii* from the Mediterranean. The incorrect synonymisation of *P. podocirra* was due to poor species descriptions combined with conservative taxonomic practices of influential taxonomists at that time which had resulted in them overlooking subtle morphological differences between species. By contrast, the mistakes in the identifications of the two *Platynereis* species may be more understandable since these species are truly cryptic (Chapters 2 and 3). These results provide evidence that the diversity of local nereidid polychaetes of South Africa has indeed been underestimated and increased the number of indigenous nereidid species reported in Day (1967) to 14, and decreased the number of pseudocosmopolitan species to 15 (Table 6.1).

Table 6.1: An updated checklist of pseudocosmopolitan nereidid species recorded from South Africa according to collections by Day (1967). The current worldwide distributional ranges and number of synonyms were extracted from the World Polychaete Database.

Species	South African distribution	Worldwide distribution	Number of synonyms
Alitta succinea Leuckhart, 1847	Plettenberg Bay, Cape St. Francis, Port Elizabeth, Richards Bay and Durban	Massachusetts to Gulf of Mexico, Uruguay, Belgium, English Channel, France, North Sea and South Africa	9
Ceratonereis (Composetia) hircinicola Eisig, 1870	St. Lucia	Mediterranean Sea, North Atlantic, Spain and South Africa	5
Dendronereis arborifera Peters, 1854	Port Elizabeth and Richards Bay	Mozambique, Madagascar and South Africa	0
Namalycastis indica Southern, 1921	Richards Bay to St. Lucia and Mozambique	India, South Africa, Mozambique, Andamans and Nicobar Islands	0
Namanereis quadraticeps Blanchard, 1849	Saldanha Bay	Southern California, North Carolina, Caribbean Sea, North Atlantic, New Zealand, Mexico, Japan and South Africa	0
Nereis coutieri Gravier, 1899	Durban, Richards Bay and Kosi Bay	Suez Canal, Red Sea, Mozambique and South Africa	0
Nereis eugeniae Kinberg, 1866	Port Nolloth, Lamberts Bay and Saldanha Bay	Chile, Falkland Islands, Argentina, Kerguelen and South Africa	1

Nereis falcaria Willey, 1905	Saldanha Bay, False Bay and Cape Agulhas	Mozambique, New Zealand, North Atlantic, South Africa,	2
Nereis falsa Quatrefages, 1865	East London and St. Lucia	France, Morocco, North Carolina, Mediterranean Sea, Madagascar, Caribbean Sea, Gulf of Mexico, South Africa, Venezuela	2
Nereis jacksoni Kinberg, 1866	Cape St. Francis, Port Elizabeth, East London and Durban	Red Sea, South Australia, New South Wales, Chatham Islands, Caribbean Sea, Cuba, Gulf of Mexico, North Atlantic Ocean, Madagascar, Mozambique, New Zealand and South Africa	1
Nereis lamellosa Ehlers, 1868	Lamberts Bay, Plettenberg Bay and East London	Morocco, Senegal, Gulf of Mexico, Mediterranean (Greece), North Atlantic, Spain and South Africa	0
Nereis pelagica Linnaeus, 1758	Saldanha Bay	Bay of Fundy, Caribbean Sea, English Channel, France, Gulf of Mexico, Ireland, Mozambique, North Atlantic, North Sea, Norway, Spain, Trinidad, United Kingdom, Venezuela and South Africa	15
Nereis persica Fauvel, 1911	Bashee and Richards Bay	Red Sea, North Atlantic, Mozambique, Madagascar, Mediterranean Sea and South Africa	2
Perinereis cultrifera Grube, 1840	Table Bay, Port Elizabeth, Durban and Richards Bay	Senegal, Mediterranean Sea, English Channel, France, Gulf of Mexico, Ireland, Madagascar, North Atlantic Ocean, South Africa, Spain and United Kingdom	7
Simplisetia erythraeensis Fauvel, 1918	Lamberts Bay, Saldanha Bay and Richards Bay	Madagascar, Japan, Mozambique, Red Sea, South Africa and Pacific Ocean	3

Chapters 2 and 3 provided updated distribution ranges for the new species. *Platynereis massiliensis s.l.* prefers the warmer parts of the coast that experience temperatures of 18 – 22 °C (Smit et al. 2013), illustrated by an increase in abundance from the Southern Benguela (Lamberts Bay) to the eastern part of the Agulhas (Kidds Beach) ecoregions. This distribution also represents an extension of its previosu known distribution from between Port Nolloth (Southern Benguela ecoregion) and Hermanus (western part of the Agulhas ecoregion) (Day 1953, 1967). Notably, this species (as *P. australis*) has never been reported in ecological studies since the publication of Day (1967) most likely owing to the confusion with the identical *Platynereis* B sp. nov. By contrast, *Platynereis* B sp.

nov. demonstrated a decrease in abundance from the Southern Benguela (Lamberts Bay) to the eastern part of the Agulhas (Kidds Beach) ecoregion, suggesting a preference for cooler parts of the coast where temperatures range between 12 – 16 °C (Smit et al. 2013). This new distribution range recorded for *Platynereis* B sp. nov. also extends the known distributional range more westward than that reported in Day (1953, 1967) where individuals were found to occur from Table Bay (Southern Benguela ecoregion) to Port Shepstone (eastern part of the Agulhas ecoregions). It is possible that specimens present in sites from Kidds Beach to Port Shepstone may actually represent *P. massiliensis s.l.* as this species prefers warmer temperatures characteristic of this stretch of coast (Smit et al. 2013). This species was also recorded in Namibia and Mozambique (Day 1967; Penrith and Kensley 1970; Branch et al. 2010). On the other hand, *P. podocirra* was found equally abundant from Lamberts Bay in the Southern Benguela ecoregion to Kidds beach in the Agulhas ecoregion, which is still within the known distribution range recorded by Day (1967), Penrith and Kensley (1970) and Branch et al. (2010) as occurring from Namibia to Mozambique. Chapters 2 and 3 demonstrated that *P. variegata, P. dumerilii* and *P. australis* do not occur in South Africa, therefore bringing into question their presence in Namibia and Mozambique. Since these species were first documented in these countries by Day (1967) and the monograph subsequently used by other researchers (e.g. Field and Mcfarlane 1968; Penrith and Kensley 1970; van Herwerden 1989; Branch et al. 2010), records of these species in Namibia and Mozambique are probably perpetuating the incorrect identifications by Day of indigenous species. In the case of *Platynereis* B sp. nov. (as *P. dumerilii* in Day (1967)), it is very unlikely that this species occurs from the cold Southern Benguela all the way up the east coast of South Africa into tropical Mozambique spanning a temperature gradient of 24 °C to 26 °C (Smit et al. 2013). Additionally, 60% of the cosmopolitan species listed in Table 6.1 have also been recorded in Namibia and Mozambique, therefore raising doubts regarding their taxonomic identifications and are highlighted as priority for further investigation. Of these species (Table 6.1), three have been investigated elsewhere in the world. *Nereis falsa* Quatrefages, 1865, was found to have a doubtful presence even in its type locality in the Black Sea (Salazar-Vallejo et al. 2017) and it is therefore probable that its junior synonym, *Nereis lucipeta* Ehlers, 1908, originally described from South Africa, represents an incorrect synonym that should be re-examined (Salazar-Vallejo et al. 2017). Similarly, *Neresi pelagica* Linnaeus, 1758 and *Perinereis cultrifera* Grube, 1840 had been

misidentified in Northeast Asia, and while the indigenous representative of the first species has not been formally described yet (Park 2018), the latter has been redescribed as *P. euiini* Park and Kim, 2017 (Park and Kim 2017).

The research conducted here clearly indicates that diversity of indigenous nereidids has been underestimated, and that further research is needed to fully resolve the problem. The high proportion of nereidid species in South Africa that require revision (>50%) is very similar to what Seddick (2018) found for the Syllidae Grube, 1850 and Simon et al. (2018) found for Spionidae Grube, 1850, suggesting that this may also apply to other families. This is further demonstrated by investigations of local eunicid, magelonid, spionid and syllid polychaetes by Lewis and Karageorgopoulos (2008), Clarke et al. (2010), Simon et al. (2017, 2018) and Seddick (2018). These studies provide evidence that many cosmopolitan species reported in the monograph for this region (Day 1967) are actually incorrect assignments. In fact, Awad et al. (2002) indicated that only 20% of polychaete species in South Africa are indigenous to the region. If only half the remaining 80% prove to be misidentifications of indigenous species, then the diversity of indigenous South African polychaete species has been severely underestimated.

The comprehensive Day (1967) monograph is an invaluable source of polychaete descriptions and distributions for the Southern African region and is thus widely used by researchers from many disciplines. It is also used widely by taxonomists working outside of the region (Hutchings and Kupriyanova 2018). Biologists using the monograph, locally and internationally, should therefore take cognisance of this fact and should use the monograph as a guideline, especially with regards to species that are considered ‘cosmopolitan’. If at least 50% of the taxa recorded in Day (1967) require revision, it is a task well beyond the current collective abilities of the polychaete taxonomists active in South Africa. As a result, more short-term solutions are proposed. Firstly, when non-taxonomists are reporting on species collected during their studies, the questionable identities of widespread species must be clearly indicated or acknowledged with the addition of ‘cf.’ to the name. This should be accompanied by the lodging of voucher specimens for taxonomic and DNA reference in museums and online databases such as GenBank and the Barcode of Life Database (BOLD). The barcoding initiative requires that a 650bp fragment of the mitochondrial cytochrome c oxidase subunit

1 gene (in a forward and reverse direction) be sequenced in order to facilitate identifications of species with incomplete taxonomy across the animal kingdom (Hebert et al. 2003). DNA barcoding thus serves as an important method to uncover biodiversity in problematic taxonomic groups (Hebert et al. 2003) such as annelids. Secondly, lists need to be generated that prioritise species of the remaining polychaete families (according to the criteria used in Chapter 1) with dubious taxonomic identifications as candidate species for further investigation so that it might serve to update both indigenous and alien polychaete lists.

What are the implications for Nereididae systematics globally?

Thorough morphological revision and molecular identification revealed that species with widespread distributions belonging to both *Pseudonereis* and *Platynereis* need revision. This is not only because each genus was found to comprise species complexes, but because both *Platynereis* species were also demonstrated to be alien in parts of their distribution. This means that it should not be taken for granted that all cosmopolitan species are examples of misidentified or incorrectly synonymised species since some may actually represent neocosmopolitan species. Furthermore, these results have reinforced the value of molecular methods for validating species identifications as the *Platynereis* species in South Africa and the Mediterranean were morphologically indistinguishable.

Chapter 2 highlighted the importance of revising the general description of *P. variegata* due to the large variation in characters of species from different places in South America that were used to compile the original description, and the fact that one of the most comprehensive descriptions of this species was based on syntype material of a junior synonym, *Nereis ferox* Hansen, 1882, from Brazil (Bakken 2007), the synonymy of which has been recently rejected (Conde-Vela 2018). Additionally, all *P. variegata* outside of Chile need to be examined further as this species is clearly not cosmopolitan as demonstrated in the present research and that by Conde-Vela (2018) and Park (2018).

Results from Chapter 3 indicate that *P. massiliensis s.l.* needs to be further investigated as the molecular data provide strong evidence indicating that South Africa is actually the native range of

this species and consequently the most likely source of invasion to the Mediterranean. In light of this, two scenarios are possible: The first scenario is that the species was described in the Mediterranean after it had been transported there. This is plausible as accidental introductions of species and their subsequent descriptions as being native to the introduced region have been recorded for other polychaete species (e.g. Sun et al. 2017a). The recognition of this species as alien in what had previously been accepted to be its native range may have a profound impact on management and conservation in the Mediterranean as it can now be listed as a species of interest and thus further spread and impact on native communities can be monitored and possibly managed. Furthermore, *P. massiliensis s.l.* sampled from the Mediterranean were concentrated in acidified areas (Wäge et al. 2017) indicating that if ocean acidification increases throughout the Mediterranean, conditions may favour this alien species, enabling it to spread. The second scenario is: the tentative identification of the Ischia clade (Clade 1) as *P. massiliensis s.l.* owing to proximity of the collection site to the type locality (Lucey et al. 2015; Wäge et al. 2017) and its brooding behaviour as previously described (Schneider et al. 1992) was incorrect and that it should be described as a new species. Furthermore, it is possible that the other brooding *Platynereis* sp. (Vulcano vent, Italy) is actually the real *P. massiliensis*. Therefore, a more detailed taxonomic revision needs to be conducted on this species using an extended sampling range from the Mediterranean which will then paint a clearer picture on the naming, distribution and pathways of invasion of this species.

Chapter 3 also revealed that *P. dumerilii*, *P. massiliensis s.l.* and *Platynereis* B sp. nov. are morphologically indistinguishable from one another. Additionally, the former two species have clearly demonstrated to be alien in parts of their distribution. Therefore, all records of the species in this complex need to be reassessed using molecular tools to confirm their identity.

The findings from the present study together with those from published literature addressing taxonomic and molecular revisions on nereidids add to the growing body of literature that emphasise the need for revisions of all nominal species and their junior synonyms that have pseudocosmopolitan distributions. Furthermore, it is also important that the new generation of nereidid taxonomists dedicate time to resolving these heterogeneous groupings designated by influential taxonomists in the past (Salazar-Vallejo et al. 2017). The idea that large morphological

variation is normal within a species must be abandoned, especially for the “highly-variable” Nereididae as it is evident from literature that this variation is most-likely due to the presence of evolutionary independent species (e.g. Scaps et al. 2000; Maltagliati et al. 2001; Audzijonyte et al. 2008; Glasby et al. 2013; Villalobos-Guerrero and Carrera-Parra 2015; Salazar-Vallejo et al. 2017). Additionally, it should not be taken for granted that morphologically identical or similar species with discontinuous global distributions represent a single species, as demonstrated for *P. dumerilii* from the Mediterranean (Wäge et al. 2017), *Platynereis* B sp. nov., and *P. massiliensis s.l.* from South Africa (Chapter 3) and *P. podocirra* from South Africa and *P. variegata* from Chile (Chapter 2).

Increasing numbers of taxonomic revisions suggest that family Nereididae is considered one of the most speciose families within the polychaete group (as of 2017, ~50 genera with 770 valid species described (Bakken et al. 2018)). In fact, in a space of 15 years, the number of species increased by one third (in 2004, ~43 genera described with just over 535 species (Bakken and Wilson 2005)). Nonetheless, the real diversity of this family has yet to be uncovered as many cryptic and misidentified species could be masked behind the remaining pseudocosmopolitan species. Thus, if the synonyms of the species considered in Table 6.1 each represent valid species, then the diversity of nereidids could potentially increase by a total of 47 species.

Research Question 2: What are the potential underlying historical and contemporary demographic factors driving the distribution, genetic structure and diversity of *Pseudonereis podocirra, Platynereis massiliensis s.l.* and *Platynereis* B sp. nov.?

This research question was addressed by investigating the population genetic structure and phylogeography of three historically misidentified nereidid polychaete worms with overlapping distribution ranges in South Africa. The genetic structure of *P. podocirra* was investigated using a high-throughput SNP dataset in addition to mitochondrial data whereas genetic structure of *Platynereis massiliensis s.l.* and *Platynereis* B sp. nov. were investigated using mitochondrial and nuclear DNA datasets. Furthermore, the phylogeography of all three species were investigated using a combined mitochondrial DNA dataset to determine whether historical events have contributed to

shaping their contemporary distribution patterns. Since all three species were considered to be part of cryptic and pseudocryptic species complexes, questions regarding speciation mechanisms were also addressed in the phylogeographic analysis.

Contemporary and historical patterns of genetic structure and connectivity

Genetic investigations revealed three contrasting patterns of population structure and connectivity for *Platynereis massiliensis s.l., Platynereis* B sp. nov. and *Pseudonereis podocirra.* According to mtCOI data, *P. massiliensis s.l.* displayed three geographically structured lineages that were separated by the Cape Point and Cape Agulhas genetic breaks whereas *Platynereis* B sp. nov. exhibited two well-mixed regional lineages that were separated by the Cape Agulhas genetic break (Chapter 3). In contrast, nuclear DNA demonstrated that both species have well-mixed populations indicating extensive gene flow, thus the intrinsic properties of each marker were used to explain the contrasting patterns of genetic structure and connectivity (Chapter 3). In contrast to these patterns of mitochondrial structure, *P. podocirra* revealed high connectivity between populations across the genetic breaks identified for *P. massiliensis s.l.* and *Platynereis* B sp. nov. using a mitochondrial dataset (Chapter 2). What was even more unexpected was that this lack of structure for *P. podocirra* was mirrored by the high-throughput SNP dataset generated for this species (Chapter 4).

The contrasting patterns of structure and connectivity observed for the three species could have been influenced by the different ages of the species (Chapter 5) in addition to larval development mode, oceanic currents and biogeography (Chapters 2 and 3). Since *P. podocirra* represents an evolutionarily older species, it is expected to be more resilient than the younger *Platynereis* species. However, resilience also depends on the intrinsic characters of a species including colour polymorphism as demonstrated by vertebrate species (Cattin et al. 2016). Since colour polymorphism enables a species to exploit different habitat types or have broader distributional ranges because of concomitant behavioural and physiological differences, resilience is enhanced (Forsman and Åberg 2008; Cattin et al. 2016), all three species are expected to be equally resilient as they are all colour polymorphic (Chapters 2 and 3). Furthermore, species with planktotrophic larvae tend to persist for longer than species with non-planktotrophic larvae, as demonstrated by the nassariid gastropod fossil record (Gili and Martinelli 1994). It was also found that species with broad

distributional ranges tend to survive the adverse conditions of multiple climatic events thus enhancing longevity and hence resilience of a species (Powell 2007). *Pseudonereis podocirra* is an evolutionarily older species and is presumed to have planktonic larvae (Chapters 2 and 4) thus, enabling it to have a broad distribution range across the South African coast. These characteristics would explain the resilience of this species and hence the lack of population structure despite the strong ecological gradients that resulted in structured lineages of the two *Platynereis* species.

Patterns of genetic and geographic structure observed for *Platynereis* B sp. nov. and *P. massiliensis s.l.*, respectively, were similar to those obtained for several other marine species in South Africa with similar larval strategies (e.g. Evans et al. 2004; Zardi et al. 2007; Teske et al. 2011; von der Heyden et al. 2013; Williams et al. 2016; Naidoo 2017). This indicates that larval mode together with the oceanographic currents and ecoregions are all important factors driving the genetic and geographic structure of marine species in South Africa (Teske et al. 2007c, 2011a; Nicastro et al. 2008). The fact that both *Platynereis* species had lineage breaks around the Cape Agulhas area, which was also found for other marine invertebrate species (e.g. Evans et al. 2004; Teske et al. 2007b, a; von der Heyden et al. 2008) indicates that this genetic break remains important in dictating the distribution of marine species in South Africa. Furthermore, the phylogeographic breaks in the Cape Point and Cape Agulhas areas are most likely so strong as they coincide with changes to two types of environmental gradients, temperature and the direction of current, that change abruptly in this area (Lutjeharms et al. 2001; Griffiths et al. 2010; Sink et al. 2012; von der Heyden et al. 2013). Since offspring of brooding species such as *P. massiliensis s.l.* (Lucey et al. 2015; Valvassori et al. 2015) spend no time in the water column, dispersal of larvae between the Southern Benguela and Agulhas ecoregions would be inhibited, resulting in the geographic structure observed (Chapter 3). The observation that thermal tolerance plays a significant role in limiting lineages and species distributions (Teske et al. 2011a, b, 2018b) are further supported by the results obtained for the *Platynereis* species (Chapter 3). Thus, if ocean temperatures continue to increase due to anthropogenically induced climate warming (Pecl et al. 2017) range shifts by genetic adaptation (e.g. Schön 2007; Maggs et al. 2008; Muteveri et al. 2015), thermally driven speciation events (e.g. Teske et al. 2018b) and or the extinction of vulnerable species (Hoffmann et al. 2011) may occur. As a

result, the findings from this research contributed to identifying thermally adapted, evolutionarily young species, that might be prone to local extinction, and thus can be used in future decision making regarding the conservation of such species and or be used as species to monitor climate change.

The west coast of South Africa was identified as an area of genetic uniqueness for *P. podocirra* as two populations here displayed the largest number of private SNPs (Chapter 4). Furthermore, the west, southwest and south coast may also serve as evolutionarily important areas for the three species as they all displayed old lineages here (Chapter 5). This also suggests that the ancestral lineages originated from these areas as they formed the origin of the subsequent diversification of more recently derived individuals in a south- and southeast-ward direction (Chapter 5). Evidence of a south and southeast-ward expansion in addition to the west being an area of genetic uniqueness provides important information for the conservation of evolutionarily unique species. The results from this study, like Nielsen et al. (2018), identified the west coast as an area of genetic uniqueness. Since the west coast was identified as an area of interest due to the increasing anthropogenic activities (i.e. fishing and mining) (von der Heyden 2009), this reinforces the need to conserve and protect the species here in the form of Marine Protected Areas.

Even though the three species exhibit contrasting patterns of contemporary genetic structure and connectivity, they experienced similar historical expansions and intraspecific radiations that were influenced by conditions during the middle to late Pleistocene (Chapter 5). This indicates that the Pleistocene served as an important period that shaped the distribution of *P. massiliensis s.l., Platynereis* B sp. nov. and *P. podocirra* as well as many other marine species (e.g. Matthee et al. 2007; Neethling et al. 2008; von der Heyden et al. 2010, 2013; Reynolds et al. 2014; Mmonwa et al. 2015; Muteveri et al. 2015). In addition to the rocky shore species investigated in this study, ~58% of the species investigated so far were rocky shore animals that also experienced Pleistocene expansions (e.g. Evans et al. 2004; von der Heyden et al. 2013; Reynolds et al. 2014; Toms et al. 2014; Mmonwa et al. 2015). This strongly indicates that rocky shore species are prone to displaying range expansions in response to Pleistocene climatic fluctuations as temperature and the shoreline during this period was altered due to fluctuations in climate and sea levels (Davies 1971, 1972, 1973; Ramsay and Cooper 2002).

These results for the first time provide insight into the patterns of, and factors responsible for, the distribution, structure, connectivity of a recently reinstated local polychaete, *P. podocirra*, and two misidentified species, *P. massiliensis s.l.* and *Platynereis* B sp. nov. Additionally, the development of a high-throughput SNP dataset for *P. podocirra* added to the small body of knowledge to date that have produced such datasets to investigate population genetics and phylogeography of marine invertebrates in South Africa (see: Bester-van der Merwe et al. 2011; Nielsen et al. 2018) and provides a baseline upon which questions about genes under natural selection can be investigated in future studies.

Evolutionary processes of cryptic species

The results from Chapter 5 indicate that the two *Platynereis* species in South Africa have undergone sympatric speciation due to reproductive isolation (differing patterns observed in population structure (Chapter 3)) followed by morphological stasis as evidenced by the identical morphologies of these species. Morphological stasis was suggested to be a result of environmentally induced stabilising selection that favoured a common morphotype as both species were observed to occupy similar ecological niches (Chapter 3). Although *Platynereis* B sp. nov. increased in abundance towards the west and *P. massiliensis s.l.* towards the east, the former species was overall more abundant at all sites (Chapter 3). This higher abundance of *Platynereis* B sp. nov. can be linked to its' presumed broadcast spawning reproductive strategy demonstrated by the patterns of genetic structure (Chapter 3). Broadcast spawning *Platynereis* species are known to release large numbers of planktotrophic larvae into the water column resulting in an increase in the fertilisation success and subsequent dispersal of this species over larger distances (Fischer and Dorresteijn 2004; Fischer et al. 2010; Lucey et al. 2015). This probably contributed to the high abundance and wider distribution of *Platynereis* B sp. nov. along the coast. By contrast, the presumed brooding strategy demonstrated by the geographic structure of *P. massiliensis s.l.* (Chapter 3), probably contributed to its overall lower abundance and limited distribution along the coast as fewer offspring with poor dispersal abilities are produced by this species in the Mediterranean (Lucey et al. 2015).

Platynereis australis, did not form part of the bigger *Platynereis* species complex (Chapter 3), despite the fact that it was mistaken for what is currently identified as *P. massiliensis s.l.* and by extension the *Platynereis* species from the Mediterranean (Chapter 3). Additionally, the comparisons between the species descriptions by Day (1967) and Read (2007) of the non-reproductive forms of *P. australis* revealed no obvious differences (Chapter 3). Similarly, the comparisons between the species descriptions of *P. australis* and *P. dumerilii* (Day, 1967) were almost identical with the exception of the absence of a particular chaetae, which is regarded as a superficial difference. Nonetheless, in New Zealand *P. australis* was found to be part of a local species complex with morphological differences only prevalent at the epitokus (reproductive form) stages (Read 2007), therefore, making it likely that these species too differ in their reproductive forms. The identical morphologies of the New Zealand, South African and the Mediterranean *Platynereis* species indicates that all species form part of global *Platynereis* pseudocryptic complex based on reproductive characters.

P. massiliensis s.l. and *P. dumerilii* most likely speciated from each other allopatrically and their ancient divergence times indicate that they are undergoing morphological stasis (Chapter 5). By contrast, *Platynereis* sp. (Vulcano, Italy) was hypothesised to have speciated from *Platynereis* sp. (Stareso, France) and *P. dumerilii* (Mediterranean) due to reproductive isolation whereas divergence times indicate that these species too are undergoing morphological stasis (Chapter 5).

Pseudonereis podocirra from South Africa and *P. variegata* from Chile also demonstrate evidence of morphological stasis or convergence (Chapters 5) reflected in their strong resemblance to each other (Chapter 2). Unfortunately, there is no species description available for *Pseudonereis* sp. from Northeast Asia, so morphology could not be compared. Nonetheless, since this species was originally mistaken for *P. variegata* from Chile such as that demonstrated for *P. podocirra* from South Africa*,* can be presumed that these species are part of a global pseudocryptic species complex.

Considering that only one additional study (i.e. Struck et al. 2017) addressed the mechanisms of cryptic speciation of polychaete worms, it is a difficult task to draw conclusions regarding factors that are responsible for driving cryptic speciation. Nonetheless, these results demonstrate the importance of understanding historical processes that could have contributed to shaping the diversity of species giving us clues into their morphological evolution. Furthermore, the present results provide possible

mechanisms of speciation that occurred in the cryptic and pseudocryptic species complexes from South Africa, further highlighting the importance of using genetic methods in addition to traditional taxonomy to correctly distinguish between species.

In conclusion, the results from this research have demonstrated that the overall indigenous diversity of polychaete worms in South Africa has been underestimated owing to incorrect taxonomic identifications of cosmopolitan species. Consequently, two indigenous (*Platynereis* B sp. nov. and *P. massiliensis s.l.*) and one reinstated species (*P. podocirra*) are recorded for South Africa which belong to global cryptic and pseudocryptic species complexes. Additionally, historical climatic oscilations, oceanographic currents, ecoregions and presumed larval development mode were demonstrated to play a role in the historical and contemoporary structure of these polychaete species. Finally, allopatry and sympatry were identified as the most likely drivers of speciation whilst morphological stasis was found to be the mechanism of cryptic speciation of the pseudocryptic and cryptic species complexes in South Africa.

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