# Taxonomy and distribution of moonshine worms (*Diopatra* sp.) in Knysna Estuary

by

**Hendré van Rensburg**

*Thesis presented in fulfilment of the requirements for the degree of*

***Master of Science in Zoology***

*Department of Botany and Zoology*

*Faculty of Science*

*Stellenbosch University*

Supervisor: Prof. Carol Anne Simon

Co-supervisor: Prof. Conrad Matthee

December 2019

# Declaration

By submitting this thesis 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.

# Abstract

Polychaetes as fish bait have become increasingly popular in the Knysna Estuary over the last decade. The presence of an unknown polychaete, *Diopatra* sp. was first reported in the Knysna Estuary ten years ago, when it was harvested as fish bait in small quantities by local fishermen who called it the moonshine worm. Since this very conspicuous species was not detected by intensive biodiversity sampling in the estuary in the 1950s and 1990s, it should thus be considered new to the estuary. A preliminary morphological investigation showed that *Diopatra* sp. may be *Diopatra neapolitana* (Delle Chiaje, 1841). However, *D. neapolitana* is a pseudo-cosmopolitan species with local distribution restricted to Durban and Port Elizabeth. As several cosmopolitan species have recently been described as cryptic endemic species, it is likely that *Diopatra* sp. in the Knysna Estuary may also represent an undescribed cryptic endemic species. The aims of this study were firstly to identify *Diopatra* sp. using molecular and morphological techniques and secondly to determine the density and distribution of the species throughout the estuary and estimate population size and baiting pressure (the percentage of worms recently removed from an area due to baiting) for conservation management. Bayesian and maximum likelihood analysis of COI and 16S markers indicated that the species in South Africa is *Diopatra aciculata* (Knox & Cameron, 1971) from Australia. Although sequence divergence between *D. aciculata* and *D. neapolitana* is lower than among other species in the genus, it was still an order of magnitude greater than the intra-specific sequence diversity of either of these species. The separation of these species is confirmed by species delimitation analysis. Molecular similarity between *D. aciculata* and *D. neapolitana* is reflected by morphological similarity, and the two species have so many features in common that it is very difficult to tell them apart. The morphology of *D. aciculata* from South Africa closely matched descriptions from Australia. The confirmation of the moonshine worms as an alien rather than an undescribed indigenous species increases the need for understanding population densities as management focus has shifted from conservation to mitigation or extirpation. During November and December 2017, density of *D. aciculata* was determined at 18 sites in the Knysna Estuary; 13 in the intertidal zone and 5 in the subtidal zone. Five sites also fell within the invertebrate reserve where baiting is prohibited. *Diopatra aciculata* was present throughout the estuary up to where freshwater conditions dominated. Distribution was patchy, with median densities ranging from 0 ± 0.03 to 8 ± 1.03 worms.m$^{-2}$ (median ± standard error) and a maximum of 58 worms.m$^{-2}$. Despite overall low mean density 3.54 worms.m$^{-2}$, the estimated population size occupying the total potential habitable area of 6,487,600m$^{2}$ exceeds 22 million worms. Bait collecting by fishermen is unlikely to be effective for large scale removal as baiting pressure was very low (5.48% maximum). Urgent research is needed to determine the impact of this species in the estuary as the Knysna Estuary is one of the most important estuaries in South Africa and alien ecosystem engineers such as *Diopatra* can have profound physical and biological impacts on their surroundings.

# Opsomming

Gesegmenteerde wurms as vis lokaas het die afgelope dekade toenemende gewildheid ervaar in die Knysna-riviermonding. Die teenwoordigheid van ‘n onbekende wurm, *Diopatra* sp. is tien jaar gelede vir die eerste keer in die Knysna-riviermond aangemeld toe dit in klein hoeveelhede as vis lokaas geoes is deur plaaslike vissers wat na die wurms as maanskyn-wurms verwys het. Aangesien hierdie baie opvallende spesie nie deur intensiewe biodiversiteit opname in die riviermonding in die 1950's en 1990's bespeur is nie, moet dit dus as nuut in die riviermonding beskou word. ‘n Voorlopige morfologiese ondersoek het getoon dat *Diopatra* sp. mag dalk *Diopatra neapolitana* (Delle Chiaje, 1841) wees. *Diopatra neapolitana* is egter 'n pseudo-kosmopolitaanse spesie met plaaslike verspreiding beperk tot Durban en Port Elizabeth. Aangesien verskeie kosmopolitiaanse spesies onlangs as kriptiese endemiese spesies beskryf is, is dit waarskynlik dat *Diopatra* sp. in die Knysna-riviermond ook 'n onbeskryfde kriptiese endemiese spesie verteenwoordig. Die doelstellings van hierdie studie was om eerstens *Diopatra* sp te identifiseer deur die gebruik van molekulêre en morfologiese tegnieke en tweedens om die digtheid en verspreiding van die spesies regdeur die riviermonding te bepaal asook die bevolkingsgrootte en die lokaasdruk (Hoeveelheid wurms onlangs verwyder in ʼn area weens lokaas oes) vir bewaringsbestuur. Bayesiese en maksimum waarskynlikheid analise van COI en 16S merkers het aangedui dat die *Diopatra* sp. in Suid-Afrika is *Diopatra aciculata* (Knox & Cameron, 1971) van Australië. Alhoewel DNS verskille tussen *D. aciculata* en *D. neapolitana* laer is as tussen ander spesies in die genus, was dit steeds 'n orde van grootte groter as die intra-spesie DNS diversiteit van albei spesies. Die skeiding van hierdie spesies word bevestig deur spesie-afbakening analise. Molekulêre ooreenkomste tussen *D. aciculata* en *D. neapolitana* word weerspieël deur morfologiese ooreenkomste en die twee spesies het soveel kenmerke in gemeen dat dit moeilik is om tussen hulle te onderskei. Die morfologie van *D. aciculata* uit Suid-Afrika het sterk ooreenkomste met beskrywings van Australië. Die bevestiging van die maanskyn-wurms as uitheems eerder as 'n onbeskryfde inheemse spesie, intensifiseer die behoefte om die digtheid van die bevolking te verstaan, aangesien fokus van bewaring tot versagting of uitwissing verskuif is. Gedurende November en Desember 2017 is digtheid van *D. aciculata* bepaal op 18 plekke in die Knysna-riviermonding; 13 in die tussengety-sone en 5 in die subgety-sone. Vyf plekke het ook in die ongewerwelde reservaat geval waar die oes van lokaas verbied is. *Diopatra aciculata* is regdeur die riviermonding aangetref tot aar varswater toestande begin oorheers het. Verspreiding was lappend, met mediaan digthede wat wissel van 0 ± 0.03 tot 8 ± 1.03 wurms.m$^{-2}$ (mediaan ± standaard fout) en 'n reeks van 58 wurms.m$^{-2}$. Ten spyte van ‘n algehele lae gemiddelde digtheid van 3.54 wurms.m$^{-2}$, is die beraamde bevolkingsgrootte in die potensiële woonbare oppervlakte van 6,487,600m$^{2}$ meer as 22 miljoen wurms. Dit is onwaarskynlik dat lokaas oes deur vissers effektief sal wees vir grootskaalse verwydering, aangesien die lokaasdruk baie laag was (5,48% maksimum). Dringende navorsing is nodig om die impak van hierdie spesie in die riviermonding te bepaal aangesien die Knysna-riviermonding een van die belangrikste riviermondings in Suid-Afrika is en uitheemse ekosisteem-ingenieurs soos *Diopatra* diepgaande fisiese en biologiese impakte op hul omgewing kan hê.

# Acknowledgements

I would firstly like to thank my parents Wynand and Alet van Rensburg who have supported me throughout all my years at university. I dedicate this work to them.

My supervisors Prof. Carol Simon and Prof Conrad Matthee for their support, guidance and patience throughout the whole project. A special thanks to Dr. Jyothi Kara who taught me almost everything that I now know of molecular techniques and always being willing to help and answer questions.

I am eternally grateful to the National Scientific Collections Facility (NSCF) and Michelle Hamer in particular. This degree would not have been possible without the personal funding I received from the NSCF. The Foundational Biodiversity Information Program (FBIP) funded the project costs and my attendance of the Biodiversity Information Management Forum (BIMF) so a special thanks to them as well.

Then also, everybody that helped me on field trips or with field work in any of the labs I worked in. Special thanks also to Louw Claassens and Peter and Frances Smith for the times spent in Knysna. Fieldwork helpers Alheit du Toit and Sian Seymour. SANParks staff for helping with logistics and contributing information, especially Owen Govender and Kyle Smith.

My close friends Dr. H. J. R. “Snakeman” van Zyl and Jandré “Petoros” Pretorius for their encouragement and support in all things. If it is a braai or even just an online conversation, I’m always happy after interactions with you.

A very special thank you to Welri Nortje for all her love and support throughout the last couple years, it makes everything easier and more enjoyable. Lastly, my cat, Picatso, for the conditional love and bouts of attention that often came precisely as things started to get dull or difficult.

# Table of Contents

# Table of Figures

# Table of Tables

# Chapter 1: Polychaetes as bait and the gaps in South African bait research

## *1.1 Background and introduction*

Fishing is a popular sport as well as an important means of subsistence for many people in South Africa (SA). The last estimates of local subsistence fishing found that about 30,000 fishermen provide sustenance for well over 150,000 people (Arnason and Kashorte, 2006; Branch and Clark, 2006; Clark et al., 2002). However, these estimates are outdated and are likely an underestimation (Masifundise Development Trust, 2010). In addition to subsistence fishing, the popularity of recreational angling (Beckley et al., 2008; Brouwer et al., 1997; McGrath et al., 1997) have led Branch and Clark (2006) to estimate that this category now number over 750 000 individuals in South Africa, however, these estimates are also over a decade old and it is unclear how many of these are marine shore fishermen. In South Africa, most shore fishermen are likely involved in collection of bait which may include smaller fishes or a variety of marine invertebrates including  crustaceans, molluscs and polychaetes (Fielding, 2009; Griffiths and Branch, 1997; Napier et al., 2009; Simon et al., 2019a; Turpie et al., 2003; van Herwerden, 1989). Thus, recreational and subsistence fishermen can be expected to exert a high pressure on bait species harvested from the intertidal zone (Hodgson et al., 2000a; Napier et al., 2009; Turpie et al., 2003; Wynberg and Branch, 1994). Furthermore, seasonal influxes of tourists to popular holiday destinations in the summer months can further exacerbate the regional exploitation of marine resources (Hodgson et al., 2000a; Nel and Branch, 2014; Simon et al., 2019a).

### *1.2 South African research bias*

Despite the frequent extraction of bait species from the intertidal zone, only the sandprawn, *Callichirus kraussi* (Stebbing, 1900), and the mudprawn, *Upogebia africana* (Ortmann, 1894) have received significant scientific attention. Studies targeting these species have focused on themes such as reproduction and development (Cretchley, 1996; Hanekom and Baird, 1992; Hanekom and Erasmus, 1989; Hill, 1977; Mendes et al., 2001), physiology (Harris et al., 1991; Schaefer, 1970; Thompson and Pritchard, 1969), population dynamics and distribution (Dubula and Lasiak, 2003; Emmerson, 1983; Hanekom and Erasmus, 1988; Hanekom and Russell, 2015; Hodgson et al., 2000b; Nel and Branch, 2013; Wooldridge and Loubser, 1996), anthropogenic exploitation (Cretchley, 1996; Hanekom and Baird, 1992; Hodgson et al., 2000a, 2000b; Nel and Branch, 2014; Wynberg and Branch, 1997, 1994, 1991) and interactions with surrounding environment or biota (Forbes et al., 2007; Henninger and Froneman, 2013; Pillay et al., 2012, 2011, 2007; Siebert and Branch, 2006, 2005). These studies either directly or indirectly contributed towards creating more effective and efficient management strategies for both species, specifically to protect and preserve stock levels and promote sustainable use (Fielding, 2009; Griffiths and Branch, 1997; Napier et al., 2009; Nel and Branch, 2013; Simon et al., 2019a).

The scope and magnitude of studies targeting *U. africana* and *C. kraussi* likely reflect their popularity; prawns have repeatedly been recognized as the most frequently collected bait species among shore fishermen in South Africa (Hodgson et al., 2000a; Napier et al., 2009; Nel and Branch, 2014; Wynberg and Branch, 1991). However, recurring studies investigating fishermen's use and preference of bait in the Knysna Estuary indicate a growing preference for polychaetes (Hodgson et al., 2000a; Napier et al., 2009; Simon et al., 2019a). Yet, despite bait worms being well studied topics in other countries (Arias et al., 2013; Arias and Paxton, 2014;

Cohen, 2012; Cohen et al., 2001; Cohen and Carlton, 1995; Conti and Massa, 1998; Costa et al., 2006; Dağli et al., 2005; De Carvalho et al., 2013; Kenny, 1969; Sypitkowski et al., 2009; Younsi et al., 2010), it remains a relatively understudied topic in South Africa; and is restricted to bloodworm, musselworm and estuarine wonderworm (Barham, 1979; Gaigher, 1979; Lewis, 2005a, 2005b; Lewis and Karageorgopoulos, 2008; van Herwerden, 1989).

### *1.3 Global use of polychaetes*

Polychaetes are harvested as bait by recreational and subsistence fishermen globally, including in the Mediterranean, Japan, India, Canada, Australia and the United States of America (Cole et al., 2018; Cunha et al., 2005; De Carvalho et al., 2013; Gambi et al., 1994; Miller and Smith, 2012; Watson et al., 2016; Younsi et al., 2010). The taxa utilized globally are quite diverse, with 12 of the 81 families being represented, with Arenicolidae, Eunicidae, Nereididae and Onuphidae being the most popular (Cole et al., 2018). At least 60 species are utilized, and use of individual species mostly reflect their natural distributions around the world (see Cole et al., 2018 supplementery material for summary of global use trends). The presence of undiscovered cryptic endemic species (i.e., genetically distinct but morphologically indistinguishable species), however, are likely to increase the number of utilized species (Bickford et al., 2007; Brasier et al., 2016; Nygren, 2014). Recently, many such species have been discovered among what are now considered pseudo-cosmopolitan species *sensu* Darling and Carlton (2014). A good example is *Marphysa sanguinea* (Montagu, 1833) that was previously considered cosmopolitan; taxonomic investigations have revealed that this nominal species comprises at least six endemic species, *Marphysa mullawa* Hutchings & Karageorgopoulis, 2003 (Hutchings and Karageorgopoulos, 2003), *Marphysa acicularum* Webster, 1884, *Marphysa nobilis* Treadwell, 1917, *Marphysa viridis* Treadwell, 1917 (Molina-acevedo and Carrera-parra, 2016) and *Marphysa victori* Lavesque, Daffe, Bonifácio & Hutchings, 2017 (see more below) (Lavesque et al., 2017).

### *1.4 Value and exploitation of bait polychaetes*

The popularity of polychaetes as bait species is further reflected in their value. Watson et al. (2016) found that when using retail price per kg, the five most expensive marine species sold on the global market are the bait polychaetes, *Glycera dibranchiata* Ehlers, 1868, *Diopatra aciculata* Knox & Cameron, 1971, *Alitta virens* (M. Sars, 1835), *Arenicola defodiens* Cadman & Nelson-Smith, 1993, and *M. sanguinea*. They also estimated that globally 121 000 metric tons of polychaetes are collected annually at a value of 7.63 billion USD (United-States dollar). It is thus clear that bait worm fisheries are of global importance and economic significance. Furthermore, the use of polychaetes for fish bait may also be growing in popularity. A 2009 study investigating *G. dibranchiata* harvesting in Maine, USA, found that the number of worms harvested per bait collector in 2007 was more than twice the mean harvests for 2004 and 2005 (Sypitkowski et al., 2009). Similarly in Turkey, harvesting of *D. neapolitana* Delle Chiaje, 1841 increased while stocks plummeted, with local collectors claiming that their catch per unit effort decreased tenfold in the preceding decade due to over exploitation caused by the increasing popularity of these worms (Dağli et al., 2005). Moreover, Gambi et al. (1994) voiced concern over the commercial exploitation of bait polychaetes in Italy and the Mediterranean while very little is known about their biology. Given the high value and importance of bait polychaetes in many other countries, it is understandable why the topic has received so much more attention in places such as the U.S.A. (Cohen, 2012; Cohen et al., 2001; Cohen and Carlton, 1995; Coleman et al., 2004; Sypitkowski et al., 2009), Argentina (Bruschetti et al., 2016, 2009, 2008; Etchegoin et al., 2012; Schwindt and Iribarne, 2000) or Australia (Knox and Cameron, 1971;

McPhee et al., 2002; Paxton, 1993, 1986, 1979). Yet, in South Africa there are almost no data on the stocks or exploitation trends of bait polychaetes.

### *1.5 Local research on bait polychaetes*

According to the marine recreational activity brochure issued by the Department of Agriculture, Forestry and Fisheries (DAFF), South African fishermen may legally collect wonder-, shingle-, moonshine-, coral-, pot-, pudding-, rock- and bloodworms for bait. Furthermore, the Cape reef worm and mussel worm are not to be harvested (Department of Agriculture Forestry and Fisheries, 2018). However, in this guide common names of only bloodworm and the two prohibited species, musselworm and Cape reefworm, are linked to genera; there are no pictures or other guidelines to help with identification of the bait species that can be collected. The identities of some species may be gleaned from research articles and identification keys. Day (1969) mentions that *Diopatra cuprea* (Bosc, 1802) (case worm) makes good bait and in another publication also reported *Arenicola loveni* Kinberg, 1866 (bloodworm) and *Pseudonereis podocirra* (Schmarda, 1861) (musselworm, as *P. variegata* (Grube, 1857)) as bait species (Day, 1969). Eight years later, van der Westhuizen and Marais (1977) reported *D. neapolitana* being used as bait by fishermen in the Swartkops Estuary in Port Elizabeth in the Eastern Cape Province. Furthermore, a field guide for the Eastern and Southern Cape coasts mentioned *A. loveni* and *P. podocirra* (as *P. variegata*), together with *Eunice aphroditois* (Pallas, 1788) (wonderworms), as bait (Lubke and De Moor, 1998). A South African fishermen’s guide listed blood-, mussel-, wonder and moonlight (likely moonshine) worms as being used but provided no scientific names and included only one illustration of a bloodworm (Whibley, 2003). A study by Napier et al. (2009) mentions a moonshine worm as *Diopatra* sp. being used as bait in the Knysna Estuary. Finally, the popular Two Oceans guide to marine life in southern Africa (Branch et al., 2017) explicitly identifies five species used as bait; *E.*

*aphroditois*, *A. loveni*, *P. podocirra* (as *P. variegata*) and then *Marphysa elityeni* Lewis & Karageorgopoulos, 2008 (estuarine wonderworm) and *Gunnarea gaimardi* (Quatrefages, 1848) (as *G. capensis* (Schmarda, 1861)) (Cape reef-worm). Thus, only the bloodworm (*A. loveni*), wonderworm (*E. aphroditois*), musselworm (*P. podocirra*) and Cape reef-worm (*G. gaimardi*) that are listed in the DAFF brochure as worms allowed for baiting, can be reliably traced to species names in the literature. This leaves the shingle-, pot-, pudding-, rock-, coral- and moonshine worms without any confirmed species identification.

As opposed to the more than 30 scientific studies that specifically targeted the two prawn bait species in South Africa, only eight studies have focused on three bait polychaete species. *Arenicola loveni*, the most popular bait polychaete in South Africa (Cockcroft et al., 2002; Fielding, 2009; Napier et al., 2009; Nel and Branch, 2014), is the only species that has been the subject of several research topics. Studies that focused on reproduction and development (Barham, 1979; Lewis, 2005a, 2005b) and population dynamics (Gaigher, 1979) could contribute to understanding the sustainability of its use and others may provide insight for commercialization (Yearsley et al., 2011). By contrast, a single study by van Herwerden (1989) showed that harvesting of daily quotas of musselworm destroyed more than the daily allowance of mussels and Cape reef worms, leading to the prohibition of collection of both worm species as bait (Department of Agriculture Forestry and Fisheries, 2018). The two remaining studies showed that two of the bait species, previously thought to have cosmopolitan distributions, were in fact misidentified indigenous species. The indigenous *P. podocirra* was previously misidentified as the pseudo-cosmopolitan *Pseudonereis variegata* (see Kara et al., 2018) and *M. elityeni* was another endemic species previously wrongly identified as *M. sanguinea* (Lewis and Karageorgopoulos, 2008). The remaining three species, *E. aphroditois*, *D. cuprea*, and *D. neapolitana*, have never been the focus of ecological, reproductive or taxonomic studies in

South Africa and similarly, all three of these species have been described as having cosmopolitan distributions. Therefore, these species records in SA may also represent pseudo-cosmopolitan misidentifications of indigenous species.

From the above it can be seen how insight provided by a single study (van Herwerden, 1989) can change baiting regulations (Department of Agriculture Forestry and Fisheries, 2018). In fact, most species that have enjoyed some scientific focus had regulations tailored to their individual biology by either banning collections or lowering daily allowed limits. For the remaining worms, a generic daily limit of ten individuals has been set, even though no information on reproductive cycles, population dynamics, dispersal and distribution, physiology, exploitation or biological interactions exist for any of these worms. Most importantly, accurate identification of these species are required before more meaningful studies could be undertaken, especially since some understudied species may be cryptic endemics which may have significant impacts on management.

### *1.6 Rationale for study*

The shortage of research on bait polychaetes in South Africa may be reflective of its use and perceived value. However, the value of polychaete bait as perceived by fishermen (Napier et al., 2009) and researchers (Turpie et al., 2003) has increased. In South Africa, the trade of polychaetes is not legal and as such, it is difficult to provide accurate estimates of the value of such worms. Nonetheless, Turpie et al. (2003) estimated the value of bait polychaetes at about R1 per worm. Six years later, Napier *et al.* (2009) found that subsistence fishers charged about R2 per worm (excluding *A. loveni* sold at R3 off-season and R5–10 in season) that was sold on the illegal market. Recent personal observations in this area suggest the value of these worms has at least doubled as asking prices varied between R4 – R8 per worm. Thus, bait worms hold value both as a means of income and subsistence as it can either be sold or used as bait for catching fish to eat or sell.

Recent studies investigating bait preference among fishers in the Knysna Estuary also demonstrated a proportionate increase in utilization of polychaetes as bait by both subsistence and recreational fishermen (Hodgson et al., 2000a; Napier et al., 2009; Simon et al., 2019a). Additionally, there has been a shift in polychaete species preference for moonshine worms, *Diopatra* sp., which is now the most sought-after bait polychaete among fishermen in Knysna Estuary (Simon et al., 2019a). Globally, *Diopatra* are widely distributed, very popular as bait in several countries and even contains one of the top five most expensive marine species (Cole et al., 2018; Watson et al., 2016). Yet, this genus has been mentioned only a few times in South African literature (Branch et al., 2017; Day, 1967, 1960, 1957, 1934; Field, 1971; Macnae, 1957, 1956; McIntosh, 1925; Mclachlan et al., 1984; Napier et al., 2009; van der Westhuizen and Marais, 1977) with the van der Westhuizen and Marais (1977) study being the only one confirming the use of a *Diopatra* species as bait in a South African estuary (Swartkops Estuary).

The Knysna Estuary is a popular tourist destination where several scientific studies have been conducted since the 1950s (Allanson et al., 2014, 2016, 2000b; Barnes, 2014; Claassens et al., 2018; Hodgson et al., 2000b; Largier et al., 2000; Marker, 2000; Marker and Maree, 2004; Napier et al., 2009; Russell, 1996; Schumann, 2000; Simon et al., 2019a; Teske et al., 2003). Yet, despite knowing that *Diopatra* sp. has been harvested as bait here for at least ten years (Napier et al., 2009), the identity of the species has not been conclusively determined. Similarly, the identification of *D. neapolitana* in the Swartkops Estuary by van der Westhuizen and Marais

(1977) cannot be confirmed since they did not deposit any type material, nor did they provide a description of the species. From previous studies and reports it can be inferred that the *Diopatra* sp. in Knysna may represent *Diopatra neapolitana* or *D. cuprea* as both these species were previously reported to be used as bait in South Africa (Branch et al., 2017; Day, 1969; van der Westhuizen and Marais, 1977). However, based on a superficial observation of *Diopatra* sp. it does not conform well with the description of *D. cuprea* in Day (1976). The length of *D. cuprea* is given as 120mm whereas *Diopatra* sp. is considerably longer. Also, *D. cuprea* is described with a uniformly brown anterior with no pigmentation patterns while *Diopatra* sp. has clear black markings on the mid dorsum of the anterior and varying colour morphs. Thus, *Diopatra* sp. likely represents either *D. neapolitana* or an undescribed indigenous species previously misidentified as *D. neapolitana*. Furthermore, although the species is increasingly exploited as bait in Knysna, there is no information regarding its densities or distribution in the estuary that may inform management strategies that would enable sustainable use. Thus, it is important to collect density and distribution data of the *Diopatra* sp. in the Knysna Estuary to inform conservation management (currently under the custodianship of South African National Parks) of its sustainable use once an accurate identification has been confirmed.

## *1.7 Aims*

The first aim of this study was to identify and clarify the taxonomy of the *Diopatra* sp. used as bait in Knysna and Swartkops estuaries (Chapter Two).

The second aim was to determine the distribution and densities of the *Diopatra* sp. throughout the Knysna Estuary and whether current baiting activities are having appreciable impacts on worm densities (Chapter Three).

# Chapter 2: Taxonomic clarification of common bait species *Diopatra* sp. used as bait in the Knysna and Swartkops estuaries

## 2.1 Introduction

*Diopatra neapolitana*, was originally described as a large, tube building polychaete occurring in the region of Naples in the Mediterranean. However, this description was rather poor (being brief and lacking detail). Using this description, the species was considered cosmopolitan, being reported from many places including Japan, India, Angola, Philippines, Arabian Peninsula and South Africa (Augener, 1931; Augener and Michaelsen, 1918; Choe, 1960; Day, 1967, 1934, Fauvel, 1953, 1932, 1930, 1923a; McIntosh, 1925; Monro, 1936, 1930; Parameswaran, 1973; Treadwell et al., 1943; Wehe and Fiege, 2002). The *Diopatra* sp. first reported in the Knysna Estuary ten years ago (Napier et al., 2009) is likely *Diopatra neapolitana*, or a morphologically similar cryptic endemic species (see Chapter One). As such it is worth considering the historical reports and accounts of *D. neapolitana* in Southern Africa.

### *2.1.1 Historical reports of D. neapolitana in southern Africa*

*Diopatra neapolitana* was first recorded in Angola in 1923 (Fauvel, 1923b). Soon after this, McIntosh (1925) recorded it in the Cape Province after which two more reports followed from Angola (Augener, 1931; Monro, 1930). In 1934, Day reported the species from dredged samples in shallow waters (1–99m) from the Agulhas Bank and Mozambique. However, he noted that there was some confusion over the taxonomy of the species although he didn't cite any authorities (Day, 1934). Two more accounts from Angola followed, one from the subtidal zone (Monro, 1936) and one from the intertidal zone (Treadwell, 1943). The earliest records of

*D. neapolitana* being reported in more specific locations was in the 1950s. In South Africa, *D. neapolitana* was reported from the Swartkops Estuary, near Port Elizabeth (Macnae, 1957, 1956) and Durban Bay (Day, 1957), while in Mozambique it was reported from Morrumbene Estuary, near Inhambane and Inhaca Island, near Maputo (Day, 1957).

In 1960, Day published part five of his “Polychaete fauna of South Africa” series in which he discussed the genus *Diopatra* Audouin & Milne Edwards, 1833, and specifically *D. neapolitana* in detail. In particular, Day (1960) discussed the taxonomic uncertainty associated with the genus and the controversy over whether it comprised several localised species or a single widely distributed species with many variations. He confirmed the validity of several species after examining his own collection together with several specimens from the British Museum. Furthermore, due to the lack of type material and the poor original description of *D. neapolitana*, he collected specimens from its type locality (Region of Naples) and re-described it so that it could be better defined and distinguished from other species. Subsequently, referring to his redescription of *D. neapolitana*, he stated that: “specimens in the British Museum labelled *D. neapolitana* from South Africa, West Africa, West Indies, various parts of the Indian Ocean and Australia were not this species” (Day, 1960). He concluded that all *D. neapolitana* that he had previously recorded in South Africa were in fact *Diopatra cuprea*, except for specimens collected from Durban Bay, which were indeed *D. neapolitana*. Thus, he confined the distribution of *D. neapolitana* in South Africa to Durban Bay alone. Furthermore, he described a subspecies *Diopatra neapolitana capensis* Day, 1960 occurring in deeper waters, originally recovered from a depth of 38.5m, from dredges in Algoa Bay, though it was also recovered from dredges along most of the SA coast from Port Nolloth on the west coast to Port St. John’s on the east coast in depths ranging from 13 – 117m (Day, 1960).

Seven years later, Day published his Monograph on the Polychaeta of Southern Africa (Day, 1967). In the introduction he used *D. neapolitana* as an example to oppose claims of polychaetes not being restricted to zoogeographical regions and stated that the cosmopolitan distributions for many has been overstated. He predicted that if cosmopolitan species were to be studied in more detail, many of them would probably have been previously misidentified. Later in the text he expanded occurrence records of *D. neapolitana* in southern Africa to include South-West Africa (Namibia today) but still listed the distribution of the species as Mediterranean only.

In the 1970s, *D. neapolitana capensis* was reported in False Bay (Field, 1971) in line with the distribution given by Day (1960, 1967). Despite the previous restriction of distribution to Durban Bay, *D. neapolitana* was once again reported in Swartkops Estuary (van der Westhuizen and Marais, 1977) and later, both *D. neapolitana* and *D. neapolitana capensis* (previously only in deeper waters of the coast) were reported in the Sundays River Estuary, just 25 km northeast of the Swartkops Estuary (Mclachlan et al., 1984). Thus, these were either misidentifications of *D. neapolitana* or Day (1960, 1967) underestimated its distribution implying the Swartkops Estuary reports from Macnae (1956, 1957) were accurate. Otherwise, *D. neapolitana* were indeed originally only present in Durban Bay (Day, 1960) but spread to Sundays River Estuary by the early 1980's. In 1989, *D. neapolitana* was reported from Langstrand in Namibia (Donn and Cockcroft, 1989) in congruence with Day (1967). Presently, Two Oceans, a guide to marine life of southern Africa (Branch et al., 2017) list both *D. neapolitana* and *D. cuprea* although their descriptions do not match those of Day (1967). Rather, the descriptions of *D. cuprea* and *D. neapolitana* in Branch et al. (2017) matches those of *D. neapolitana* and *D. n. capensis* respectively *sensu* Day (1967). Furthermore, the distribution of both are given as along the South African coast in sheltered bays and estuaries, rather than the punctuated distributions as described in Day (1967). From the above it is clear that accounts of *D. neapolitana* in southern Africa are very confusing and often conflicting.

### *2.1.2 Crypticity and pseudo-cosmopolitanism of D. neapolitana*

Recent advances in *Diopatra* taxonomy suggest that Day (1960) might have been right about *D. neapolitana* all along. This species was recently re-described (Arias et al., 2016) with its native range confirmed as the Mediterranean Sea and north-east Atlantic coasts, with the authors casting doubt on the occurrence of *D. neapolitana* beyond the current confirmed range. As morphological and molecular techniques have advanced it has become more apparent that several polychaete species previously thought of as cosmopolitan are in fact part of cryptic endemic species (Hutchings and Kupriyanova, 2018; Nygren, 2014; Nygren et al., 2018). This was recently demonstrated for *D. neapolitana* in two ways. The first involves the discovery of a cryptic species, *Diopatra biscayensis* Fauchald, Berke & Woodin, 2012 (Fauchald et al., 2012), occurring in sympatry with *D. neapolitana* in its native range. The second involves the discovery of another localised cryptic species *D. sugokai* Izuka, 1907 from Japan, originally reported as *D. neapolitana*, occurring outside the native range of *D. neapolitana* (see Paxton, 1998). Thus, *D. neapolitana* should be considered pseudo-cosmopolitan *sensu* Darling and Carlton (2018) until identifications outside of the Mediterranean can be confirmed molecularly. The idea of pseudo-cosmopolitanism and underestimated endemism is further supported by other polychaete species that were previously considered cosmopolitan, but are now known to be indigenous to South Africa (Kara et al., 2018; Simon et al., 2019b, 2018). Therefore, it may be likely that worms previously reported as *D. neapolitana* in South Africa are instead also a distinct native species.

Sometimes a single species truly is widespread and this is usually a consequence of human-mediated vectors (Çinar, 2013; Zenetos et al., 2012). Therefore, there is a possibility that the *Diopatra* sp. used as bait in Knysna and Swartkops can be a non-indigenous species. At least 292 polychaete species are reported to have been transported by humans around the world's oceans and 180 of these species have become established (Çinar, 2013). As discussed in Chapter One, many countries trade polychaetes as bait and some of this trade has led to introductions of species in non-native areas (Arias et al., 2013; Çinar, 2013; Cohen and Carlton, 1995; Costa et al., 2006; Nishi and Kato, 2004; Zenetos et al., 2012). However, it is unlikely that the *Diopatra* sp. was transported to South Africa in this way as trade in live polychaetes is prohibited. Still, it is possible that it could have arrived via an alternative vector, such as shipping, especially since the four species of Onuphidae: *Epidiopatra hupferiana hupferina* Augener, 1918, *Epidiopatra hupferiana monroi* Day, 1957, *Longibranchium atlanticum* (Day, 1973) and *Onuphis eremita oculata* Hartman 1951 listed as invasive by Cinar (2013) are suggested to have been introduced in this manner.

### *2.2.3 Identification approach*

Both morphological and molecular data are increasingly included in species descriptions (Arias et al., 2016; Pires et al., 2010; Rodrigues et al., 2009), and when clarifying the identities of pseudo-cosmopolitan species (Kara et al., 2018; Lavesque et al., 2017; Zanol et al., 2016). For example, Simon et al. (2019) showed that *Spirobranchus kraussii* (Baird, 1865), a species indigenous to SA but with an apparently wide distribution, actually comprises a complex of genetically distinct species. Given that a cryptic species as well as another species morphologically similar to *D. neapolitana* have been detected in its native range (Arias and Paxton, 2014; Fauchald et al., 2012; Pires et al., 2010), it is possible that the *Diopatra* sp. in

Knysna and Swartkops is also a similar but distinct species. It is therefore imperative that this species is investigated using morphological and molecular information.

For animals, one of the most widely used molecular markers for phylogenetic analysis and species delimitation is the mitochondrial Cytochrome c Oxidase subunit 1 (henceforth COI) (Hebert et al., 2003b; Patwasdhan et al., 2014; Pentinsaari et al., 2016). The COI marker has been shown to be effective in distinguishing 94–95% of tested animal species from one another (Hebert et al., 2016; Stoeckle and Thaler, 2018). Thus, the usefulness of the COI marker has led to it being considered the barcoding marker for animals (Folmer et al., 1994; Hebert et al., 2003b, 2003a; Stoeckle, 2003). Barcoding works by amplifying a small stretch of DNA and comparing it to a reference library of known species. Thus, a single sequence from an unknown organism can be used to identify it to species level, much like scanning a barcode on a product in the supermarket, greatly minimizing the effort required to identify some species. For this technique to work, interspecific variation needs to be greater than intraspecific variation to create the so called "barcoding gap" used to delimit species (Hebert et al., 2016, 2003b; Meyer and Paulay, 2005; Stoeckle and Thaler, 2018). There is, however, no universal standard for the size of the gap; while most experts would consider a 2–3% interspecific difference as adequate, others deem an order of magnitude difference necessary (Hebert et al., 2003b; Lobo et al., 2016). Thus, each taxon should be considered within its own context as some taxa may display greater inter-species variation than others.

For phylogenetic analysis it is standard practice to use more than one marker as different markers evolve at different rates in different taxa and so the COI marker cannot provide the same insight for all taxa (Vences et al., 2005). The 16S mitochondrial marker has also been used as an effective marker and has been popularly used in phylogenetic studies (Budaeva et al., 2016; Novo et al., 2010; Patwasdhan et al., 2014; Pires et al., 2010; Simon et al., 2019b; Struck et al., 2006; Vences et al., 2005; Zanol et al., 2010; Zhou et al., 2010). Furthermore, there are several COI and 16S sequences available in public sequence libraries allowing for better comparison among *Diopatra* species. COI and 16S are linked as both are mitochondrial genes. Thus, they only provide information on the maternal line of evolution. Ideally, a marker of nuclear origin would also be amplified. However, the applicable sequences available for comparison are currently limited to mitochondrial markers.

### *2.1.4 Aims and hypothesis*

The main aim of this study was to identify the *Diopatra* species used as bait in the Knysna and Swartkops estuaries, using morphological and molecular techniques. I hypothesised that a single species occurs in both estuaries, and this species is not reciprocally monophyletic with *D. neapolitana* and will instead represent an indigenous but previously undescribed species.

## 2.2 Materials and Methods

### *2.2.2 Specimen collection and storage*

As permitted by sampling permit RES2017-27 issued by the Department of Agriculture, Forestry and Fisheries to Prof. Carol A. Simon; a minimum of 20 animals per site were collected from the Knysna Estuary (34°03'37.4"S 23°02'52.5"E) in Knysna and the Swartkops Estuary (33°51'37.9"S 25°37'03.9"E) in Port Elizabeth using the same methods used by bait collectors. This involved pushing a thin wire with a hooked tip into the burrow, turning it a few times to hook the worm and extracting by slowly pulling out the wire. After harvesting, specimens were kept alive in tanks with aerated fresh seawater, for no longer than 16 hours, before euthanasia.

Animals were anaesthetized in a 7% Magnesium Chloride solution in tap water after which specimens were photographed. Close-up photos of anterior and mid-sections of live worms were taken using a Samsung Galaxy Note 4 with LIEQI macro lens attachment for mobile telephones. A section of the mid body was removed and stored directly in 96% ethanol for molecular analysis. The rest of the animal was fixed in 4% seawater formalin and stored in 70% ethanol for future reference. All formalin-fixed specimens will be lodged at the IZIKO South African Museum, Cape Town.

### *2.2.3 DNA extraction, sequencing*

DNA was isolated from sampled tissues using Quick-DNA miniprep plus kit (Zymo Research) following the stipulated protocol. A small piece of preserved tissue was cut from the worm and washed in distilled water to remove the ethanol. This was done to prevent the inhibition of Proteinase-K by excess ethanol. Care was taken to remove as much gut contents from sample material as possible to minimize risk of contamination. Tissue was cut into smaller pieces to ensure maximum DNA yield. During the final elusion step, at least 50µl elusion buffer were left to incubate for about 5 minutes at room temperature and then centrifuged at high speed for 30 seconds for the elution of DNA. This step was repeated up to two more times to extract all possible DNA.

To check for successful extraction a 5µl sample of each extracted elusion was loaded into a 1% Agarose gel together with 1µl gel loading dye batter (Thermo Scientific™) for electrophoresis at 100V for 90 minutes. Loading dye batter consisted of 30ml glycerol, 0.250g bromophenol blue and 0.250g xylene cyanol and diluted with distilled water to 100ml. Images of gels were taken using Labnet Enduro™ GDS imaging system. The DNA was then stored in -80° freezer until needed for Polymerase Chain Reaction (PCR).

Genomic DNA was amplified using PCR. Primers are listed in the Appendix. The total PCR volume of 25µl contained 12.5µl of EconoTaq® PLUS GREEN 2X Mastermix (Lucigen™), 8.5µl of Ambion Nuclease-free water (Invitrogen™), 0.5µl of forward and reverse primers at 10µM concentration each, 1µl bovine serum albumin (BSA) and 2µl of template DNA. An Applied Biosystems GeneAmp® PCR system 2700 was used for PCR and cycle conditions are in the Appendix. PCR products were stored in a refrigerator at 12 °C.

PCR products were run on a 1% agarose gel on 100V for 90mins using 2–5µl of PCR product with 1µl gel loading dye batter as described above. Images of gels were taken using Labnet Enduro™ GDS imaging system. Finally, PCR products were sequenced at the Central Analytical Facility of Stellenbosch University. Sequence data will be deposited on Genbank and Barcode of Life Database (BOLD).

### *2.2.4 Genetic analysis*

Sequences were processed and trimmed in BioEdit v.7.2.6 (Hall, 1999) and aligned with ClustalX® v.2.1 (Larkin et al., 2007). Trimmed sequences were BLASTed on Genbank to look for sequence similarity and to confirm the authenticity of the data. Comparative sequences were downloaded from BOLD for the COI marker and from Genbank for the 16S marker. BOLD is a specialized database for the COI sequences which is used as a barcoding reference library. Thus, it has stricter sequence uploading rules and therefore more reliable sequences. Outgroups were chosen from the BLAST results as the closest matching taxa that is in a different genus but same family. Downloaded sequences included 107 sequences from eight species for the

COI marker and 14 sequences from eight species for the 16S marker (downloaded sequence information in the Appendix). After the addition of the downloaded sequences both datasets were aligned and trimmed again if necessary, before further analysis.

Intra-species sequence diversity and inter-species sequence divergences were determined in MEGA X® ver. 10.0.5 (Kumar et al., 2018) using uncorrected p-distances for both markers. Species delimitation was analysed using the automatic barcode gap discovery (ABGD) method (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) with Pmin set to 0.001, Pmax set to 0.019 and barcode gap threshold (X) set to 3 and ran for 40 steps. The optimal models that described molecular evolution of both markers were determined in MEGA X® (Kumar et al., 2018) using the corrected Akaike Information Criterion (AICc) (Akaike, 1973; Burnham and Anderson, 2004). Maximum likelihood trees were constructed in MEGA X® (Kumar et al., 2018) and node support calculated with 10 000 bootstrap iterations. Bayesian inference trees were generated with the Bayesian Evolutionary Analysis by Sampling Trees (BEAST) package (Suchard et al., 2018) using a strict clock with a clock rate of 0.011 (Chevaldonné et al., 2002; Jolly et al., 2006; Nygren, 2014) for 10 000 000 chains and discarding the first 25% of trees generated as burn-in.

### *2.2.5 Morphological analysis*

Permanent slides were prepared of individual segments to observe chaetal structure. This was done by mounting a thin section of representative segments from the anterior, mid- and posterior body on a microscope slide using mounting fluid and sealing with clear nail polish. Whole preserved animals and sections were examined on dissecting (Leica MZ 7.5) and light (Leica DM1000) microscopes and images were captured using a Leica EC3 microscope camera and the Leica Application Suite EZ (LAS EZ) software. Live animals were photographed using a Samsung Galaxy S6 smartphone.

For scanning electron microscopy, specimens were dehydrated according to a protocol developed by Dr L. M. Joubert (Central Analytic Facility, Stellenbosch University); two washes in 100% ethanol of 10 minutes each, then one wash in a 1:1 mixture of 100% ethanol and Hexamethyldisalazane (HMDS) for 15 minutes, and finally two washes in HMDS for 30 minutes each. The HMDS was then poured off and the specimens left overnight for residual HMDS to evaporate off. Specimens were sputter-coated with gold palladium and viewed on a Zeiss Merlin scanning electron microscope at the Stellenbosch University Central Analytical Facility.

The following diagnostic features were considered: the arrangement and structure of branchiae, number of rings of the ceratophores of the antennae, length of antennae, presence and structure of pseudo-compound hooks in anterior chaetigers, serrated blades, number of teeth on comb chaetae, general colouration and pigmentation patterns and size of the animal. Tube structure was also described. Specimens were identified using published descriptions or identification keys for species of this genus (Arias et al., 2016; Budaeva and Fauchald, 2011; Day, 1967; Paxton, 1979).

# 2.3 Results

## *2.3.1 Molecular results*

### *2.3.1.1 PCR yield*

Extraction, PCR and sequencing yielded 15 COI sequences from Knysna specimens and ten from Swartkops specimens. Amplification of 16S marker yielded 16 sequences from Knysna specimens and 19 from Swartkops specimens. After alignment and trimming the COI dataset contained 547 base pairs and the 16S dataset had 483 base pairs.

### *2.3.1.2 Intra-specific sequence diversity and inter-specific sequence divergence*

Intra-specific COI sequence are summarised in *Table 2.1*. Mean intra-specific distances for all species were under 0.5% and the maximum range was 1.9% for *Diopatra neapolitana*. Inter-specific COI sequence divergences are summarised in *Table 2.2*. The lowest sequence divergence of 0.3% was between all RSA *Diopatra* sp. and both *Diopatra aciculata* and *Diopatra dentata* Kinberg, 1865. The divergence between these three were all 4.5% from *D. neapolitana*. The largest distance of 22.4% was between RSA *Diopatra* sp. and *Diopatra ornata*. Excluding *D. aciculata* and *D. dentata* the rest of the identified *Diopatra* species all had interspecific distances between 16.0% – 22.4%.

Mean intra-specific distances for 16S could only be calculated for four species as the remaining species were only represented by single sequences. There was 0.1% intra-specific sequence diversity among RSA *Diopatra* sp. and 0.0% within each *D. neapolitana* and *Diopatra tubercalantennata* Budaeva & Fauchald, 2008 and finally, 0.2% within *Diopatra sugokai*. Inter-specific 16S sequence divergences are summarised in *Table 2.3*. The smallest sequence divergence between species was between *Diopatra* sp. and both *D. aciculata* and *D. dentata* at

0.1%. The largest divergence of 22.3% was between *D. ornata* Moore, 1911, and *Diopatra micrura* Pires, Paxton, Quintino & Rodrigues, 2010. Excluding *D. aciculata* and *D. dentata*, the rest of the identified *Diopatra* species all had interspecific divergences between 9.9 – 22.3%.

*Table 2.1: Estimated evolutionary sequence diversity within species for the COI marker. Number of base substitutions per site is shown in the diversity column with estimated standard errors in second column. RSA Diopatra includes all specimens from Knysna and Swartkops estuaries.*

| | **Diversity** | **Standard error** | **Range** |
|---|---|---|---|
| **RSA *Diopatra*** | 0.003 | 0.002 | 0.006 |
| ***D. dentata*** | N/A | N/A | N/A |
| ***D. aciculata*** | N/A | N/A | N/A |
| ***D. ornata*** | N/A | N/A | N/A |
| ***D. micrura*** | 0.002 | 0.002 | 0.002 |
| ***D. marocensis*** | 0.004 | 0.001 | 0.015 |
| ***D. cuprea*** | 0.002 | 0.002 | 0.004 |
| ***D. neapolitana*** | 0.001 | 0.000 | 0.019 |

*Table 2.2: Estimated evolutionary sequence divergence between species at the COI marker. Number of base substitutions per site is shown below the diagonal with estimated standard errors above the diagonal. Analysis was conducted using uncorrected p-distances in MEGA X. RSA Diopatra includes all specimens from Knysna and Swartkops estuaries. Eunice chicasi is the outgroup.*

| | **RSA *Diopatra*** | ***D. aciculata*** | ***D. dentata*** | ***D. neapolitana*** | ***D. micrura*** | ***D. marocensis*** | ***D. cuprea*** | ***D. ornata*** | ***E. chicasi*** |
|---|---|---|---|---|---|---|---|---|---|
| **RSA *Diopatra*** | | 0,001 | 0,001 | 0,008 | 0,016 | 0,016 | 0,017 | 0,017 | 0,018 |
| ***Diopatra aciculata*** | 0,003 | | 0,003 | 0,008 | 0,017 | 0,016 | 0,017 | 0,017 | 0,018 |
| ***Diopatra dentata*** | 0,003 | 0,005 | | 0,008 | 0,016 | 0,016 | 0,017 | 0,017 | 0,018 |
| ***Diopatra neapolitana*** | 0,045 | 0,045 | 0,045 | | 0,016 | 0,015 | 0,017 | 0,017 | 0,018 |
| ***Diopatra micrura*** | 0,174 | 0,176 | 0,173 | 0,160 | | 0,014 | 0,016 | 0,015 | 0,017 |
| ***Diopatra marocensis*** | 0,189 | 0,190 | 0,188 | 0,183 | 0,166 | | 0,016 | 0,015 | 0,017 |
| ***Diopatra cuprea*** | 0,198 | 0,201 | 0,196 | 0,196 | 0,188 | 0,193 | | 0,016 | 0,017 |
| ***Diopatra ornata*** | 0,224 | 0,223 | 0,225 | 0,208 | 0,171 | 0,190 | 0,187 | | 0,018 |
| ***Eunice chicasi*** | 0,255 | 0,255 | 0,255 | 0,251 | 0,245 | 0,230 | 0,233 | 0,262 | |

*Table 2.3: Estimated evolutionary sequence divergence between species at the 16S marker. Number of base substitutions per site is shown below the diagonal with estimated standard errors above the diagonal. Analysis was conducted using uncorrected p-distances in MEGA X. RSA Diopatra includes all specimens from Knysna and Swartkops estuaries. Eunice norvegica is the outgroup.*

| | **RSA *Diopatra*** | ***D. aciculata*** | ***D. dentata*** | ***D. neapolitana*** | ***D. micrura*** | ***D. marocensis*** | ***D. sugokai*** | ***D. tuberculantennata*** | ***D. ornata*** | ***E. norvegica*** |
|---|---|---|---|---|---|---|---|---|---|---|
| **RSA *Diopatra*** | | 0,001 | 0,001 | 0,003 | 0,014 | 0,015 | 0,017 | 0,018 | 0,017 | 0,019 |
| ***Diopatra aciculata*** | 0,001 | | 0,002 | 0,003 | 0,014 | 0,015 | 0,017 | 0,018 | 0,017 | 0,019 |
| ***Diopatra dentata*** | 0,001 | 0,002 | | 0,003 | 0,014 | 0,015 | 0,017 | 0,018 | 0,018 | 0,019 |
| ***Diopatra neapolitana*** | 0,005 | 0,004 | 0,006 | | 0,014 | 0,015 | 0,017 | 0,018 | 0,017 | 0,019 |
| ***Diopatra micrura*** | 0,107 | 0,106 | 0,108 | 0,108 | | 0,016 | 0,018 | 0,018 | 0,019 | 0,020 |
| ***Diopatra marocensis*** | 0,129 | 0,128 | 0,131 | 0,133 | 0,146 | | 0,017 | 0,018 | 0,019 | 0,019 |
| ***Diopatra sugokai*** | 0,180 | 0,180 | 0,182 | 0,180 | 0,201 | 0,188 | | 0,018 | 0,014 | 0,021 |
| ***Diopatra tuberculantennata*** | 0,197 | 0,198 | 0,195 | 0,202 | 0,188 | 0,186 | 0,182 | | 0,017 | 0,021 |
| ***Diopatra ornata*** | 0,184 | 0,183 | 0,186 | 0,183 | 0,223 | 0,203 | 0,099 | 0,176 | | 0,020 |
| ***Eunice norvegica*** | 0,229 | 0,228 | 0,230 | 0,232 | 0,230 | 0,246 | 0,287 | 0,279 | 0,281 | |

#### *2.3.1.3 Species delimitation*

The COI distance analysis showed inter-specific divergences between *Diopatra* sp., *D. aciculata*, *D. dentata* and *D. neapolitana* were at least three times smaller than other inter-specific divergences of species analysed. Thus, only these four species were considered for species delimitation analyses. Using the COI dataset, the ABGD method consistently returned two putative species groups (PSGs). The first (PSG-1) contained *Diopatra* sp., *D. aciculata* and *D. dentata* with a maximum sequence diversity between these sequences of 0.6%. The second group (PSG-2) contained all of the *D. neapolitana* sequences and had a maximum intraspecific sequence diversity of 1.9%. Thus, the sequence divergence is great enough to separate *D. neapolitana* from *Diopatra* sp. but could not separate *Diopatra* sp. from *D. aciculata* and *D. dentata*.

#### *2.3.1.4 Trees*

Model selection for COI sequences yielded the general time reversible (GTR) model with gamma distribution and invariant sites as the best suited with a 5839.998 corrected Akaike information criteria (AICc) score. The maximum likelihood (ML) tree shows *D. aciculata* and *D. dentata* as part of a monophyletic clustering with the Knysna (KN) and Swartkops (PE) specimens which supports the grouping of these into PSG-1 (*Figure 2.1*). PSG-1 and PSG-2 show well supported separation into two sister clades. Bayesian analysis showed significant support for most nodes including separating of PSG-1 and PSG-2 (*Figure 2.1*). BEAST analysis of the COI sequences revealed PSG-1 only diverged from *D. neapolitana* about 2.28 million years ago whereas other species in the genus diverged 8.2 – 12.2 million years ago.

Model selection for 16S sequences yielded the GTR model with invariant sites as the best suited with a 4058.582 corrected Akaike information criteria (AICc) score. The maximum likelihood (ML) tree similarly shows support for the separation of PSG-1 and PSG-2 though the separation is not as pronounced (*Figure 2.2*).

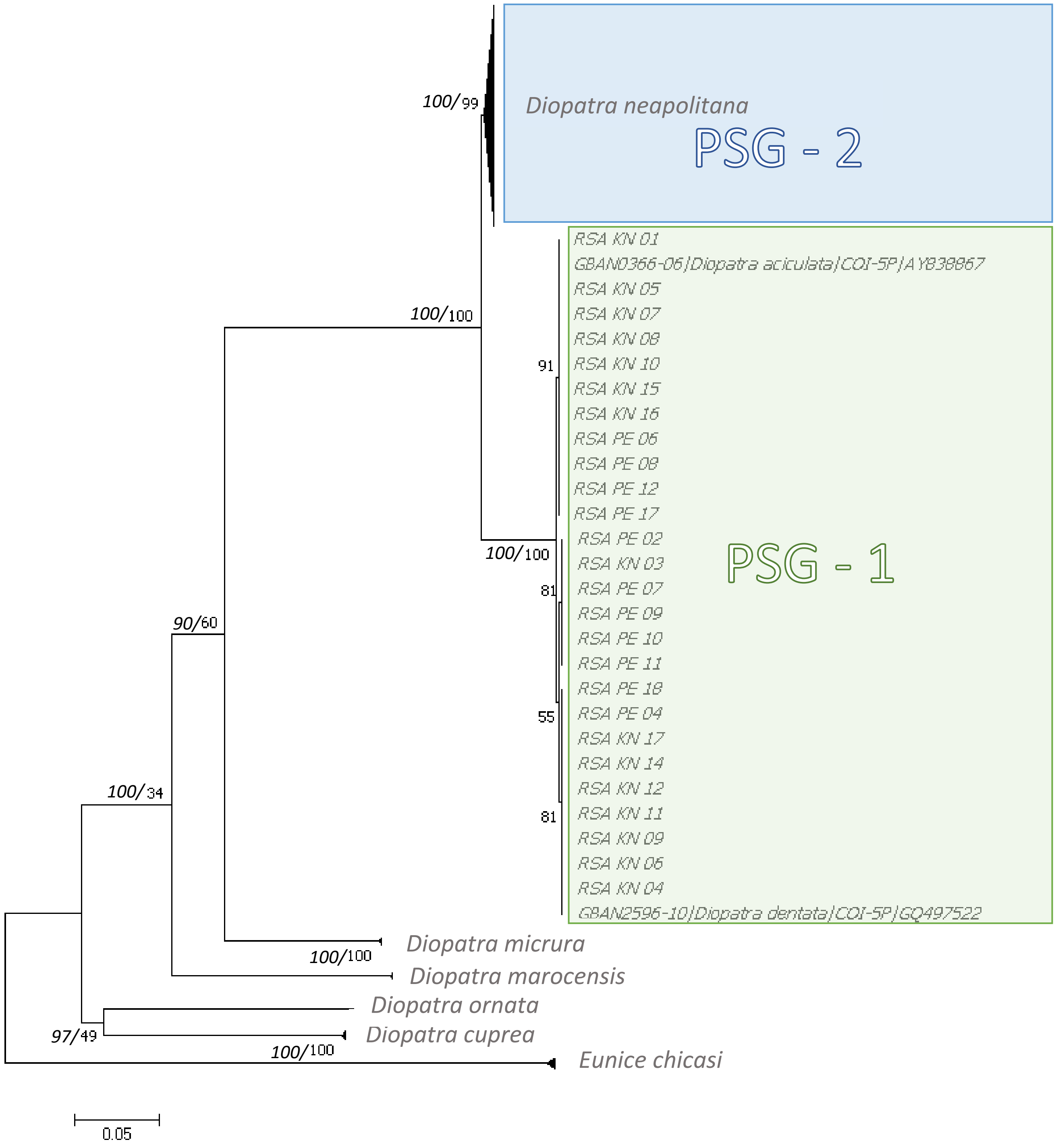


*Figure 2.1: COI maximum likelihood tree generated in MEGA X with Bayesian inference support added to nodes. South African samples together with Diopatra aciculata and Diopatra dentata samples from Australia (grouped as PSG-1 through species delimitation) sorts as a sister clade to Diopatra neapolitana (PSG-2), with high bootstrap and posterior probability (number in italics) support. Diopatra cuprea, Diopatra marocensis, Diopatra micruara, PSG-1 and PSG-2 are well supported as monophyletic groups by Bayesian inference. Eunice chicasi was the chosen outgroup. RSA = Republic of South Africa; KN = Knysna Estuary; PE = Swartkops Estuary.*

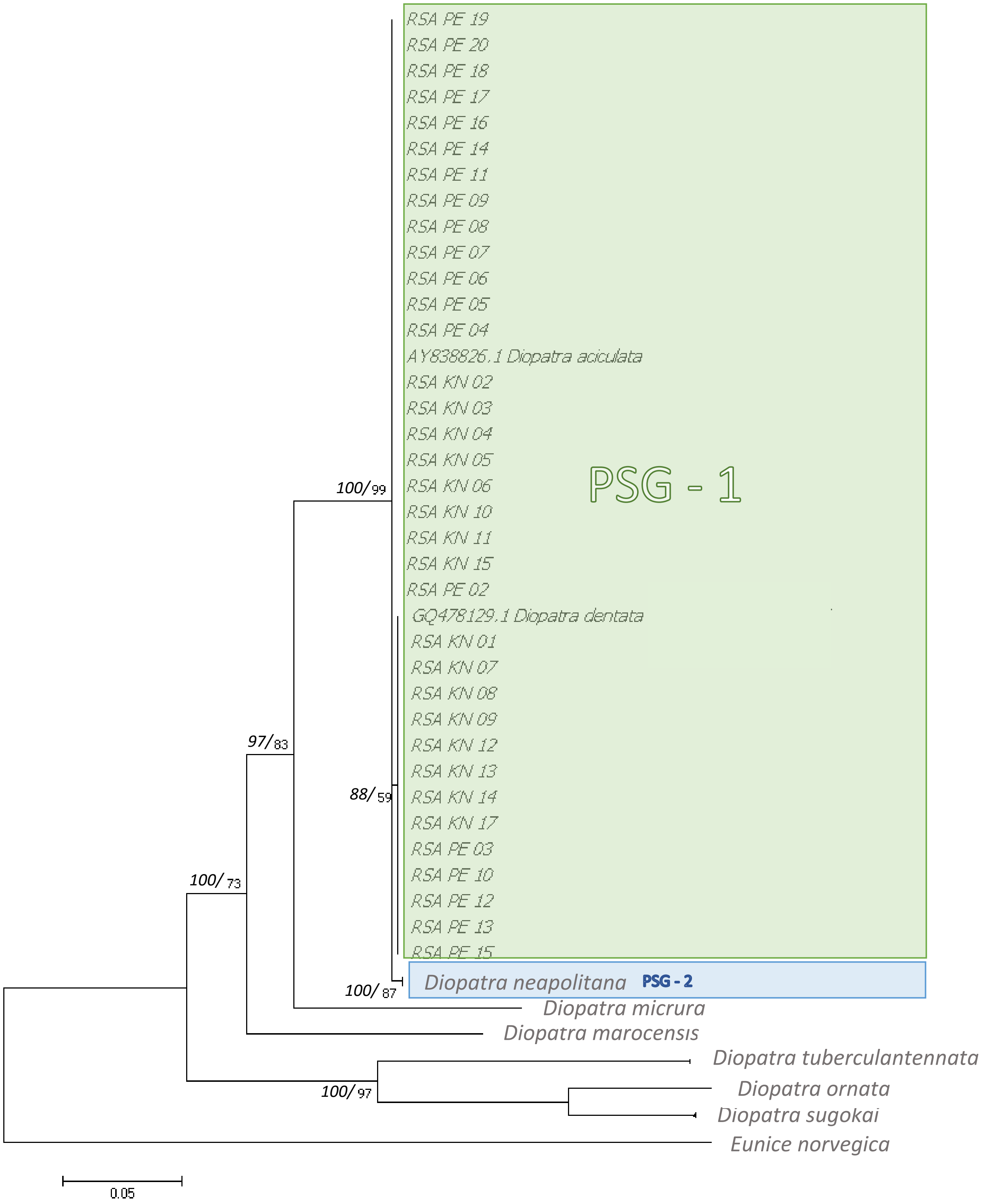


*Figure 2.2: 16S maximum likelihood tree generated in MEGA X with Bayesian inference support added to nodes. South African samples together with Diopatra aciculata and Diopatra dentata samples from Australia (grouped as PSG-1 through species delimitation) sorts as a monophyletic clustering with Diopatra neapolitana with high bootstrap and posterior probability support. Within this clustering D. neapolitana is supported as monophyletic group though PSG -1 is not. Eunice norvegica was the chosen outgroup.*

## *2.3.2 Morphological results*

### *2.3.2.1 Systematics*

Family **Onuphidae** Kinberg, 1865

Subfamily **Onuphinae** Kinberg, 1865

Genus ***Diopatra*** Audouin & Milne Edwards, 1833

Species ***Diopatra aciculata*** Knox & Cameron, 1971

(Figs 2.3 – 2.9)

| | | |
|---|---|---|
| As | *D. aciculata* | Knox & Cameron, 1971:31, figs. 20 – 25 |
| | | Day & Hutchings, 1979:118 |
| | | Paxton 1986:19,38, fig. 12 |
| | | Paxton 1993:144, figs. 6, 31 – 35 |
| As | ? *D. neapolitana* | Macnae, 1956:42 (Swartkops, Eastern Cape) |
| | | Macnae, 1957:372 (Swartkops, Eastern Cape) |
| | | Day, 1960:341, fig. 9 a – g |
| | | Day, 1967:413, fig 17.10 e – k |
| | | van der Westhuizen & Marais, 1977:501 (Swartkops, Eastern Cape) |
| | | Mclachlan et al., 1984:56 (Sundays River, Eastern Cape) |
| As | ? *D. cuprea* | Branch et al., 2016:68, fig. 26.3 |
| As | *Diopatra* sp. | Napier et al., 2009:300 (Knysna, Western Cape) |
| | | Allanson et al., 2016:53 (Knysna, Western Cape) |
| | | Simon et al., 2019 (Knysna, Western Cape) |

### *2.3.2.2 Material examined*

Non-type material: 16 specimens, incomplete, Knysna Estuary (34°04'17.6"S 23°03'30.5"E), Knysna, Western Cape, South Africa, coll. H. van Rensburg, 20 February 2017; 15 specimens, incomplete, Swartkops Estuary (33°52'00.7"S 25°36'42.5"E), Port Elizabeth, Western Cape, South Africa, coll. H. van Rensburg, 30 March 2017.

### *2.3.2.3 Description*

All specimens large adults. Maximum live length at least 60cm, maximum preserved width excluding parapodia was 11.4 mm at chaetiger 10. Longest preserved anterior fragment 158mm with 131 chaetigers. Preserved body colour pale to dark brown, anterior regions darker. In live specimens, anterior often iridescent dark blue-green (*Figure 2.3 A*) otherwise darker brown (*Figure 2.3 B*). Inside of tentacular cirri brown (*Figure 2.3 A, B*), pigment lost during preservation. The inner surface of ceratophore rings brown (*Figure 2.3 B, C*), live specimens with small white spots irregularly spaced on antennae styles (*Figure 2.3 B*). Single short black middorsal bar on anterior margin of each chaetiger in branchial region (*Figures 2.3 A, B, 2.7 A*), difficult to see on live specimens with darker anterior regions. Ceratophore and mid-dorsal pigmentations maintained during preservation. Median and posterior segments pale brown to cream in colour.

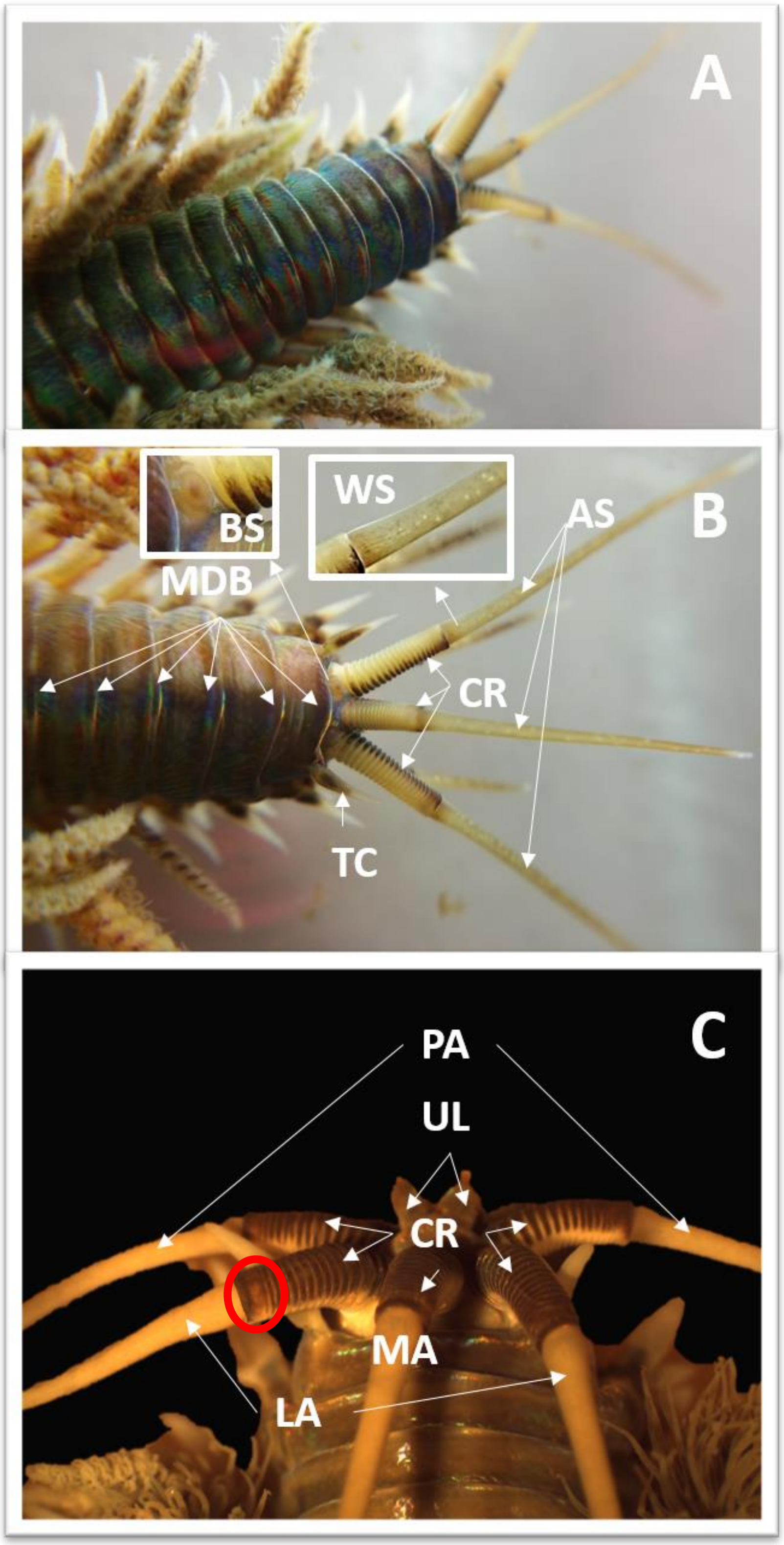


*Figure 2.3: Anterior regions of (A) A darker and (B) A lighter live Diopatra aciculata and one (C) preserved specimen. Mid-dorsal bars (MDB) very difficult to see in live individuals, especially darker specimens. Red circle shows longer distal ring of ceratophore. Live animals photographed with Samsung Galaxy S6, preserved animal photographed using EC3 microscope camera attachment. AS = Antennae styles; BS = Brown spot in center of nuchal organ; CR = Ceratophore rings; LA = Lateral antennae; MA = Median antennae; MDB = Mid-dorsal bar; PA= Palps; TC = Tentacular cirri; UL = Upper lips; WS = White spots.*

Prostomium extended anteriorly with two frontal subulate lips, cirriform, smooth and tapering. Upper ventral lips have distal lobes (*Figure 2.3 C*). Three occipital antennae and two ventro-lateral palps mounted on 12 – 20 ceratophore rings, rings equally sized proximally with a longer distal one (*Figure 2.3*). Antennae styles smooth, long, slender, tapering to blunt end, reaching to chaetiger 9 – 15 (median) or 13 – 17 (lateral). Rows of interrupted sensory buds on antennae (*Figure 2.4 B, C*), buds flattened, circular and irregularly spaced (*Figure 2.4 C, D*), serous gland pores gradually disappearing distally. Nuchal groove almost completely circular, opening toward anterior (*Figure 4 A*), some live specimens have small brown dot present in centre of nuchal organ (*Figure 2.3 B*).

Peristomium as long as succeeding chaetiger, two widely spaced tentacular cirri 1.5 – 2 times length of the peristomium mounted on anterior margin, laterally to posterior occipital antennae (*Figure 2.3 A, B; 2.4, A*).

Three or four anterior chaetigers abranchiate; parapodia larger than on branchiate chaetigers, directed antero-ventrally; dorsal cirri elongated, slender, tapering, longer than ventral cirri (*Figure 2.5 A*). Pre-chaetal lobes rounded, post-chaetal lobe long and subulate (*Figure 2.5 A*). Pseudo-compound hooks distally uni- or bi-dentate, covered in pointed hood.

Unmodified parapodia usually from fifth chaetae. Dorsal cirri slender, elongated, longest in branchial region, similar in length to branchiae. Ventral cirri pad-like (*Figure 6*). Post-chaetal lobe elongated, triangular (*Figure 2.6 B, C*). Pre- and post-chaetal lobes gradually become smaller toward posterior. Pre-chaetal lobe disappears but post-chaetal lobe remains distinct. Limbate and pectinate chaetae present with pectinate chaetae having 5–10 teeth (*Figure 2.5 B, C*), one lateral tooth often thicker than the rest. Middle region of chaetae become serrated towards end of branchial region (*Figure 2.5 D*). Chaetal serrations remain to posterior. Two bidentate subacicular hooks from chaetiger 19–23 onwards (*Figure 2.6 B, C*).

Spiraled branchiae from fourth or fifth chaetiger, up to 20 brachial whorls arranged close together, brush-like or bushy appearance tapering towards tips (*Figure 2.7*). After 20–40 segments, branchiae gradually shorten and whorls reduce until only a single filament remains, terminate shortly thereafter (*Figure 2.7*).

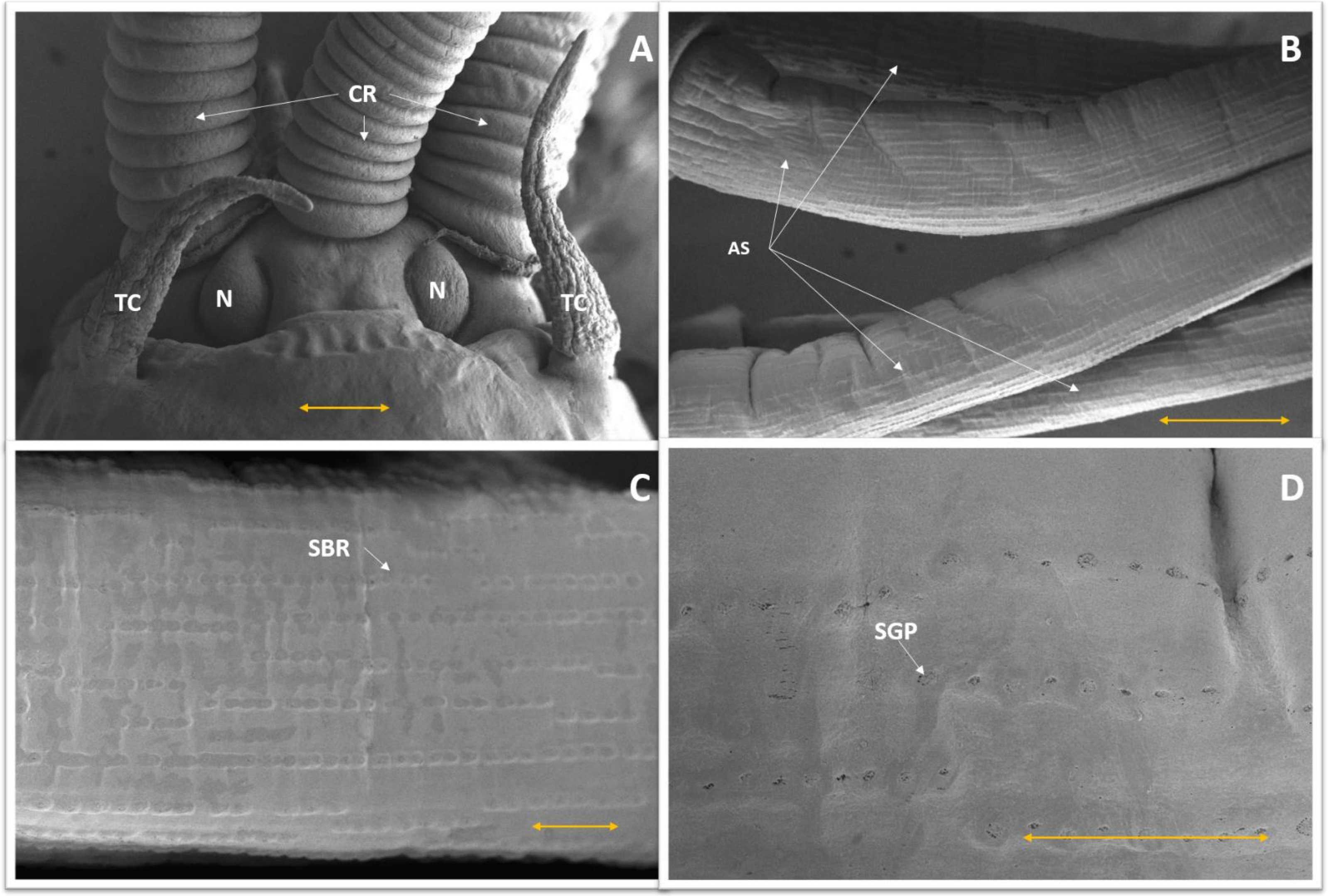


*Figure 2.4: Scanning electron micrographs of Diopatra aciculata collected in Knysna and Swartkops estuaries showing (A) Nuchal grooves and tentacular cirri; (B) Irregular rows of sensory buds on antenna styles; (C) Mid antenna area with fewer serous gland pores in sensory buds; (D) Closer view of serous gland pores in sensory buds. Yellow scale bars denote: A) 500µm; B) 500µm; C) 100µm and D) 100µm. AS = Antennae styles; CR = Ceratophore rings; N = Nuchal organ encircled by nuchal groove; SBR = Sensory bud rows; SGP = Serous gland pores; TC = Tentacular cirri.*

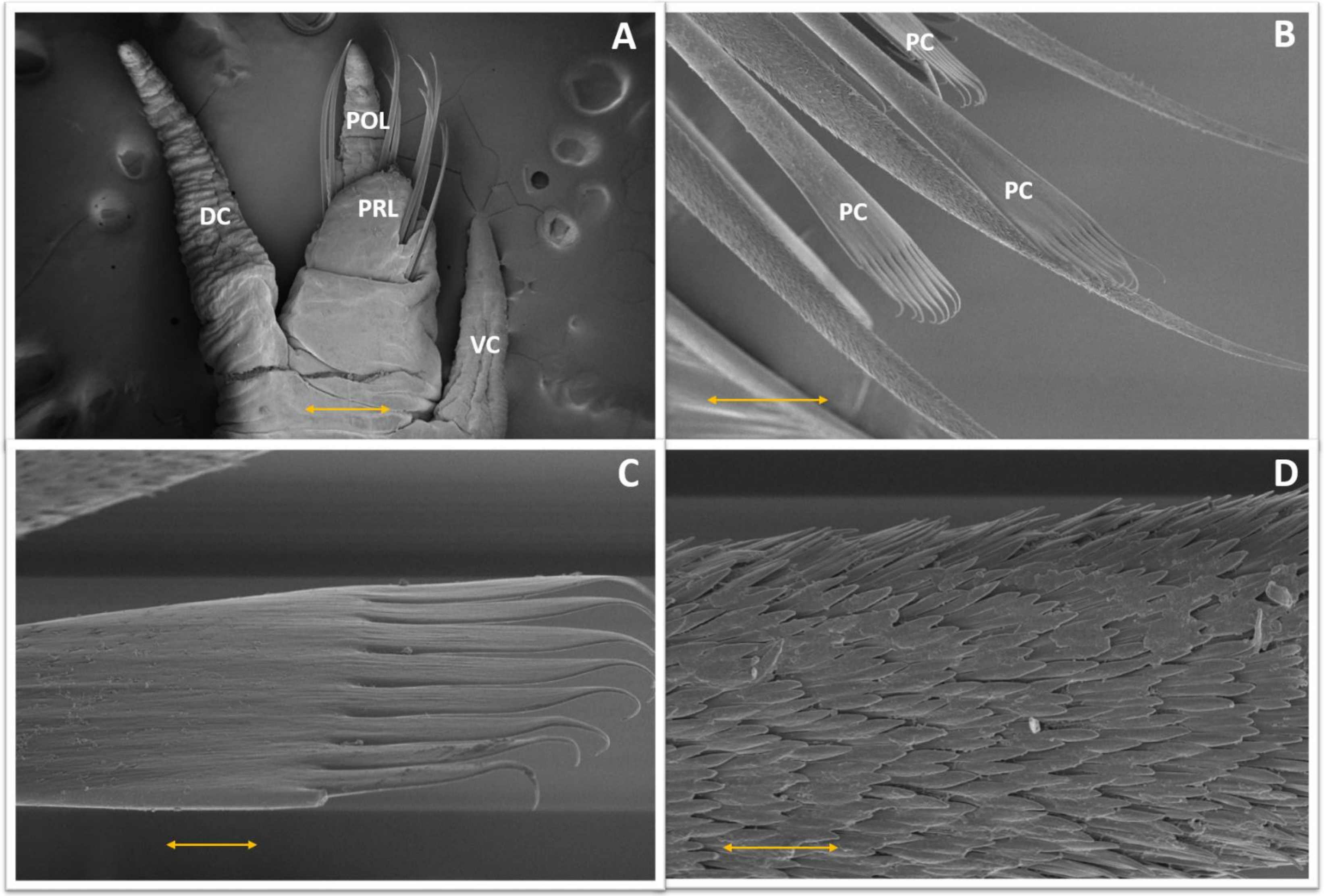


*Figure 2.5: Scanning electron micrograph of Diopatra aciculata collected in Knysna and Swartkops estuaries showing (A) Modified parapodia; and (B–D) chaetae. (A) Ventral cirri elongated and subulate; (B) Pectinate cheatae with 5–10 teeth with hooked tips; (C) Closeup of Pectinate cheatae; (D) Serrated surface of mid regions of cheatae. Yellow scale bars denote: A) 400µm; B) 50µm; C) 10µm and D) 5µm. DC = Dorsal cirri; PC = Pectinate chaetae; POL = Post-chaetal lobe; PRL = Pre-chaetal lobe; VC = Ventral cirri.*

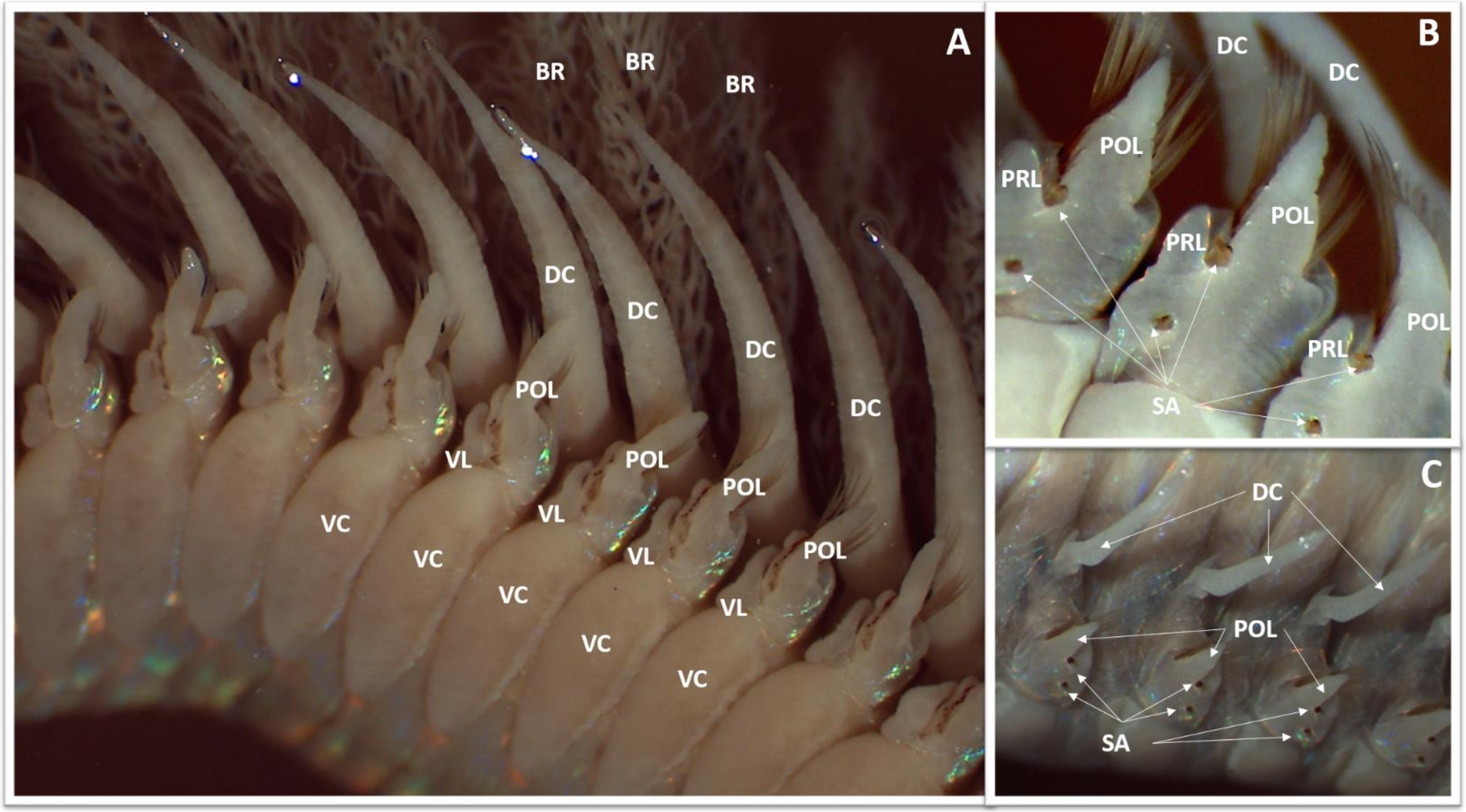


*Figure 2.6: Progression of parapodia of Diopatra aciculata collected in Knysna and Swartkops estuaries showing (A) Latero-ventral view of branchial region with very long dorsal cirri, pad-like ventral cirri and presence of a ventral lobe on parapodia and lack of subacicular hooks. (B) Ventral view towards end of branchial region, longer dorsal cirri visible in background, appearance of subacicular hooks. (C) Lateral view past branchial region dorsal cirri become reduced, ventral lobe and pre-chaetal lobe disappear, subacicular hooks remain. BR = branchiae; DC = Dorsal cirri; POL = post-chaetal lobe; PRL = Pre-chaetal lobe; SA = subacicular chaetae; VC = Ventral cirri; VL = ventral lobe.*

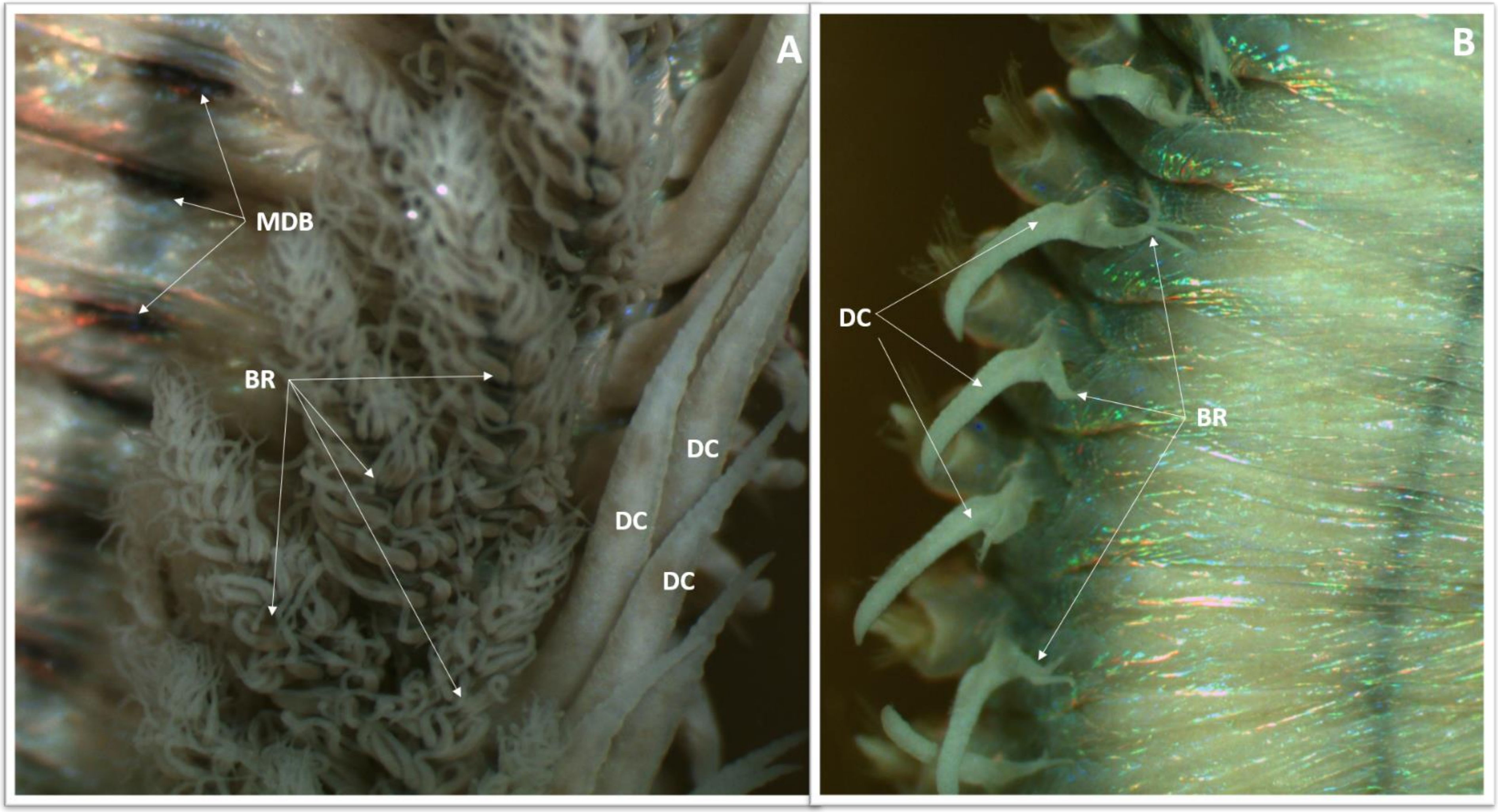


*Figure 2.7: Progression of branchiae from dorsal view of Diopatra aciculata collected in Knysna and Swartkops estuaries showing (A) Main branchial region where branchiae has several whorls, large and bushy in appearance, dorsal cirri here characteristically long, mid-dorsal bar clearly present in preserved specimens. (B) Shows branchiae reducing, branchiae eventually disspear, absence of mid-dorsal bar. BR = Branchiae; DC = Dorsal cirri; MDB = Mid-dorsal bar.*

### *2.3.2.4 Taxonomic remarks*

The specimens from South Africa resemble both *D. aciculata* and *D. neapolitana* with regards to the number of chaetigers with subulate ventral cirri (four), branchiae starting on chaetiger 4–5, circular sensory buds, horseshoe to almost complete circular shape of nuchal groove, number of rings on ceratophores (10–18), maximum number of brachial whorls (20), pectinate chaetae teeth number (5–10) and presence of uni- and bi-dentate pseudo-compound hooks.

The specimens examined here conformed best with the descriptions of *D. aciculata* by Paxton (1993, 2016). Similar features included width at tenth chaetiger (11.5mm) and lengths of palps, median and lateral antennae reaching chaetigers 2–5, 8–15 and 8–15 respectively. However, the adult specimens from South Africa differed from *D. aciculata* in that they never presented with tridentate hooks (Paxton, 1993:146 fig. 34) and were often twice as long as the 340mm length reported by Paxton (1993, 2016) though an accurate measure of total length of specimens were difficult as no complete specimens could be collected. Tentacular cirri of South African specimens were 1.5 – 2 times longer than the peristomium, in accordance with Paxton (2016) rather than the longer 2 – 3 times length described in Paxton (1993).

The South African specimens differ from *D. neapolitana* in a few ways; they were 28 – 43% wider at 10th chaetiger and had longer palps, antennae and dorsal cirri (Arias et al., 2016). Furthermore, the branchial region terminated slightly before it did in *D. neapolitana*. The observed length of live specimens collected here agreed best with newer descriptions of *D. neapolitana* (Arias et al., 2016).

Morphologically *D. aciculata* and *D. neapolitana* have a lot in common and are exceedingly difficult to tell apart, although the longer dorsal cirri, wider bodies and longer antennae of *D. aciculata* may be the best morphological traits to use to tell the species apart. Molecular COI analysis is, however, the easiest method to differentiate between the two species.

### *2.3.2.5 Distribution*

Knysna and Swartkops estuaries.

### *2.3.2.6 Ecology*

Tubes lined with white parchment-like material. In sandy environments, tubes have protruding chimneys that are often bent horizontally (*Figure 2.8 A*). Chimneys are made of sand with a smooth texture and plant material and shell fragments usually embedded into the tube with larger shell pieces often found distally (*Figure 2.8 A*). Some chimneys had no plant or shell attachments and appeared smooth. In muddy or silty environments, openings of tubes are flush with sediment surface and off-white mucus lining clearly visible (*Figure 2.8 B*).

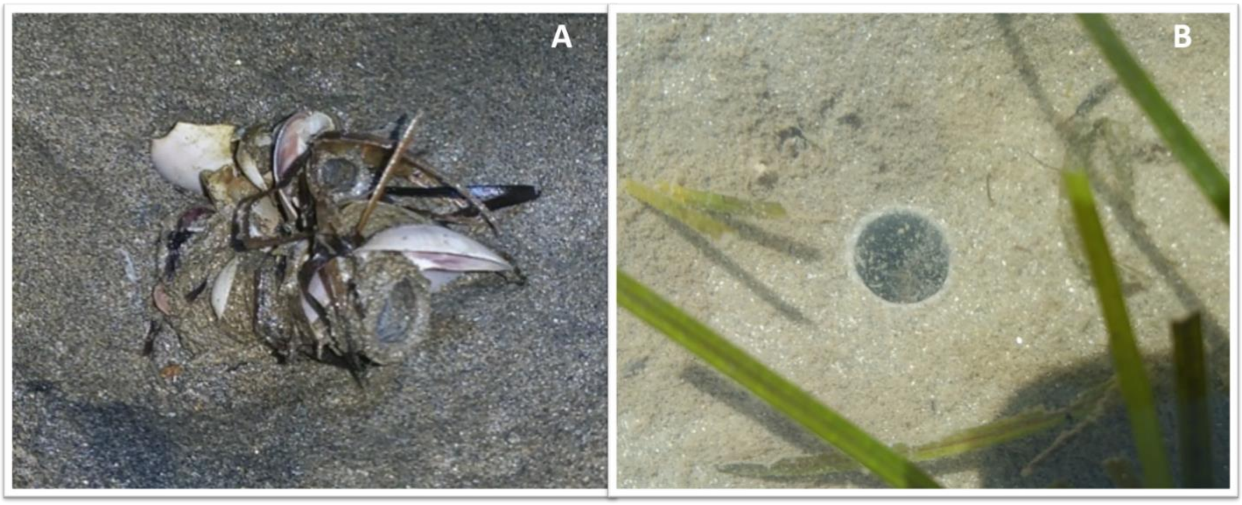


*Figure 2.8: Identification of Diopatra aciculata tubes. (A) In more sandy areas tubes protrude from substrate and are often bent in the direction of water flow with shell and plant fragments attached. (B) In areas with more muddy/silty substrates, often amongst seagrasses, tubes are flush with substrate but can be differentiated from other infauna by the presence of the off-white inner lining of the tube.*

## 2.4 Discussion

This study showed that the *Diopatra* species used as bait by fishermen in Knysna and Swartkops estuaries is a single, non-indigenous, species, *Diopatra aciculata*, that was originally described from Australia (see Knox and Cameron, 1971). This conclusion is based on both morphological and molecular data combined as both the morphological and molecular datasets alone are unclear. This was unexpected for two reasons. Firstly, given the history of pseudo-cosmopolitan species being re-described as indigenous species in South Africa, I expected *Diopatra* sp. to be an undescribed indigenous species previously misidentified as *Diopatra neapolitana*. Secondly, I expected that if the *Diopatra* sp. proved to be non-indigenous, it would be *D. neapolitana* from the Mediterranean.

### *2.4.1 D. aciculata or D. dentata*

Intra- and inter-specific distances for COI and 16S show the same trends; inter-specific distances between the South African *Diopatra* sp. and *D. aciculata* and *D. dentata* fall within the intra-specific distance among South African specimens for both markers, providing strong evidence that these are all a single species. The South African specimens examined in the current study strongly agree with the description of *Diopatra aciculata*, especially that provided by Paxton (2016) with regards to width and lengths of palps, median antenna, lateral antennae and dorsal cirri. By contrast, *D. dentata* also originally described from Australia, differs from *D. aciculata* as it is much shorter (max 245mm) and thinner (7mm width) and has shorter lateral antennae (reaching chaetiger 7–10) and much shorter dorsal cirri (only reaching 2nd – 3rd branchial whorl). Furthermore, *D. dentata* has more teeth on its pectinate chaetae (11 – 20) and no ventral lobe on the anterior parapodia. Thus, the inclusion of a sequence of *D. dentata* within the clade with *D. aciculata* most likely represents an error in identification or labelling (Zanol et al., 2010).

### *2.4.2 Comparison of D. aciculata and D. neapolitana*

Several researchers have noted the morphological similarity between *D. aciculata* and *D. neapolitana* (Dağli et al., 2005; Paxton, 2016, 1993; Rodrigues et al., 2009). *Diopatra aciculata* was first described in 1971 by Knox and Cameron though they only provided two distinguishing features that could be considered unique for the species. These were dorsal cirri that are as long as, or longer than, the branchial whorls and a knob-like secondary tooth on the pseudo-compound hooks. In 1993, Paxton noted only the longer dorsal cirri and smoother burrow tube of *D. aciculata* as distinctly different from *D. neapolitana* and suggested that the knob-like appearance of the secondary tooth on the pseudo-compound hooks, as described by Knox and Cameron (1971), was likely an optical illusion. From the current study I agree with Paxton (1993) on the knob-like secondary tooth and longer dorsal cirri in *D. aciculata*. However, I observed burrow chimneys covered in shells and plant material as well as some with no “decorations” that apear smooth. Furthermore, in other areas burrows did not have chimneys at all and were flush with the ground. Therefore, I’m not confident that this can be considered a diagnostic feature. I suspect that burrow chimneys are not built out from the ground but rather appear when sediment is moved through hydrodynamic forces as burrows appeared flush with sediment in areas with less tidal influence, more silty sediment or more vegetation. When sand moves and a bare tube is exposed it appears smooth until shell and plant material are added on, most likely to provide structural integrity. Later this will provide refugia for other infuanal or meifuana. Still, Paxton (1993) argued for keeping the two species separate until more evidence to the contrary is produced. Dağli et al. (2005) also commented on the morphological similarities of these two species and suggested molecular analysis be undertaken to reliably conclude the taxonomic validity of *D. aciculata* and molecular results in this study have confirmed that *D. aciculata* and *D. neapolitana* are separate but very similar species.

Rodrigues et al. (2009) were the first to molecularly compare *D. aciculata* and *D. neapolitana*. They compared a single sequence of *D. aciculata* to 45 sequences of *D. neapolitana* obtained from one site and found 5% and 1% distances between COI and 16S respectively. In this study with regards to COI, I compared 27 *D. aciculata* sequences from four sites to 80 *D. neapolitana* sequences from at least 17 sites and found that these differences first reported by Rodrigues *et al*. (2009) are consistent. The distances between these two species are an order of a magnitude greater than their respective intra-specific distances. However, the distance between *D. aciculata* and *D. neapolitana* is also an order of a magnitude lower than distances among other congenerics tested. But, there is no set gap threshold which defines when species are valid, although when using COI sequences to differentiate species most agree the inter-specific distance needs to be either a minimum of 2 – 3% higher or at least an order of magnitude greater than the intra-specific distances (Hebert et al., 2003b; Lobo et al., 2016). Therefore, the 4.6% inter-specific distance between *D. aciculata* and *D. neapolitana* reported here is at least 3% higher and an order of magnitude greater than the 0.6% intra-specific distance of *D. aciculata*. Furthermore, the ABGD species delimitation analysis and Bayesian analysis both support the existence of two separate species with PSG-1 representing *D. aciculata* and PSG-2 representing *D. neapolitana*. Finally, the BEAST timescales show the recent divergence of *D. aciculata* from *D. neapolitana* not overlapping with other species divergences. Thus, *D. aciculata* and *D. neapolitana* are closely related sister taxa that diverged more recently than any of the other *Diopatra* sequences considered here. It is therefore clear that *D. aciculata* is very closely related to, but a distinct species from, *D. neapolitana*.

### *2.4.3 Cryptic invasion of D. aciculata in South Africa*

A cryptic invasion may occur when a non-indigenous species is misidentified as an indigenous species (Morais and Reichard, 2018). The reason *D. aciculata* has never been reported from SA could be that it was misidentified as *D. neapolitana*, a pseudo-cosmopolitan species as opposed to an indigenous species. Certainly, when Day was identifying *Diopatra* from SA during the 1930s to 1960s period, *D. aciculata* had not been described yet so despite his reservations he reported *D. neapolitana*. This misidentification is understandable as the differences between *D. neapolitana* and *D. aciculata* are subtle. Furthermore, Day, as the leading authority on South African polychaetes at the time, likely influenced subsequent studies regarding the presence of *D. neapolitana* (e.g., Macnae, 1957, Napier et al. 2009). Indeed, even the hypothesis of this study was predicated based on Day's outputs, especially Day (1967) which is still a highly regarded taxonomic reference for polychaete taxonomists in South Africa (Griffiths et al., 2010).

This study provides the first record of *D. aciculata* as a non-indigenous species anywhere in the world. It is notable that despite all the work being conducted in Knysna Estuary, no study ever explored the identity of a large polychaete that builds very conspicuous burrows which had not been reported there before but has become increasingly popular as a bait species. However, the reason for this might be that *D. aciculata* closely resembles *D. neapolitana*, a species which has previously been described from SA (Day, 1967). Furthermore, Knysna falls within the supposed distribution range of *D. neapolitana* in SA (Branch et al., 2017) so the identity of the moonshine worm was never investigated as nobody thought its presence unusual.

This led to the cryptic invasion of *D. aciculata* and uncertainty of when the species arrived in the Knysna Estuary or how far it has spread into the estuary.

# Chapter 3: Opening a can of worms: distribution, densities and population estimate of *D. aciculata* in the Knysna Estuary.

## 3.1 Introduction

The Knysna basin supports substantial recreational and subsistence fisheries that are increasingly relying on polychaetes as bait as described in Chapter One (but see also Hodgson et al., 2000; Napier et al., 2009; Simon et al., 2019). As such, valid permit holders may collect up to ten polychaete and other marine worms for bait per day, depending on the species (Department of Agriculture Forestry and Fisheries, 2018). Apart from these general restrictions, the Knysna Estuary falls within a large protected area under the management jurisdiction of South African National Parks (SANParks). This estuary therefore has additional restrictions on activities, most of which apply to an area demarcated as an invertebrate reserve where swimming, fishing and bait collecting are prohibited with the aim to preserve a natural refuge for many fauna and flora, including invertebrate species harvested as bait (van der Walt et al., 1990).

### *3.1.1 Shift in study perspective*

In Chapter Two I demonstrated that a popular bait polychaete species known locally as the moonshine worm is an invasive species, *Diopatra aciculata,* originally described from Australia (Knox and Cameron, 1971; Paxton, 1993). Currently very little is known of the ecology or impact of the species in the Knysna Estuary. As my initial hypothesis predicted the identification of an indigenous species, this study began intending to understand the distribution and exploitation of the species in view of conservation. However, the perspective shifted to understanding the potential impact of a non-indigenous species with a view to management and potential extirpation. Invasive species are usually, but not always, considered major contributors to biodiversity loss (Charles and Dukes, 2007; Lovell et al., 2006; Lowe et al., 2000; Miehls et al., 2009; Mooney and Cleland, 2001; Pimentel et al., 2005; Vilà et al., 2011). However, in rare cases invasive species can facilitate improved fitness of some native species (Robinson et al., 2005; Rodriguez, 2006). Finally, invasive species can have no net negative or positive effects and considered neutral, at least as far as can be measured (Mabin, 2017).

### *3.1.2 Potential impacts of D. aciculata in Knysna Estuary*

Species of the genus *Diopatra* have been described as ecosystem engineers as they construct parchment-like burrows that may protrude from the sediment, altering the surrounding environment (Arias and Paxton, 2015; Berke et al., 2010; Harwell and Orth, 2001; Volkenborn et al., 2009). *Diopatra* burrows in particular are known to stabilize sediment (Bailey-brock, 1984; Luckenbach, 1986), facilitate the dispersal and growth of eelgrass and algae (Harwell and Orth, 2001; Thomsen and McGlathery, 2005), provide refuge to other invertebrates (Ban and Nelson, 1987) as well as increase richness and abundance of surrounding invertebrates and infauna (Thomsen et al., 2011; Woodin, 1981). Thus, if an ecosystem engineer such as *D. aciculata* invades an ecosystem, it may cause compounding problems through cascading effects on resident biota as the environment is altered (Crooks, 2002; Cuddington and Hastings, 2004; Volkenborn et al., 2009). However, it could also provide benefits within the Knysna Estuary such as facilitating the recovery of native eelgrass. Furthermore, *D. aciculata* could become a new important link in the local food chain as benthic polychaetes are important prey items for many southern African fish and birds (Coetzee, 1986; Harrison and Cherry, 1997; Heemstra and Heemstra, 2004; Kalejta, 1992; Macpherson, 1989; Marais, 1984; van der Westhuizen and

Marais, 1977). Thus, *D. aciculata* presence could potentially have either positive or negative effects on the Knysna Estuary ecosystem.

### *3.1.3 Importance of density information*

The first step towards understanding the impact that *D. aciculata* might have is to determine their density and distribution within the estuary. Then, informed decisions can be made towards either extirpation from the estuary or mitigation of impacts within the estuary. Extirpation of invasive species can depend on the size of the invading population and the stage of the invasion (Allendorf and Lundquist, 2003; Blackburn et al., 2011; Zavaleta et al., 2001). Thus, if the moonshine worm population is localised and small, extirpation might still be feasible. However, once invading populations are large and integrated into the native ecosystem, usually by outcompeting and replacing native biota in ecosystem roles, complete extirpation can be impossible or even have undesired negative effects (Allendorf and Lundquist, 2003; Bergstrom et al., 2009; Zavaleta et al., 2001).

If extirpation is not feasible, mitigation options need to be considered. “Maintenance management” is a mitigation strategy whereby invader densities are kept low enough to mitigate severe negative impacts (Simberloff, 2002). This is known as density maintenance and can be done through chemical, mechanical or biological control (Simberloff et al., 2002). To help control population numbers of *Diopatra aciculata*, SANParks may capitalize on the fact that it is currently the second most popular polychaete species harvested as bait in the Knysna Estuary (Simon et al., 2019a). This may seem counterintuitive since baiting activity can have significant direct and indirect negative impacts on target and non-target species through overexploitation, by-catch and habitat destruction (Cunha et al., 2005; De Carvalho et al., 2013; Griffiths and

Branch, 1997; McPhee et al., 2002; Napier et al., 2009; Pillay et al., 2010; Shepherd and Boates, 1999; Watson et al., 2007). For example, in Europe, *Diopatra* are usually harvested using hand rakes, which efficiently removes the worms, but causes physical destruction of the associated environment (Dağli et al., 2005; De Carvalho et al., 2013). Luckily, moonshine worms in Knysna are harvested in an environmentally friendly manner, directly from their burrows using a thin wire with a hooked tip, causing no by-catch and little physical destruction to the surroundings (Chapter Two).

### *3.1.4 Baiting pressure*

The way *D. aciculata* is harvested in Knysna usually leaves an intact empty tube that may persist for several days before collapsing (Pers. Obs.). This provides a proxy to measure the relative impact that baiting may have on the *D. aciculata* population as only human bait harvesting can leave such pristine intact burrows. This is very different to the way fish and birds feed on worms. Benthivorous fish usually feed using digging and/or suction mechanisms that greatly disturb surrounding sediment (Gerking, 2014; Hoogenboezem, 1991; Roozen et al., 2007) and birds would destroy the worm burrows either through bill probing or pecking behaviour (Elner et al., 2005; Le and Durell, 2000). This difference in collection methods meant that I could use empty tubes as a proxy for recent removal due to baiting. Thus, relative numbers of unoccupied *Diopatra* tubes enables the measurement of recent baiting extraction rate (ER), while the baited area (BA) could be determined by the prevalence of unoccupied tubes throughout the estuary. Together, extraction rate and baited area can provide an estimate of baiting pressure (BP). Such a measurement can help inform feasibility of using bait collectors to help control *D. aciculata* populations in a maintenance management scenario.

### *3.1.5 Aims and hypothesis*

The aims of the study were therefore to determine the current distribution and densities of *D. aciculata* in the Knysna Estuary and to estimate its population size. Furthermore, I aimed to ascertain whether baiting activity can have an appreciable impact on densities of *D. aciculata* by calculating the baiting pressure where applicable. As fishermen can't collect bait in the subtidal zone, I hypothesised baiting pressure here to be zero. I also hypothesised baiting pressure to be lower in the reserve (where bait collecting is prohibited) than in the public areas.

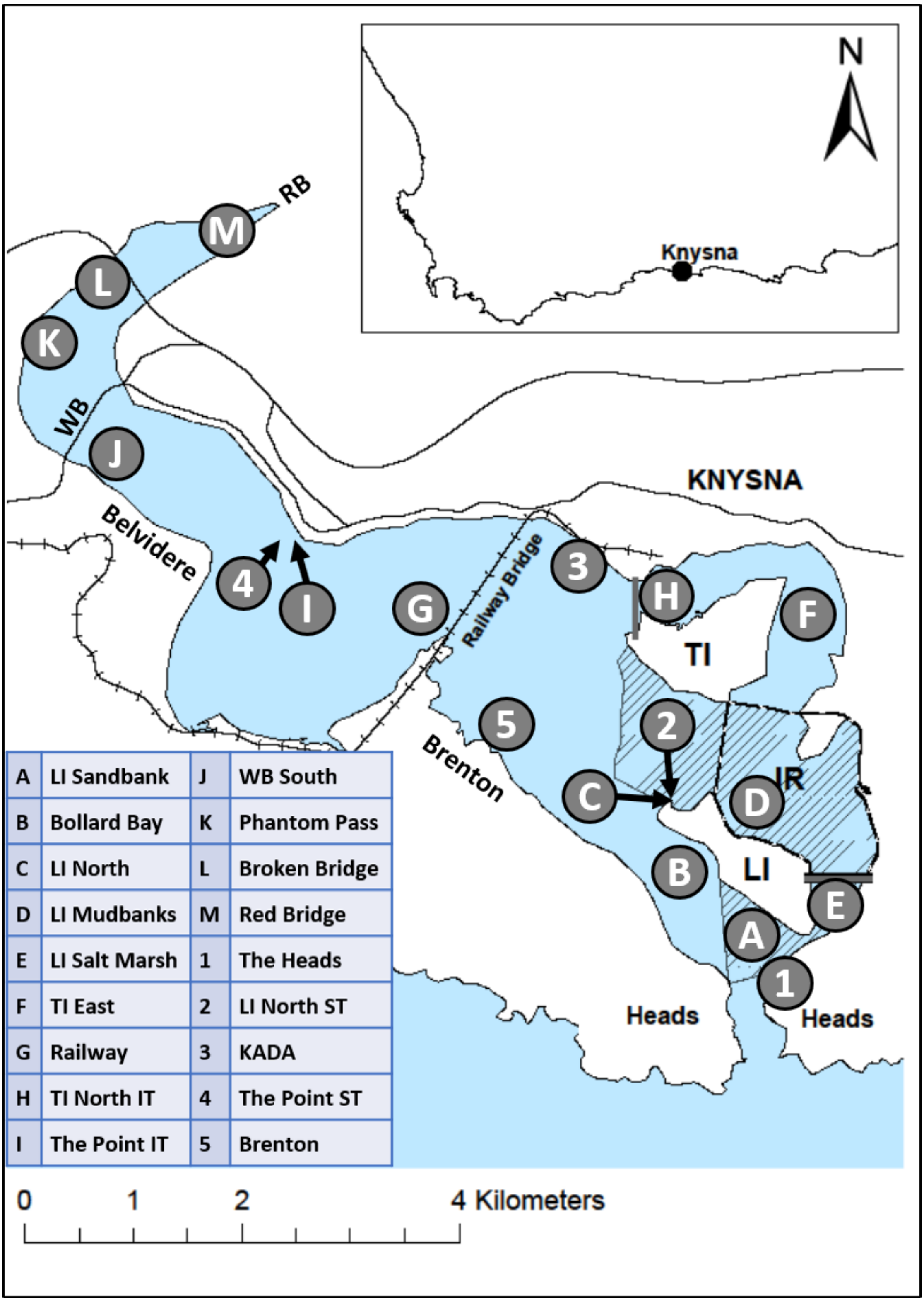
## 3.2 Materials & Methods

### *3.2.1 Study area*

The Knysna Estuary meets all the criteria of being an estuary although it is more accurately described as a marine or estuarine embayment as most of the estuary is entirely marine dominated (Allanson et al., 2000a). During high water spring tide, the tidal flow covers an area of 1827 hectares with a tidal range of about 1.8m and a spring tide prism of 19 million $m^3$. Tidal and saline influence extends 19 km from the mouth, also known as "The Heads", along the main winding channel (Allanson et al., 2000a; Largier et al., 2000). The estuary contains two inhabited islands, Leisure Island (LI) and Thesen Island (TI), and is bordered to the east by the Knysna central business district (CBD).

### *3.2.2 Sampling sites*

I sampled 18 sites in the Knysna Estuary from the mouth (The Heads) to the Red Bridge about 14 km upstream (*Figure 3.1*). These sites cover most of the estuary and include sites previously surveyed by Day (1950) and Allanson et al. (2000) and are popular bait collecting sites sampled by Hodgson et al. (2000), Napier et al. (2009) and Simon et al. (2019). Thirteen sites were in the low intertidal zone (i.e., at the spring low-water mark ± 0.5m) and five were in the subtidal zone (i.e., below the intertidal zone) (*Figure 3.1*). Three intertidal and two subtidal sample sites, Leisure Island (LI) sandbanks, LI mudbanks, LI salt marsh, The Heads and both LI north sites respectively, fell within the invertebrate reserve, while the rest were open to the public (*Figure 3.1*). SANParks officials reported that illegal baiting activity regularly took place at two locations in the Ashmead channel (between Thesen Island and Leisure Island) within the invertebrate reserve, so these areas were avoided (M.K. Smith, Unpublished data). Sampling was conducted during spring tides in November 2017.



| | | | |
|---|---|---|---|
| A | LI Sandbank | J | WB South |
| B | Bollard Bay | K | Phantom Pass |
| C | LI North | L | Broken Bridge |
| D | LI Mudbanks | M | Red Bridge |
| E | LI Salt Marsh | 1 | The Heads |
| F | TI East | 2 | LI North ST |
| G | Railway | 3 | KADA |
| H | TI North IT | 4 | The Point ST |
| I | The Point IT | 5 | Brenton |

*Figure 3.1: Map of the Knysna Estuary showing all the sites where sampling for Diopatra aciculata took place. Intertidal sites are given as letters A–M and subtidal sites are denoted 1–5. The invertebrate reserve is shown as the shaded area. WB = White Bridge, TI = Thesen Island, LI = Leisure Island, IR = Invertebrate reserve, RB = Red Bridge, IT = Intertidal, ST = Subtidal.*

### 3.2.3 Sampling methodology

All sampling took place for four hours around low tide. Using a $1m^2$ steel quadrat, a minimum of $20m^2$ were sampled at each site within the defined sampling zones. In each quadrat the number of visible *Diopatra aciculata* tubes were recorded first, then the number of worms present in the tubes (i.e., density). Worms were detected by luring them to the openings of their burrows using bait bags (frozen sardines (Clupeidae) in 44 decitex nylon stockings) that were squeezed near the burrow entrances. Subtidal sampling was conducted by two free-divers following the same protocol.

### 3.2.4 Extraction rate, Baited area and Baiting pressure

Extraction rate (ER) represents the rate at which worms are extracted in a given area. This calculation was made for each quadrat containing *D. aciculata* according to the equation:

$$ER\left(\frac{\%worms\ extracted}{m^2}\right) = 100 - \left(\left(\frac{Number\ of\ worms\ in\ qudrat}{Number\ of\ tubes\ in\ quadrat}\right)\times 100\right) \qquad \text{(Equation 1)}$$

The baited area (BA) represents the extent of baiting at a given site. This calculation is made for each sampled site where at least 20 quadrats contained worms as follows:

$$BA = \frac{Number\ of\ quadrats\ containing\ empty\ burrows}{Total\ number\ of\ quadrats\ containing\ burrows} \quad . \quad . \quad . \qquad \text{(Equation 2)}$$

The baiting pressure (BP) represents the impact of baiting activities on the *D. aciculata* population. The BP is given as a percentage where 0% would indicate no baiting activity in the area and 100% would mean complete removal of all observable individuals in an area. The BP is calculated for each quadrat using the BA value for the relevant site according to the equation:

$$BP = ER \times BA \quad . \quad . \quad . \quad . \quad . \quad . \quad . \qquad \text{(Equation 3)}$$

The BP of subtidal and intertidal sites as well as public and reserve sites were compared.

### *3.2.5 Population estimate*

To calculate a population estimate for the estuary we used the following equations (Wheater et al., 2011):

$$Population\ estimate\ \left(\check{P}\right) = \frac{\bar{x} \times n}{SF} = \frac{mean\ per\ sample \times number\ of\ samples}{Sampling\ Factor}$$ (Equation 4)

$$Sampling\ Factor\ (SF) = \frac{Area\ sampled}{Total\ area}$$ . . . . (Equation 5)

$$95\%\ Confidence\ Interval = \frac{2 \times Standard\ Error \times n}{SF}$$ . . . (Equation 6)

The mean number of worms per sample necessary for *Equation 5* was calculated using data from all sites where *D. aciculata* was present. Area sampled in *Equation 6* was calculated using data from these same sites where *D. aciculata* occurred. The total area used for *Equation 5* was calculated using a conservative estimate of the area likely occupied by moonshine worms based on the area covered by water during neap low tide as *D. aciculata* has been found in deeper waters than the deepest part of Knysna Estuary (c.f. Knox and Cameron, 1971; Largier et al., 2000; Paxton, 1993).

### *3.2.6 Statistical analysis*

All statistical analyses were performed in R-STUDIO run in the R v.1.0.153 environment. In all instances data were tested for normality using Shapiro-Wilks tests. Differences in density between sampling sites were calculated using Kruskal-Wallis rank sum test followed by Dunn's post hoc test for multiple comparisons using rank sums with Bonferroni correction. Differences in BP among 1) subtidal and intertidal sites and 2) sites in- and outside of the reserve were tested using Mann-Whitney U-tests.

## 3.3 Results

A total of 860m$^2$ were sampled at the eighteen sites resulting in a mean of 47.8m$^2$ covered per site. The maximum number of samples at a single site covered 88m$^2$ at the site south of Leisure Island while a minimum of 20m$^2$ was sampled at the three sites north of the White Bridge (Sites L, M & N, *Figure 3.1*). In total, 458m$^2$ were sampled in the intertidal zone, of which 203m$^2$ were in the invertebrate reserve. In the subtidal zone, a total of 402m$^2$ were sampled, of which 160m$^2$ were in the invertebrate reserve. No worms or holes were found in the three sites north of the White Bridge (Sites L, M & N, *Figure 3.1*), and these sites, totaling 60m$^2$, were therefore excluded from further analyses. Of the 800 quadrats sampled from the remaining fifteen sites, 443 quadrats had no worms in them.

### *3.3.1 Densities and distribution*

The median density was above zero at only seven sites: Bollard Bay, Railway Bridge, Thesen Island (TI) east, Leisure Island (LI) mudbanks, LI north, White Bridge (WB) and Knysna Angling and Diving Association (KADA). Densities were highest at Bollard Bay and Railway Bridge with medians of 8 worms.m$^{-2}$ at each site, and a maximum of 52 worms.m$^{-2}$ at the former. At the remaining five sites, median density ranged between 3–5 worms.m$^{-2}$. Densities varied significantly by site (Kruskal-Wallis $H_{(14,\ 800)} = 376.55$; $p << 0.01$, *Figure 3.2*). Post-hoc analysis revealed six overlapping homogenous groups (*Figure 3.2*). All the data could be divided into two exclusive groups, one with high densities and one with low densities (I and II, respectively, *Figure 3.2*). The high-density group (I) contained the seven sites with median densities of 3–8 mentioned above, while the low-density group contained the remaining eight sites. Overall, there were two groups containing high-density sites (I and III), two groups containing low-density sites (II and VI) and two intermediate groups containing a mixture of high- and low-density sites (IV and V). All but one site (KADA) from the high-density groups were from the intertidal zone. Despite all the overlap, two sites in groups I and III (Bollard Bay and Railway) never overlapped with sites in groups II and VI. *Diopatra aciculata* densities appeared to be patchily distributed throughout the estuary (*Figure 3.3*).

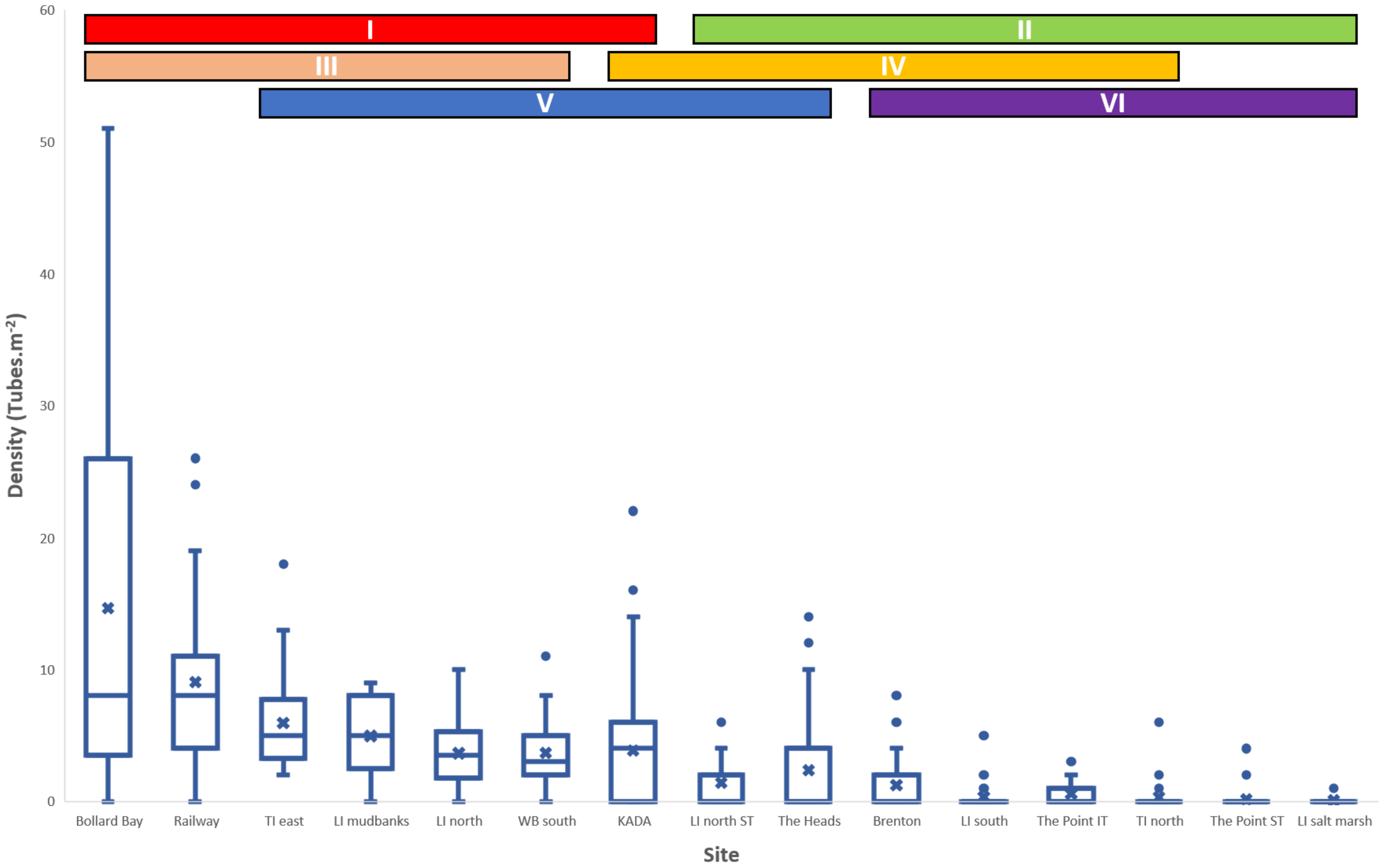


*Figure 3.2: Boxplots showing densities of* *__Diopatra aciculata__* *in Knysna Estuary at all sampled sites where worms were found. Results of post hoc Dunn's test showing homogenous groups (I – VI) are shown visually as bars above boxplots. Crosses (X) denote means while centre bars show medians (indistinguishable in groups II & VI). Box and whiskers shows quartiles with minimum and maximum values. Dots represent outliers.*

### *3.3.2 Population estimate*

As worms were absent beyond the White Bridge, these sites were excluded from the population estimate and area calculations**.** The mean density per sample was 3.47 worms.m$^{-2}$ for 800 samples, covering a total area of 800m$^2$. A conservative estimate of the total area that could be occupied by *D. aciculata* was determined as the area submerged during neap low tide south of the White Bridge (*Figure 3.1*). This amounted to 6,487,600m$^2$ of the 18,270,000m$^2$ area of the Knysna Estuary. The sampling factor (SF) was therefore 1.233 x 10$^{-4}$, and the population size estimate was calculated as 22,514,193 worms with a 95% confidence interval of 2,338,229 worms. Thus, the estimated population size of *D. aciculata* in the Knysna Estuary is between 20 and 24 million worms if distribution throughout the habitable part of the estuary is continuous.

### *3.3.3 Baiting pressure*

Baiting pressure (BP) is summarised in *Table 3.1* and displayed visually in *Figure 3.3*. Only Bollard Bay, Railway, TI east, LI mudbanks, LI north intertidal, WB, KADA, LI north subtidal, The Heads, Brenton, LI sandbank contained enough quadrats with tubes to be considered for baiting pressure analysis. The overall BP was very low; in 292 of the 357 quadrats with tubes, BP was 0%. The channel east of Thesen Island had the highest BP, 5.48%, of all sites. This was followed closely by LI north intertidal with a BP of 5.35%. All subtidal sites had zero BP.

*Table 3.1: Baiting pressure given as percentage for eleven sites that had at least 20 quadrats containing Diopatra aciculata tubes in Knysna Estuary. Arranged in decreasing Baiting Pressure.*

| Site | | Access | Tidal Zone | Baiting Pressure (%) | Standard error |
|---|---|---|---|---|---|
| F | TI east | Public | Intertidal | **5.48** | **1.16** |
| C | LI north IT | Reserve | Intertidal | **5.35** | **1.14** |
| B | Bollard Bay | Public | Intertidal | **3.16** | **0.79** |
| D | LI Mudbanks | Reserve | Intertidal | **1.5** | **0.6** |
| G | Railway | Public | Intertidal | **0.87** | **0.32** |
| J | White Bridge | Public | Intertidal | **0.53** | **0.26** |
| A | LI south | Reserve | Intertidal | **0.33** | **0.23** |
| 3 | KADA | Public | Subtidal | **0.0** | **0.0** |
| 2 | LI north | Reserve | Subtidal | **0.0** | **0.0** |
| 1 | The Heads | Reserve | Subtidal | **0.0** | **0.0** |
| 5 | Brenton | Public | Subtidal | **0.0** | **0.0** |

Baiting pressure was significantly higher in intertidal than subtidal sites (Wilcoxon W (n = 335) = 18,197; p << 0), but there was no significant difference between public and reserve sites (Wilcoxon W (n = 335) = 14,003; p = 0.453). When only intertidal public and reserve sites were compared, no significant difference was found (Wilcoxon W (n = 190) = 4,136; p = 0.9). Baiting pressure did vary significantly by site (Kruskal-Wallis $H_{(10,\ 335)}$ = 95.03; p << 0.01), but no meaningful homogenous groups could be recovered from post-hoc analysis.

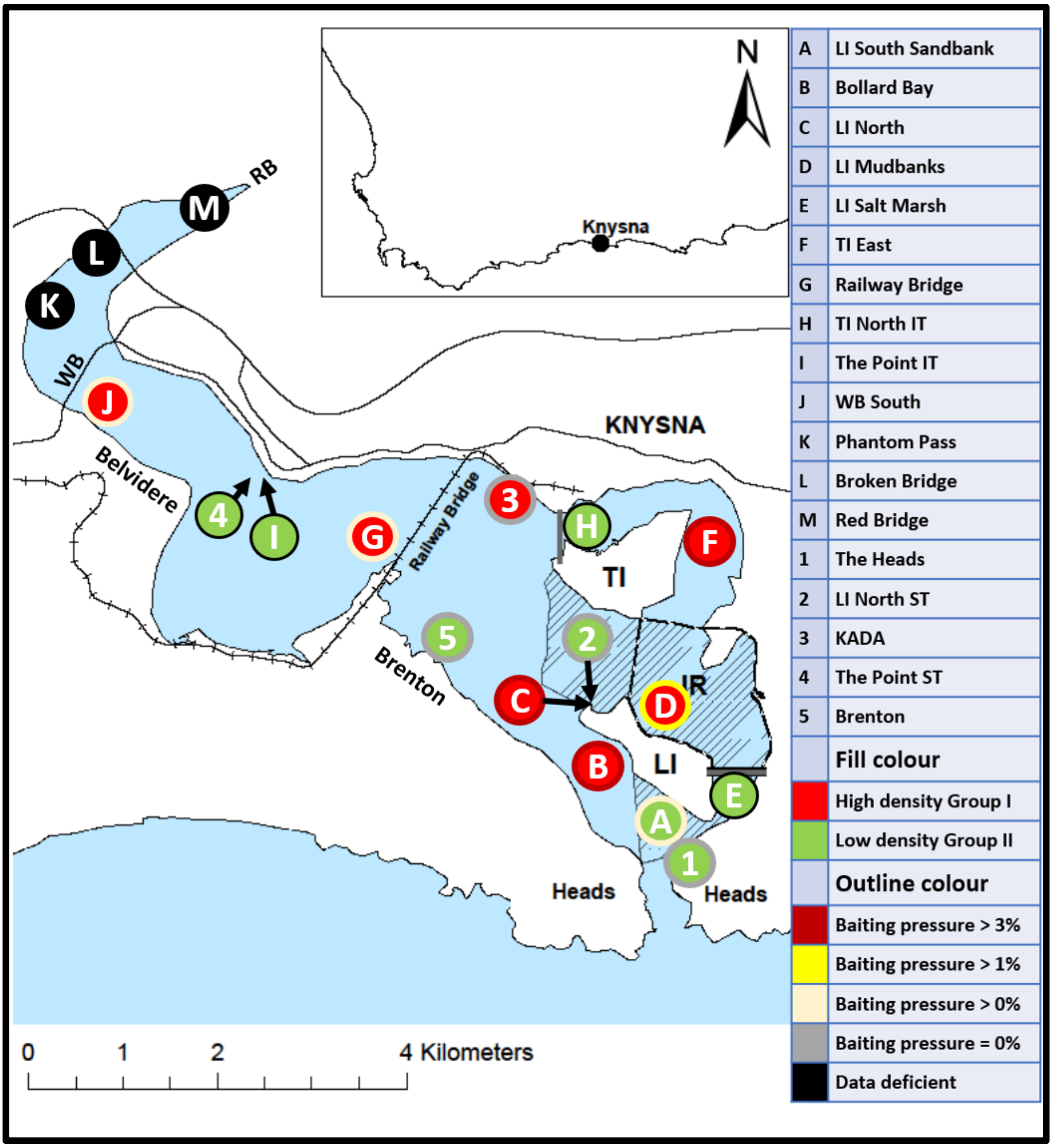


*Figure 3.3: Density of Diopatra aciculata and baiting pressure at sampling sites in Knysna Estuary. Intertidal sites are given as letters A–M and subtidal sites are denoted 1–5. Circle fill colour represents density, high density representing medians of 3 – 8 worms.m*$^{-2}$ *and low density representing a median density of 0 worms.m*$^{-2}$*, circle outline represents baiting pressure. Black denotes no/insufficient data. WB = White Bridge, TI = Thesen Island, LI = Leisure Island, IR = Invertebrate reserve, RB = Red Bridge, IT = Intertidal, ST = Subtidal*

# 3.4 Discussion

### *3.4.1 Densities of D. aciculata in Knysna Estuary*

*Diopatra aciculata* was found throughout the estuary at all of the sites from the Heads near the mouth up to the White Bridge (WB) about 12km upstream. Although species in the genus *Diopatra* are marine-adapted, many occur in estuaries around the world (Arias and Paxton, 2014; Arias et al., 2010; Cunha et al., 2005; de Carvalho et al., 2013b; Pires et al., 2010; Pires et al., 2012; Rodrigues et al., 2009; van der Westhuizen and Marais, 1977; this study). Paxton (1993) reported *D. aciculata* from several large, marine-dominated estuaries around southern Australia and thus the conditions predominating in most of the Knysna Estuary (Allanson et al., 2000a; Largier et al., 2000) provided a favourable environment that facilitated the establishment and spread of *D. aciculata*. However, the absence of the species from sites north of the White Bridge may be related to the fact that high freshwater input here frequently results in salinities that drop below 25g.L$^{-1}$, a level linked to increased mortality in the closely related *Diopatra neapolitana* (Freitas et al., 2015; Pires et al., 2015). Additionally, dissolved oxygen concentrations start dropping rapidly beyond the White Bridge (Allanson et al., 2000a; Largier et al., 2000), probably further contributing to the unsuitability of this part of the estuary for the species.

Species of the genus *Diopatra* can often attain high densities (Arias et al., 2016; Arias and Paxton, 2015; Berke et al., 2010; Conti and Massa, 1998; Cunha et al., 2005; Dağli et al., 2005; Harwell and Orth, 2001; Rodrigues et al., 2009). For example, the mean densities of *D. neapolitana,* a similarly sized and closely related species, may be as low as 20 and as high as 200 worms.m$^{-2}$ in different parts of the Mediterranean (Arias et al., 2016; Arias and Paxton, 2015; Berke et al., 2010; Dağli et al., 2005). At a mean of 3.47 worms.m$^{-2}$ (and maximum of 51 worms.m$^{-2}$), densities of *D. aciculata* in Knysna are well below the densities reported for *D.*

*neapolitana*, but it is not known whether densities have reached saturation. As *D. aciculata* only arrived in the Knysna Estuary sometime between 10 – 20 years ago, it is likely that the population is still growing. Furthermore, the current distribution and densities throughout the estuary suggest a rather rapid dispersal and growth of the *D. aciculata* population in Knysna since settlement. Dispersal through the estuary would probably be facilitated by broadcast spawning and the production of planktonic larvae, as is found in other large *Diopatra* species (Arias et al., 2016; Pires et al., 2012). However, because this is the first study that measures densities and distribution of *D. aciculata* in Knysna it is impossible to know at what rate the population is growing. Furthermore, there is no information regarding threshold densities for ecological impacts so without further studies it is impossible to say if *D. aciculata* is having any significant ecological impacts in the estuary. Nevertheless, if the published densities of *D. neapolitana* indicate biological potential, the population of *D. aciculata* in Knysna may still increase.

### *3.4.2 Patchy distribution of D. aciculata*

Density of *D. aciculata* was not consistent throughout the Knysna Estuary. Instead, the patchy distribution of dense aggregations suggests that density may be related to site-specific environmental conditions. Unfortunately, there is no information on environmental preferences of *D. aciculata*, and I can only speculate that water flow rate may be influencing *D. aciculata* settlement within the estuary (c.f., Mangum et al. (1968)). Previous studies showed that densities of *Diopatra cuprea* increased with current velocity (Mangum et al., 1968), and this may apply to *D. aciculata* in Knysna Estuary too. At Bollard Bay, where the highest density per quadrat occurred, quadrats in the shallow intertidal banks were empty or had very few worms, but more clusters of *D. aciculata* were present in the deeper intertidal, towards the main channel where water flowed markedly faster. At the Railway site, where all quadrats sampled were close to the main channel in moving water, the densities of worms were more consistent. Similarly, north of Thesen Island (TI) I only found worms in deeper areas (>15 cm under water) with faster flowing water that formed part of the main channel. Thus, I predict if this area were to be resampled with efforts focused toward this middle channel a higher density would be recorded. By contrast, low densities were found at sites with very weak water currents during most of the low tide, such as at LI salt marsh and Leisure Island (LI) south sites. The salt marsh is characterized by stagnant warm water with long residency times throughout the tidal cycle (Pers. Obs.). During spring low tides, there is little water movement here and the four worms that I found all occurred within the only small stream of permanently flowing water in this area. Similarly, the LI sandbank to the south of the island is exposed for long periods during the tidal cycle. During spring low tide there are very few pockets and streams of water in this area. Thus, lack of moving water for large periods of the tidal cycle may make it difficult for worms to survive as, in general, they appeared to mainly occupy well-flushed areas closer to the main channels or other faster moving streams within the estuary (Pers. Obs.). This preference for faster flowing water may be related to feeding habits. As a discretely motile omnivorous scavenger (Fauchald and Jumars, 1979; Jumars et al., 2015), fast-flowing water is more likely to move plant or detrital material to within feeding range of the burrow. Alternatively, the worms may avoid areas where water flows slowly and at risk of having low oxygen levels. However, two studies on congeneric *D. cuprea* indicated that they used unusually low amounts of oxygen (Mangum et al., 1968; Mangum and Cox, 1971) which would refute this.

The reasons for low densities at other sites in group II were not always obvious but may be related to competitive interactions with other species. Interactions with other biota may keep *D. aciculata* populations from growing in certain areas. For example, the bloodworm, *Arenicola loveni*, which is also present in the estuary (Branch et al., 2017; Smith and Smith, 2012) may potentially exclude *D. aciculata* from its surrounding environment. The former species is also an environmental engineer, but as a bioturbator it is the functional opposite of a sediment stabiliser such as *D. aciculata* (Berke et al. 2010). Arenicolid-rich patches are characteristically devoid of algal and vascular plants and harbour lower diversities of infaunal species (Berke et al., 2010). It has been shown that invertebrate tubes, such as those of *Diopatra*, can have negative impacts on burrowing species such as arenicolids but how it translates to fitness of the animal is not known (Brenchley, 1982). Nevertheless, opposing ecosystem functions and limited space in the benthic community may cause exclusion of one of these species by the other (Volkenborn et al., 2009).

### *3.4.3 Population size estimate*

I estimated that 20 to 24 million *D. aciculata* occur in the Knysna Estuary. However, this estimate should be considered within the context of the study. My sampling effort only represents 0.01% of the total area of the estuary, and I found distribution of *D. aciculata* to be patchy. Such patchy distributions can lead to gross misrepresentations when not sampled thoroughly. Therefore, using the mean density of 3.47 worms.m$^{-2}$ from such a small portion of the total area to estimate population size may be crude. Consequently, it is important that further sampling take place to get a more accurate estimate, especially in light of *D. aciculata* being an alien species. Still, the data that I gathered shows that *D. aciculata* likely already plays a role in the Knysna Estuary ecosystem. I hypothesise that a mean of 3.47 worms.m$^{-2}$ is not high enough to cause cascading ecosystem engineering effects so those risks can still be mitigated. However, the presence of 20 million individuals could undoubtedly have an impact on other biota if only by serving as extra food for benthivorous fish.

### *3.4.4 Baiting pressure*

The baiting pressure (BP) throughout the estuary was quite low and unevenly distributed, but this should not be disregarded. A baiting pressure of 5% means that 5% of the detectable *D. aciculata* were removed from that site in the days immediately preceding sampling only. Unfortunately, it is not known exactly how long empty tubes remain before disappearing and so it is difficult to quantify over which time period worms were extracted. Furthermore, because the BP only reflects very recent baiting, the calculated BP for each site may not be an accurate reflection of the mean BP a site may experience throughout a year. Sampling for this study was done before the peak baiting season determined by Simon et al. (2019a) and results may be different if sampled again during or after December or Easter holidays.

In some places such as Bollard Bay, LI north IT, LI mudbanks and TI east high densities of *D. aciculata* may explain the comparatively high baiting pressure in these areas. Some of these sites also correspond with popular bait collecting sites determined in Simon et al. (2019). However, sites such as Railway and WB south also harbour high *D. aciculata* densities, yet baiting pressure here is very low. Here, the low baiting pressure may reflect difficulty of access to these sites. For example, bait collectors need to walk about 1 km on the dangerous abandoned railway bridge to reach the Railway bait collecting site. Similarly, the White Bridge site is about 8km from the Knysna CBD, and even further from the large informal settlements, which are much farther than the other sites. Most subsistence fishermen walk to their bait collecting sites (du Toit and Simon, Unpublished data) so the White Bridge site will only be baited by those living nearby. Extraction from this site may be further discouraged, as it is very muddy, requiring much more effort to move around. Thus, recreational fishers would rather walk or drive to LI and subsistence fishers would walk to the nearest place that is convenient in concordance with what Simon et al. (2019) found.

No subtidal sites experienced any baiting pressure. This was expected as baiters need to see the worm and burrow clearly to extract it and so they cannot extract *D. aciculata* from deeper waters. Unexpectedly, there were no significant differences in baiting pressure between public and reserve sites. Since I specifically did not sample at sites in the reserve where SANParks had reported regular illegal baiting, this suggests that baiting activity within the reserve is more prevalent than previously thought.

# Chapter 4: Study overview with management and research recommendations for Knysna estuary

The Knysna Estuary is the largest clear-water estuary along the coast of South Africa (Allanson et al., 2000a), and based mainly on its size and the many habitats and high biodiversity which it supports, it was ranked the most important estuary in the country (Turpie and Clark, 2007). As a consequence, the Knysna Estuary has been, and still is, a hotspot for research focusing on topics such as diversity mapping (Allanson et al., 2014, 2000b; Barnes, 2014, 2004; Day et al., 1951; Hodgson et al., 2000a, 2000b), hydrology and sand bank movement (Allanson et al., 2000a; Largier et al., 2000; Marker and Maree, 2004), pollution (Allanson et al., 2016), conservation (Claassens et al., 2018; Teske et al., 2003) and bait collecting (Hodgson et al., 2000a; Napier et al., 2009; Simon et al., 2019a). Furthermore, Knysna Estuary falls within the Garden Route National park and is therefore managed by South African National Parks (SANParks). Additionally, the Knysna Basin Project (KBP) (http://knysnabasinproject.co.za/) runs several programs such as Knysna Estuary Management Platform (KEMP) and ShoreSearch that aim to aid in the conservation of the estuary. However, despite all the conservation efforts and research, nobody recognized the significance of the appearance and spread of the moonshine worm. This study therefore aimed to identify this species and determine its distribution within Knysna Estuary.

## *4.1 Study overview*

In Chapter Two, I identified the *Diopatra* sp. from Knysna Estuary as *Diopatra aciculata* and showed that the non-indigenous *Diopatra aciculata* has invaded two estuaries on the South African south-east coast. *Diopatra aciculata* from South Africa was described and shown to be a different but closely related species to *D. neapolitana* using both morphological and molecular techniques.

In Chapter Three, I established that the alien *D. aciculata* is widely distributed within the Knysna Estuary from the White Bridge all the way to The Heads with densities reaching as high as 51 worms.m$^{-2}$ in places. The species seems to prefer low intertidal areas with higher water flow rates and is also present in subtidal areas. Furthermore, I calculated a crude population estimate for *D. aciculata* in Knysna to be between 20 – 24 million worms. Lastly, I found that overall baiting pressure was very low, except at two popular baiting sites, and baiting activity inside the invertebrate reserve appears to be more widespread than expected.

## *4.2 Factors that led to the cryptic invasion of D. aciculata*

The earliest reference to *D. aciculata* in Knysna (as *Diopatra* sp.) was only published in 2009 (Napier et al., 2009). Yet, nobody recognized that the species was new to the estuary or that it was not indigenous. Instead, it was misidentified as the indigenous wonderworm (Allanson et al., 2016) or *Marphysa elityeni* (see Smith and Smith, 2012), while descriptions of pseudo-cosmopolitan *Diopatra* species in a popular field guide (Branch et al., 2017) are too vague to enable accurate identification. However, accurate species identification was probably inhibited by the taxonomic difficulties of the *Diopatra* genus (Day, 1960; Paxton, 1993). Thus, the cryptic invasion by *D. aciculata* was possible not because of failed detection but rather of failed identification. This cryptic invasion was probably further facilitated by the decrease in professional and amateur taxonomists (Griffiths et al., 2010; Herbert et al., 2001; Hopkins and Freckleton, 2002; Martens and Hamer, 1999; Smith et al., 2008) active in the region, who may have been able to identify the species.

The re-examining of previous accounts of *D. neapolitana* outside of the Mediterranean should be made a priority as such specimens may represent an alien presence of *D. aciculata* as it did here. This may also be true for other apparently cosmopolitan species which emphasizes the need to re-examine all such species. If a non-indigenous species is wrongfully identified as an indigenous species, a cryptic invasion may even be facilitated through management strategies aimed towards protecting the falsely identified species.

### *4.3 Feasibility of mechanical removal of D. aciculata from Knysna Estuary*

The unified framework for biological invasions suggests that once a species has become invasive, management should focus on extirpation, containment or mitigation to avoid negative effects on surrounding native biota (Blackburn et al., 2011). Though there have been numerous cases of successful eradication of invasive species, most involved removing vertebrates from islands as it is easier to meet conditions for extirpation for small populations of isolated species that have no dormant life stages and relatively low reproductive rates (Veitch and Clout, 2002). The situation is quite different for marine species. In an attempt to remove the invasive crab *Carcinus maenas* (Linnaeus, 1758) from Hout Bay harbour, a total of 36,244 crabs were removed, six times the most recent population estimate, yet extirpation was still deemed unsuccessful (Mabin, 2017). Given that I estimated that there are between 20 and 24 million worms in the Knysna Estuary, extirpation may prove impossible or even counterproductive (see also Bergstrom et al., 2009; Veitch and Clout, 2002; Zavaleta et al., 2001).

Due to the value of the estuary (Turpie and Clark, 2007), extirpation efforts need to be non-destructive. Luckily, *D. aciculata* are removed by a wire inserted into individual tubes, which does not disturb the sediment or inadvertently kill non-target species. With practice, it is possible to collect a worm within 20 seconds (Pers. Obs.), but it can take several minutes to find worm tubes and moving between patches containing worms. It is therefore difficult to estimate a realistic extraction rate, though most fishermen to whom I spoke said they could collect their daily bag limit (10 worms) within 20–60 minutes (Unpublished data). Thus, 10–30 worms may be collected within an hour if the collector already knows where the worms are. Currently there are about 500 full–time and part-time subsistence fishermen active in the Knysna Estuary (Simon et al., 2019a). If they were all employed on a full-time basis for four hours a day (only extractable during day-time low tide), five days a week, and extract 30 worms per hour, it would take more than 20 months to remove the maximum estimated 24,852,422 worms that occur in the estuary. However, not all worms are extractible since many occur in the subtidal zone. Additionally, worms that were removed may be replaced by new recruits spawned by worms that have not yet been removed. Thus, the worms in the subtidal zone could act as a source of recruits for the rest of the estuary. Thus, this effort would have to be ongoing and complete removal may not be possible.

Eradication programs such as these also have financial implications (Mabin, 2017). The recommended minimum wage in South Africa is R20 per hour excluding farm- and domestic workers (National Minimum Wage Panel, 2016). Thus, if 500 workers received minimum wage during the removal scenario described above, it would cost more than R18 million over 20.7 months. However, as extirpation would undoubtedly be an ongoing process, costs would not end after 20.7 months.  Costs can be cut nearly in half by classifying the extirpation effort as a public works program cutting minimum compensation almost in half to R11 per hour (National Minimum Wage Panel, 2016). Costs may be further offset due to the commercial value of *D. aciculata*. In the global fisheries market, *D. aciculata* has been sold at US$141,62 per kilogram. Those employed for extirpation efforts could be allowed to keep what they remove for their own use or to sell, though this may have unintended consequences (Fielding, 2009). For example, baiters may then want to use more efficient but destructive methods, such as shoveling or pumping, to collect worms at a greater tempo. Also, if baiters are paid to remove worms from the estuary for as long as they are there, it would be in their best interest to not remove all the worms as this would bring an end to their income. Thus, paying baiters for the removal of *D. aciculata* in Knysna may not be a permanent or final solution but rather a means of maintenance management as part of a mitigation strategy.

### *4.4 Management recommendations*

The failure to detect the presence and spread of a conspicuous alien species such as *D. aciculata* could have been avoided if well-designed and properly executed biodiversity monitoring programs were implemented (Lindenmayer et al., 2012; Niemelä, 2000; van der Zanden et al., 2010). Lindenmayer et al. (2012) provided five outcomes that such biodiversity monitoring programs should provide, 1) information on trends in key aspects of biodiversity (e.g., population shifts), 2) early warning of problems that might be difficult or too expensive to reverse if left unattended (such as biological invasions), 3) evidence of success or failure of conservation effort, 4) information regarding management inefficiencies, and 5) information on the return on conservation investment. A monitoring program that can deliver all these outcomes could incur considerable cost, however, the return on such an investment could be immeasurable. Furthermore, a program such as this may require manpower that isn't always available to SANParks and thus such monitoring efforts may need to be outsourced or approached more efficiently. There are several ways to improve efficiency and cut costs of traditional biodiversity monitoring programs, including using citizen scientists and molecular techniques (Bohmann et al., 2014; Darling and Mahon, 2011; Goldberg et al., 2015; Schmeller et al., 2008; Thomsen and Willerslev, 2015).

The use of volunteers and citizen science could add value to such a program without adding much cost. Evaluation of large-scale monitoring programs in Europe has shown that, contrary to popular belief, volunteer-based data can be reliable and deliver unbiased results (Schmeller et al., 2008). However, the precision of such programs is a function of volunteer involvement (Schmeller et al., 2008) so it would be important to promote a culture of reporting biodiversity among those who frequently make use of the estuary which could likely be done through workshops and education initiatives although it can be difficult to implement these in ways that have measurable positive outcomes (see Simon et al., 2019a). Furthermore, an online data portal or smartphone application would make it easy and convenient for volunteers to log reports, thereby promoting its use.

In an estuary as large and important such as Knysna, a biodiversity monitoring program should be routine. The Knysna Estuary should have a structured monitoring program that would measure key aspects of biodiversity, such as presence and abundance of keystone species, at regular intervals on both coarse and finer scales. The Knysna Basin Project (KBP) developed the ShoreSearch biodiversity monitoring program that assesses biodiversity in ten sites in the estuary annually on a seasonal basis (Knysna Basin Project, 2019). However, the program is mostly citizen science- and volunteer-based and thus likely do not have the necessary taxonomic expertise to accurately identify all organisms and furthermore, would not have access to molecular techniques. These circumstances could explain why *D. aciculata* was never detected during ShoreSearch monitoring.

With taxonomy at the basis of any biodiversity monitoring program, the decline in taxonomic expertise is concerning. Without clear and accurate taxonomic references any biodiversity monitoring program would be meaningless. However, molecular techniques (such as DNA barcoding) has made it easier than ever for non-taxonomists to accurately identify species. Unfortunately, there are many pitfalls related to barcoding (see Da Silva and Willows-Munro, 2016), the main one being the need for accurate reference libraries which may not always be available. This was seen in this study where a sequence of *D. aciculata* was uploaded as *D. dentata.* Another molecular technique that has seen a rise in popularity, especially in biodiversity monitoring, is the collection of environmental DNA (eDNA) (Bohmann et al., 2014; Darling and Mahon, 2011; Goldberg et al., 2015; Thomsen and Willerslev, 2015). Environmental DNA are trace DNA molecules shed from plants and animals through blood, sperm, fecal matter, mucus etc. into the water or sediment which, when analyzed, can show which species had recently been present in the area. This method has been shown to be effective at detecting rare and elusive species as well as alien species (Bohmann et al., 2014; Thomsen and Willerslev, 2015). The use of molecular techniques such as eDNA can provide cost effective ways to monitor biodiversity and detect alien species in the future. Collection of eDNA is a purely molecular technique and does not involve collection of organisms that can be stored as reference in a museum. Thus, it heavily relies on an accurate comparative library of identified sequences as there is no deposited material to re-examine at a later point. Such reliable reference libraries should ideally be generated by taxonomic experts using a total evidence approach. So, while molecular techniques can hasten and simplify identification of species, the reference library required for its use takes a lot of time and effort to compile.

### *4.5 Research recommendations*

Other sheltered bays and estuaries, especially well-flushed ones, should be thoroughly surveyed to get a more complete record of *D. aciculata* distribution in SA. These could start in areas where *Diopatra neapolitana* has been previously reported, such as Durban Bay and Sundays River. Furthermore, it might be helpful to examine records of *D. neapolitana* from South Africa stored in the British Museum (Day, 1960) as it may provide insight on the historical aspects of the invasion. Lastly, Branch et al. (2017) should modify the description of *D. neapolitana* to reflect that of the subspecies, *Diopatra neapolitana capensis*, and include an entry for *D. aciculata*. *Diopatra aciculata* should be included in the register of alien species held by the South African National Biodiversity Institute and its current distribution be given as Knysna and Swartkops in both sandy and silty sediment. This record can then be updated as new information emerges. Additionally, DAFF needs to update their annual guide to recreational fishing to include more specific descriptions of bait which would ideally include pictures.

Given the projected cost, effectiveness and duration of the hypothetical extirpation strategy outlined, it is likely that complete extirpation is unachievable at this point. Though, if it can be managed sustainably, a baiting incentive strategy may be pursued to mitigate possible impacts of the non-indigenous species by keeping population numbers low. However, it is still not known what kind of impact this species has or will have in the estuary as neither this species nor a closely related one have ever been reported as invasive from elsewhere.

In the above scenarios it is assumed that *D. aciculata* has negative impacts on surrounding native biota, which would justify the extirpation effort. However, invasive species do not always have negative impacts on surrounding biota and can have no discernable effects (Mabin, 2017). Nevertheless, *D. aciculata* should still be treated with caution and prioritized for further in-depth studies.

Given the potential ecological and financial implications of extirpation, the first goal of future research should be to determine the nature of direct or indirect impacts of *D. aciculata* on the surrounding environment, ecological structure or trophic levels (Alexander et al., 2016). For example, determine if *D. aciculata* is facilitating spread of native eelgrass or increasing biodiversity within its surroundings. The species may also be competing with other native species for food, while it may also be a source of food for others. Furthermore, determine if a displacement interaction exists between *D. aciculata* and a functionally opposite ecosystem engineer such as the native *Arenicola loveni*. Furthermore, information regarding reproductive cycles and population structure could be informative in developing management strategies that will enable effective mitigation or removal with minimum re-colonization.

Studies that follow up on this one should be done to discern the rate of change in population size and/or densities, especially also during a year, such as right after peak baiting season.

Future research should thus, as a priority, focus on generating information that can clarify the urgency of necessary management strategies. Furthermore, information should be generated that would help inform more efficient and effective management strategies.

# References

Akaike, H., 1973. Maximum Likelihood Identification of Gaussian Autoregressive Moving Average Models. *Biometrika* 60, 255–265.

Alexander, M.E., Simon, C.A., Griffiths, C.L., Peters, K., Sibanda, S., Miza, S., Groenewald, B., Majiedt, P., Sink, K.J., Robinson, T.B., 2016. Back to the future: reflections and directions of South African marine bioinvasion research. *African J. Mar. Sci.* 38, 141–144.

Allanson, B., Smith, F., Smith, P., 2014. The invertebrate macrobenthos of artificial rocky shores in the Knysna Estuary. *Trans. R. Soc. South Africa* 69, 19–33.

Allanson, B.R., Human, L.R.D., Claassens, L., 2016. Observations on the distribution and abundance of a green tide along an intertidal shore, Knysna Estuary. *South African J. Bot.* 107, 49–54.

Allanson, B.R., Maree, B., Grange, N., 2000a. An introduction to the chemistry of the water column of the knysna estuary with particular reference to nutrients and suspended solids. *Trans. R. Soc. South Africa* 55, 141–162.

Allanson, B.R., Nettleton, J., De Villiers, C.J., 2000b. Benthic macrofauna richness and diversity in the Knysna Estuary: a 50 year comparison. *Trans. R. Soc. South Africa* 55, 177–185.

Allendorf, F.W., Lundquist, L.L., 2003. Introduction: population biology, evolution, and control of invasive species. *Conserv. Biol.* 17, 24–30.

Arias, A., Anadón, N., Paxton, H., 2010. New records of Diopatra marocensis (Annelida: Onuphidae) from northern Spain. *Zootaxa* 68, 67–68.

Arias, A., Paxton, H., 2014. First record of the polychaetous annelid Diopatra micrura Pires et al., 2010 in the Mediterranean Sea. *Mediterr. Mar. Sci.* 15, 5–8.

Arias, A., Paxton, H., 2015. The cryptogenic bait worm Diopatra biscayensis Fauchald etal., 2012 (Annelida: Onuphidae) - Revisiting its history, biology and ecology. *Estuar. Coast. Shelf Sci.* 163, 22–36.

Arias, A., Paxton, H., Budaeva, N., 2016. Redescription and biology of Diopatra neapolitana (Annelida: Onuphidae), a protandric hermaphrodite with external spermaducal papillae. *Estuar. Coast. Shelf Sci.* 174, 1–17.

Arias, A., Richter, A., Anadón, N., Glasby, C.J., 2013. Revealing polychaetes invasion patterns: Identification, reproduction and potential risks of the korean ragworm, perinereis linea (treadwell), in the western mediterranean. *Estuar. Coast. Shelf Sci.* 131, 117–128.

Arnason, R., Kashorte, M., 2006. Commercialization of South Africa's Subsistence Fisheries? Considerations, Criteria and Approach. *Int. J. Ocean. Oceanogr.* 1, 45–65.

Augener, H., 1931. Die bodensässigen Polychäten nebst einer Hirudinee der Meteor-Fahrt. Zoologisches Staatsinstitut und Zoologisches Museum, Zoologisches Staatsinstitut und Zoologisches Museum.

Augener, H., Michaelsen, W., 1918. Beitrage zur Kenntnis der Meeresfauna Westafrikas. *L. Friederichsen Co. eds* 2, 472–474.

Bailey-brock, J.H., 1984. Ecology of the tube-building polychaete Diopatra leuckarti Kinberg, 1865 (Onuphidae) in Hawaii: community structure, and sediment stabilizing properties. *Zool. J. Linn. Soc.* 80, 191–199.

Ban, S.M., Nelson, W.G., 1987. Role of Diopatra cuprea Bosc ( Polychaeta: Onuphidae) tubes in structuring a subtropical infaunal community. *Bull. Mar. Sci.* 40, 11–21.

Barham, W.T., 1979. Spawning of Arenicola loveni Kinberg in the Heunings River Estuary, Bredasdorp. *S. Afr. J. Sci.* 75, 262–264.

Barnes, R.S.K., 2004. The distribution and habitat in the Knysna Estuary of the endemic South African mudsnail Hydrobia knysnaensis and the influence of intraspecific competition and ambient salinity on its abundance. *African J. Aquat. Sci.* 29, 205–211.

Barnes, R.S.K., 2014. The nature and location of spatial change in species assemblages: a new approach illustrated by the seagrass macrofauna of the Knysna estuarine bay, South Africa. *Trans. R. Soc. South Africa* 69, 75–80.

Beckley, L.E., Fennessy, S.T., Everett, B.I., 2008. Few fish but many fishers: A case study of shore-based recreational angling in a major South African estuarine port. *African J. Mar. Sci.* 30, 11–24.

Bergstrom, D.M., Lucieer, A., Kiefer, K., Wasley, J., Belbin, L., Pedersen, T.K., Chown, S.L., 2009. Indirect effects of invasive species removal devastate World Heritage Island. *J. Appl. Ecol.* 46, 73–81.

Berke, S.K., Mahon, A.R., Lima, F.P., Halanych, K.M., Wethey, D.S., Woodin, S.A., 2010. Range shifts and species diversity in marine ecosystem engineers: Patterns and predictions for European sedimentary habitats. *Glob. Ecol. Biogeogr.* 19, 223–232.

Bickford, D., Lohman, D.J., Sodhi, N.S., Ng, P.K.L., Meier, R., Winker, K., Ingram, K.K., Das, I., 2007. Cryptic species as a window on diversity and conservation. *Trends Ecol. Evol.* 22, 148–155.

Blackburn, T.M., Pyšek, P., Bacher, S., Carlton, J.T., Duncan, R.P., Jarošík, V., Wilson, J.R.U., Richardson, D.M., 2011. A proposed unified framework for biological invasions. *Trends Ecol. Evol.* 26, 333–339.

Bohmann, K., Evans, A., Gilbert, M.T.P., Carvalho, G.R., Creer, S., Knapp, M., Yu, D.W., de Bruyn, M., 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends Ecol. Evol.* 29, 358–367.

Branch, G.M., Clark, B.M., 2006. Fish stocks and their management: The changing face of fisheries in South Africa. *Mar. Policy* 30, 3–17.

Branch, G.M., Griffiths, C.L., Branch, M.L., Beckey, L.E., 2017. Two oceans: a guide to the marine life of southern Africa. Penguin Random House South Africa, Cape Town.

Brasier, M.J., Wiklund, H., Neal, L., Jeffreys, R., Linse, K., Ruhl, H., Glover, A.G., 2016. DNA barcoding uncovers cryptic diversity in 50% of deep-sea antarctic polychaetes. *R. Soc. Open Sci.* 3.

Brenchley, G.A., 1982. Mechanisms of spatial competition in marine soft-bottom communities. *J. Exp. Mar. Bio. Ecol.* 60, 17–33.

Brouwer, S.L., Mann, B.Q., Lamberth, S.J., Sauer, W.H.H., Erasmus, C., 1997. A survey of the South African shore-angling fishery. *South African J. Mar. Sci.* 18, 165–177.

Bruschetti, M., Bazterrica, C., Luppi, T., Iribarne, O., 2009. An invasive intertidal reef-forming polychaete affect habitat use and feeding behavior of migratory and locals birds in a SW Atlantic coastal lagoon. *J. Exp. Mar. Bio. Ecol.* 375, 76–83.

Bruschetti, M., Luppi, T., Fanjul, E., Rosenthal, A., Iribarne, O., 2008. Grazing effect of the invasive reef-forming polychaete Ficopomatus enigmaticus (Fauvel) on phytoplankton biomass in a SW Atlantic coastal lagoon. *J. Exp. Mar. Bio. Ecol.* 354, 212–219.

Bruschetti, M., Luppi, T., Iribarne, O., 2016. Effect of an invasive filter-feeder on the zooplankton assemblage in a coastal lagoon. *J. Mar. Biol. Assoc. United Kingdom* 96, 1201–1210.

Budaeva, N., Fauchald, K., 2011. Phylogeny of the Diopatra generic complex with a revision of Paradiopatra Ehlres, 1887 (Polychaeta: Onuphidae). *Zool. J. Linn. Soc.* 163, 319–436.

Budaeva, N., Schepetov, D., Zanol, J., Neretina, T., Willassen, E., 2016. When molecules support morphology: Phylogenetic reconstruction of the family Onuphidae (Eunicida, Annelida) based on 16S rDNA and 18S rDNA. *Mol. Phylogenet. Evol.* 94, 791–801.

Burnham, K.P., Anderson, D.R., 2004. Multimodel inferance: Understanding AIC and BIC in Model Selection. *Sociol. Methods Res.* 33, 261–304.

Charles, H., Dukes, J.S., 2007. 13 Impacts of Invasive Species on Ecosystem Services. *Biol. Invasions* 193, 217–237.

Chevaldonné, P., Jollivet, D., Desbruyeres, D., Lutz, R.A., Vrijenhoek, R.C., 2002. Sister-species of eastern Pacific hydrothermal vent worms (Ampharetidae , Alvinellidae , Vestimentifera) provide new mitochondrial COI clock calibration. *Cah. Biol.* 43, 367–370.

Choe, S., 1960. On the life history of the polychaete worm, Diopatra neapolitana Delle Chiaje. *Bull. Japanese Soc. Sci. Fish.* 26, 430–437.

Çinar, M.E., 2013. Alien polychaete species worldwide: Current status and their impacts. *J. Mar. Biol. Assoc. United Kingdom* 93, 1257–1278.

Claassens, L., Booth, A.J., Hodgson, A.N., 2018. An endangered seahorse selectively chooses an artificial structure. *Environ. Biol. Fishes* 101, 723–733.

Clark, B.M., Hauck, M., Harris, J.M., Salo, K., Russell, E., 2002. Identification of subsistence fishers, fishing areas, resource use and activities along the South African coast. *South African J. Mar. Sci.* 24, 425–437.

Cockcroft, A.C., Sauer, W.H.H., Branch, G.M., Clark, B.M., Dye, A.H., Russell, E., 2002. Assessment of resource availability and suitability for subsistence fishers in South Africa, with a review of resource management procedures. *South African J. Mar. Sci.* 24, 489–501.

Coetzee, P.S., 1986. Diet composition and breeding cycle of blacktail, Diplodus sargus capensis (Pisces: Sparidae), caught off St. Croix Island, Algoa Bay, South Africa. *African Zool.* 21, 237–243.

Cohen, A.N., 2012. Live Saltwater Bait and the Introduction of Non-native Species into California. Sacramento, CA.

Cohen, A.N., Carlton, J.T., 1995. Nonindigenous aquatic species in a United States estuary.

Cohen, A.N., Weinstein, A., Emmett, M.A., Lau, W., Carlton, J.T., 2001. Investigations into the Introduction of Non-indigenous Marine Organisms via the Cross-Continental Trade in Marine Baitworms, U.S. Fish and Wildlife Service San Francisco Bay Program. Sacramento.

Cole, V.J., Chick, R.C., Hutchings, P.A., 2018. A review of global fisheries for polychaete worms as a resource for recreational fishers: diversity, sustainability and research needs. *Rev. Fish Biol. Fish.* 543–565.

Coleman, F.C., Figueira, W.F., Ueland, S.J., Crowder, L.B., 2004. The Impact of United States Recreational Fisheries on Marine Fish Populations. *Science* 305, 1958–1960.

Conti, G., Massa, F., 1998. Experienze de allavamento del polichete Diopatra neapolitana Delle Chiaje, 1841 nella Laguna di S. Gilla (Sardegna Meridionale). *Biol. Mar. Meditteranea* 5, 1473–1480.

Costa, P.F.E., Gil, J., Passos, A.M., Pereira, P., Melo, P., Batista, F., Da Fonseca, L.C., 2006. The market features of imported non-indigenous polychaetes in Portugal and consequent ecological concerns. *Sci. Mar.* 70S3, 287–292.

Cretchley, R., 1996. Exploitation of the bait organism Upogebia africana (crustacea: Anomura) in the Knysna Estuary. Rhodes University.

Crooks, J.A., 2002. Characterizing ecosystem-level consequences of biological invasions: The role of ecosystem engineers. *Oikos* 97, 153–166.

Cuddington, K., Hastings, A., 2004. Not Invasive engineers. *Ecol. Modell.* 178, 335–347.

Cunha, T., Hall, A., Queiroga, H., 2005. Estimation of the Diopatra neapolitana annual harvest resulting from digging activity in Canal de Mira, Ria de Aveiro. *Fish. Res.* 76, 56–66.

Da Silva, J.M., Willows-Munro, S., 2016. A Review of over a Decade of DNA Barcoding in South Africa: A Faunal Perspective. *African Zool.* 51, 1–12.

Dağli, E., Ergen, Z., Çinar, M.E., 2005. One-year observation on the population structure of Diopatra neapolitana Delle Chiaje (Polychaeta: Onuphidae) in Izmir Bay (Aegean sea, eastern Mediterranean). *Mar. Ecol.* 26, 265–272.

Darling, J.A., Mahon, A.R., 2011. From molecules to management : Adopting DNA-based methods for monitoring biological invasions in aquatic environments. *Environ. Res.* 111, 978–988.

Darling J.A. and Carlton J.T., 2018. A Framework for Understanding Marine Cosmopolitanism in the Anthropocene. Front. Mar. Sci. 5:293. doi: 10.3389/fmars.2018.00293

Day, J.H., 1934. On a collection of South African Polychaeta, with a catalogue of the species recorded from South Africa, Angola, Mosambique, and Madagascar. *J. Linn. Soc. London, Zool.* 39, 15–82.

Day, J.H., 1957. The Polychaete Fuana of South Africa: Part 4 New species and records from Natal and Mozambique. *Ann. South African Museum* 14, 59–129.

Day, J.H., 1960. The Polychaete Fuana of South Africa: Part 5 Errant Species Dredged Off Cape Coasts. *Ann. South African Museum* 45, 375–548.

Day, J.H., 1967. A Monograph on the Polychaeta of Southern Africa Part 1: Errantia. *Ann. South African Museum* 45, 375–548.

Day, J.H., 1969. A guide to marine life on South African shores, Marine life on South African shores. Cape Town : Balkema, Cape Town.

Day, J.H., Millard, N.A.H., Harrison, A.D., 1951. The Ecology of South African Estuaries. *Trans. R. Soc. South Africa* 33, 367–413.

De Carvalho, A.N., Vaz, A.S.L., Sérgio, T.I.B., Dos Santos, P.J.T., 2013. Sustainability of bait fishing harvesting in estuarine ecosystems – Case study in the Local Natural Reserve of Douro Estuary, Portugal. *Rev. Gestão Costeira Integr.* 13, 157–168.

Department of Agriculture Forestry and Fisheries, 2018. Marine Recreational Activity Information Brochure.

Donn, T.E., Cockcroft, A.C., 1989. Macrofuanal community structure and zonation of two sandy beaches on the central Namib coast, South West Africa/Namibia. *Madoqua* 16, 129–135.

Dubula, O., Lasiak, T.A., 2003. Spatial Variability in the Mudprawn Upogebia Africana on the South-east Coast of South Africa. *African J. Mar. Sci.* 25, 275–282.

Elner, R.W., Beninger, P.G., Jackson, D.L., Potter, T.M., 2005. Evidence of a new feeding mode in western sandpiper ( Calidris mauri ) and dunlin ( Calidris alpina ) based on bill and tongue morphology and ultrastructure. *Mar. Biol.* 146, 1223–1234.

Emmerson, W.D., 1983. Tidal exchange of two decapod larvae Palaemon pacificus (Caridea) and Upogebia africana (Thalassinidae) between the Swartkops River estuary and adjacent coastal waters. *South African J. Zool.* 18, 326–330.

Etchegoin, J.A., Merlo, M.J., Parietti, M., 2012. The role of the invasive polychaete Ficopomatus enigmaticus (Fauvel, 1923) (Serpulidae) as facilitator of parasite transmission in Mar Chiquita coastal lagoon (Buenos Aires, Argentina). *Parasitology* 139, 1506–1512.

Fauchald, K., Berke, S.K., Woodin, S.A., 2012. Diopatra (Onuphidae: Polychaeta) from intertidal sediments in southwestern Europe. *Zootaxa* 3395, 47–58.

Fauchald, K., Jumars, P.A., 1979. The diet of worms: a study of polychaete feeding guilds. *Oceanogr. Mar. Biol. An Annu. Rev.* 17, 193–284.

Fauvel, P., 1923a. Polychètes errantes. Kraus reprint.

Fauvel, P., 1923b. Sur quelques Polychetes de Angola Portugaise. *Messag. Gotebg. Museum Zool.* 20.

Fauvel, P., 1930. Annelida Polychaeta of the Madras Government Museum. *Bull. Madras Gov. Museum* 1, 1–75.

Fauvel, P., 1932. Annelida Polychaeta of the Indian Museum, Calcutta. *Mem. Ind. Mus.* 12, 1–262.

Fauvel, P., 1953. Annelida Polychaeta. *Fauna India, Incl. Pakistan, Ceylon, Burma Malaya* 12, 507.

Field, J.G., 1971. A Numerical Analysis of the Changes in the Soft-Bottom Fuana Along a Transect Acress False Bay, South Africa. *J. Exp. Mar. Bio. Ecol.* 7, 215–253.

Fielding, P.J., 2009. The subsistence bait fishery in the Swartkops Estuary, Eastern Cape stock assessment of principal bait organisms. East London.

Folmer, O., Black, M., Hoeh, W., Lutz, R., Vrijenhoek, R., 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol. Mar. Biol. Biotechnol.* 3, 294–299.

Forbes, D., Pillay, G.M., Branch, A.T., 2007. Experimental evidence for the effects of the thalassinidean sandprawn Callianassa kraussi on macrobenthic communities. *Mar. Biol.* 152, 611–618.

Freitas, R., Pires, A., Velez, C., Almeida, Â., Wrona, F.J., Soares, A.M.V.M., Figueira, E., 2015. The effects of salinity changes on the Polychaete Diopatra neapolitana: Impacts on regenerative capacity and biochemical markers. *Aquat. Toxicol.* 163, 167–176.

Gaigher, C.M., 1979. Aspects of the population dynamics and ecology of the Bloodworm (Arenicola loveni Kinberg). University of Cape Town.

Gambi, M.C., Castelli, A., Giangrande, A., Lanera, P., Prevedelli, D., Vandini, R.Z., 1994. Polychaetes of commercial and applied interest in Italy: an overview. *Mèmoires du Musèum Natl. dHistoire Nat.* 162, 593–603.

Gerking, S.D., 2014. Feeding ecology of fish. Elsevier.

Goldberg, C.S., Strickler, K.M., Pilliod, D.S., 2015. Moving environmental DNA methods from concept to practice for monitoring aquatic macroorganisms. *Biol. Conserv.* 183, 1–3.

Griffiths, C.L., Branch, G.M., 1997. The exploitation of coastal invertebrates and seaweeds in south africa: Historical trends, ecological impacts and implications for management. *Trans. R. Soc. South Africa* 52, 121–148.

Griffiths, C.L., Robinson, T.B., Lange, L., Mead, A., 2010. Marine biodiversity in south africa: An evaluation of current states of knowledge. *PLoS One* 5(8): e12008.

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

Hanekom, N., Baird, D., 1992. Growth, production and consumption of the thalassinid prawn *Upogebia africana* (Ortmann) in the Swartkops estuary. *South African J. Zool.* 27, 130–139.

Hanekom, N., Erasmus, T., 1988. Variations in size compositions of populations of Upogebia africana ( Ortmann ) ( Decapoda , Crustacea ) within the Swartkops estuary and possible influencing factors. *South African J. Zool.* 23.

Hanekom, N., Erasmus, T., 1989. Determinations of the reproductive output of populations of a thalassinid prawn Upogebia africana ( Ortmann ) in the Swartkops estuary. *African Zool.* 24, 244–250.

Hanekom, N., Russell, I.A., 2015. Temporal changes in the macrobenthos of sandprawn ( Callichirus kraussi ) beds in Swartvlei Estuary , South Africa beds in Swartvlei Estuary , South Africa. *African Zool.* 50, 41–51.

Harris, J.M., Seiderer, L.J., Lucas, M.I., 1991. Gut Microflora of Two Saltmarsh Detritivore Thalassinid Prawns , Upogebia africana and Callianassa kraussi. *Microb. Ecol.* 21, 277–296.

Harrison, J.A., Cherry, M., 1997. The atlas of southern African birds. BirdLife South Africa Johannesburg.

Harwell, M.C., Orth, R.J., 2001. Influence of a tube-dwelling polychaete on the dispersal of fragmented reproductive shoots of eelgrass. *Aquat. Bot.* 70, 1–7.

Hebert, P.D.N., Cywinska, A., Ball, S.L., DeWaard, J.R., 2003a. Biological identifications through DNA barcodes. *Proc. R. Soc. B Biol. Sci.* 270, 313–321.

Hebert, P.D.N., Hollingsworth, P.M., Hajibabaei, M., 2016. From writing to reading the encyclopedia of life. *Philos. Trans. R. Soc. B Biol. Sci.* 371.

Hebert, P.D.N., Ratnasingham, S., De Waard, J.R., 2003b. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. B Biol. Sci.* 270, S96–S99.

Heemstra, P.C., Heemstra, E., 2004. Coastal fishes of southern Africa. NISC (PTY) LTD.

Henninger, T.O., Froneman, P.W., 2013. Role of the sandprawn Callichirus kraussi as an ecosystem engineer in a South African temporarily open / closed estuary. *African J. Aquat. Sci.* 38, 101–107.

Herbert, D.G., Smith, G.F., Hamer, M., Scholtz, C., 2001. Taxonomy and systematics research in South Africa: vital research facing a crisis in capacity and resources. Pretoria.

Hill, B.J., 1977. The effect of heated effluent on egg production in the estuarine prawn Upogebia africana (Ortman). *J. Exp. Mar. Bio. Ecol.* 29, 291–302.

Hodgson, A.N., Allanson, B.R., Cretchley, R., 2000a. The exploitation of Upogebia africana (Crustacea: Thalassinidae) for bait in the Knysna Estuary. *Trans. R. Soc. South Africa* 55, 197–204.

Hodgson, A.N., Cretchley, R., Allanson, B.R., 2000b. An estimation of the standing stock and population structure of upogebia africana (crustacea: Thalassinidae) in the knysna estuary.

*Trans. R. Soc. South Africa* 55, 187–196.

Hoogenboezem, W., 1991. The analysis of the pharyngeal-sieve mechanism and the efficiency of food intake in the bream (Abramis brama. Cyprinidae). Agriculture University of Wageningen.

Hopkins, G.W., Freckleton, R.P., 2002. Declines in the numbers of amateur and professional taxonomists : implications for conservation. *Anim. Conserv.* 5, 245–249.

Hutchings, P., Kupriyanova, E., 2018. Cosmopolitan polychaetes - Fact or fiction? Personal and historical perspectives. *Invertebr. Syst.* 32, 1–9.

Hutchings, P.A., Karageorgopoulos, P., 2003. Designation of a neotype of Marphysa sanguinea ( Montagu , 1813 ) and a description of a new species of Marphysa from eastern Australia. *Hydrobiol. 496* 497, 87–94.

Jolly, M.T., Viard, F., Gentil, F., Thiébauts, E., Jollivet, D., 2006. Comparative phylogeography of two coastal polychaete tubeworms in the Northeast Atlantic supports shared history and vicariant events. *Mol. Ecol.* 15, 1841–1855.

Jumars, P.A., Dorgan, K.M., Lindsay, S.M., 2015. Diet of Worms Emended : An Update of Polychaete Feeding Guilds. *Ann. Rev. Mar. Sci. 7: 497–520*

Kalejta, B., 1992. Time Budgets and Predatory Impact of Waders at the Berg River Estuary, South Africa. *Ardea -Wageningen-* 80, 327–342.

Kara, J., Macdonald, A.H.H., Simon, C.A., 2018. Integrative taxonomic methods reveal an incorrect synonymisation of the South African Pseudonereis podocirra (Schmarda) as the widespread Pseudonereis variegata (Grube) from Chile. *Invertebr. Syst.* 32, 1282–1297.

Kenny, R., 1969. Temperature tolerance of the polychaete worms Diopatra cuprea and Clymenella torquata. *Mar. Biol.* 4, 219–223.

Knox, G.A., Cameron, D.B., 1971. Port Phillip Bay Survey 1957-1963, Victoria, Australia. Part 2(4) Polychaeta. In: Memoirs of the National Museum of Victoria. Melbourne, pp. 21–42.

Knysna Basin Project, 2019. ShoreSearch [WWW Document]. URL http://knysnabasinproject.co.za/conserve/shoresearch/ (accessed 4.28.19).

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X : Molecular Evolutionary Genetics

Analysis across Computing Platforms. *Mol. Biol. Evol.* 35, 1547–1549.

Largier, J.L., Attwood, C., Harcourt-Baldwin, J.L., 2000. The hydrographic character of the knysna estuary. *Trans. R. Soc. South Africa* 55, 107–122.

Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., Mcgettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23, 2947–2948.

Lavesque, N., Daffe, G., Bonifácio, P., Hutchings, P., 2017. A new species of the Marphysa sanguinea complex from French waters ( Bay of Biscay , NE Atlantic ). *Zookeys* 17, 1–17.

Le, S.E.A., Durell, V.D., 2000. Individual feeding specialisation in shorebirds : population consequences and conservation implications. *Biol. Rev.* 75, 503–518.

Lewis, C., 2005a. Fertilization, post-fertilization development and larval biology of the south african polychaete, arenicola loveni loveni (kinberg 1866). *Invertebr. Reprod. Dev.* 48, 19–30.

Lewis, C., 2005b. Aspects of the reproductive biology of the South African polychaete, Arenicola loveni loveni (Kinberg 1866). *Invertebr. Reprod. Dev.* 48, 147–160.

Lewis, C., Karageorgopoulos, P., 2008. A new species of Marphysa (Eunicidae) from the western Cape of South Africa. *J. Mar. Biol. Assoc. United Kingdom* 88, 277–287.

Lindenmayer, D.B., Gibbons, P., Bourke, M.A.X., Burgman, M., Dickman, C.R., Ferrier, S., Fitzsimons, J., Freudenberger, D., Garnett, S.T., Groves, C., Hobbs, R.J., Kingsford, R.T., Krebs, C., Legge, S., Lowe, A.J., Lean, R.O.B.M., Montambault, J., Possingham, H., Radford, J.I.M., Robinson, D., Smallbone, L., Thomas, D., Varcoe, T., Vardon, M., Wardle, G., 2012. Improving biodiversity monitoring. *Austral Ecol.* 37, 285–294.

Lobo, J., Teixeira, M.A.L., Borges, L.M.S., Ferreira, M.S.G., Hollatz, C., Gomes, P.T., Sousa, R., Ravara, A., Costa, M.H., Costa, F.O., 2016. Starting a DNA barcode reference library for shallow water polychaetes from the southern European Atlantic coast. *Mol. Ecol. Resour.* 16, 298–313.

Lovell, S.J., Stone, S.F., Fernandez, L., 2006. The economic impacts of aquatic invasive species: a review of the literature. *Agric. Resour. Econ. Rev.* 35, 195–208.

Lowe, S., Browne, M., Boudjelas, S., De Poorter, M., 2000. 100 of the world's worst invasive alien species: a selection from the global invasive species database. Invasive Species Specialist Group

Auckland.

Lubke, R., De Moor, I.J. (Eds.), 1998. Field guide to the Eastern & Southern Cape coasts. Juta and Company Ltd.

Luckenbach, M.W., 1986. Sediment stability around animal tubes : The roles of hydrodynamic processes and biotic activity. *Limnol. Oceanogr.* 31, 779–787.

Mabin, C.A., 2017. Carcinus maenas in South Africa : status , impacts and management. Stellenbosch University.

Macnae, W., 1956. Aspects of life on muddy shores in South Africa. *S. Afr. J. Sci.* 53, 40–44.

Macnae, W., 1957. The Ecology of Plants and Animals in the Intertidal Regions of the Zwartkops Estuary , Near Port Elizabeth , South Africa. *J. Ecol.* 45, 361–387.

Macpherson, E., 1989. Influence of geographical distribution, body size and diet on population density of benthic fishes off Namibia (South West Africa). *Mar. Ecol. Prog. Ser.* 50, 295–299.

Mangum, C.P., Cox, C.D., 1971. Analysis of the Feeding Response in the Onuphid Polychaete Diopatra cuprea ( Bosc ). *Biol. Bull.* 140, 215–229.

Mangum, C.P., Santos, S.L., Rhodes, W.R.J., 1968. Distribution and feeding in the onuphid polychaete , Diopatra cuprea ( Bosc ). *Mar. Biol.* 2, 33–34.

Marais, J.F.K., 1984. Feeding ecology of major carnivorous fish from four eastern Cape estuaries. *South African J. Zool.* 19, 210–223.

Marker, M.E., 2000. A descriptive account of sand movement in the knysna estuary. *Trans. R. Soc. South Africa* 55, 129–139.

Marker, M.E., Maree, B., 2004. The impact of the 1996 floods on a tidal sand bank in the Knysna estuary, Western Cape. *S. Afr. J. Sci.* 100, 334–336.

Martens, K., Hamer, M., 1999. Taxonomy and biodiversity. *Hydrobiologia* 397, 1–3.

Masifundise Development Trust, 2010. A handbook towards sustainable small-scale fisheries in South Africa.

McGrath, M.D., Horner, C.C.M., Brouwer, S.L., Lamberth, S.J., Mann, B.Q., Sauer, W.H.H., Erasmus, C.,

1997. An economic valuation of the South African linefishery. *South African J. Mar. Sci.* 18, 203–211.

McIntosh, W.C., 1925. A second contribution to the marine polychaetes of South Africa. *Union South Africa Fish. Mar. Biol. Surv. Cape T.* 4, 1–93.

Mclachlan, A., Cockcroft, A.C., Malan, D.E., 1984. Benthic faunal response to a high energy gradient. *Mar. Ecol. Prog. Ser.* 16, 51–63.

McPhee, D.P., Leadbitter, D., Skilleter, G.A., 2002. Swallowing the bait: Is recreational fishing in Australia ecologically sustainable? *Pacific Conserv. Biol.* 8, 40–51.

Mendes, R.N., Paci, S., Mclaughlin, P., Gherardi, F., Emmerson, W., 2001. Combined effects of temperature and salinity on the larval development of the estuarine mud prawn Upogebia africana ( Crustacea , Thalassinidea ). *Hydrobiologia* 449, 141–148.

Meyer, C.P., Paulay, G., 2005. DNA barcoding: Error rates based on comprehensive sampling. *PLoS Biol.* 3: e422.

Miehls, A.L.J., Mason, D.M., Frank, K.A., Krause, A.E., Peacor, S.D., Taylor, W.W., 2009. Invasive species impacts on ecosystem structure and function: A comparison of Oneida Lake, New York, USA, before and after zebra mussel invasion. *Ecol. Modell.* 220, 3194–3209.

Miller, R.J., Smith, S.J., 2012. Nova Scotia’s bloodworm harvest: Assessment, regulation, and governance. *Fish. Res.* 113, 84–93.

Molina-acevedo, I.C., Carrera-parra, L.F., 2016. Reinstatement of three species of the Marphysa sanguinea complex (Polychaeta: Eunicidae) from the Grand Caribbean Region. *Zootaxa* 2925.

Monro, C.C.A., 1930. Polychaete worms. Cambridge University Press.

Monro, C.C.A., 1936. Notes on some Polychaeta from the Congo coast. *Rev. Zool. Bot. Africanes* 28, 245–248.

Mooney, H.A., Cleland, E.E., 2001. The evolutionary impact of invasive species. *Proc. Natl. Acad. Sci. U. S. A.* 98, 5446–51.

Morais, P., Reichard, M., 2018. Cryptic invasions : A review. *Sci. Total Environ.* 613–614, 1438–1448.

Napier, V.R., Turpie, J.K., Clark, B.M., 2009. Value and management of the subsistence fishery at

Knysna Estuary, South Africa. *African J. Mar. Sci.* 31, 297–310.

National Minimum Wage Panel, 2016. Recommendations on Policy and Implementation. National Minimum Wage Panel Report To the Deputy President. 1–129.

Nel, P., Branch, G.M., 2013. Assessment of the abundance and distribution of burrowing sandprawns and mudprawns (Callichirus and Upogebia species) in Langebaan Lagoon, South Africa. *African J. Mar. Sci.* 35, 195–208.

Nel, P., Branch, G.M., 2014. The effect of bait collectors on stocks of Callichirus kraussi and Upogebia species in Langebaan Lagoon. *African Zool.* 49, 30–43.

Niemelä, J., 2000. Biodiversity monitoring for decision-making. *Ann. Zool. Fennici* 307–317.

Nishi, E., Kato, T., 2004. Introduced and globally invaded polychaetous annelids. *Japanese J. Benthol.* 59, 83–95.

Novo, M., Almodóvar, A., Fernández, R., Trigo, D., Díaz Cosín, D.J., 2010. Cryptic speciation of hormogastrid earthworms revealed by mitochondrial and nuclear data. *Mol. Phylogenet. Evol.* 56, 507–512.

Nygren, A., 2014. Cryptic polychaete diversity: A review. *Zool. Scr.* 43, 172–183.

Nygren, A., Parapar, J., Pons, J., Meißner, K., Bakken, T., Kongsrud, J.A., Oug, E., Gaeva, D., Sikorski, A., Johansen, R.A., Hutchings, P.A., Lavesque, N., Capa, M., 2018. A mega-cryptic species complex hidden among one of the most common annelids in the north east Atlantic, PLoS ONE 13(6): e0198356.

Parameswaran, V.S., 1973. Distribution of Diopatra neapolitana Delle Chiaje (Polychaeta) in the South-West Coast of India. *Mar. Sci.* 62–63.

Patwasdhan, A., Samit, R., Amit, R., 2014. Molecular Markers in Phylogenetic Studies-A Review. *J. Phylogenetics Evol. Biol.* 2:131 doi:10.4172/2329- 9002.1000131.

Paxton, H., 1979. Taxonomy and aspects of the life history of Australian beachworms (Polychaeta: Onuphidae). *Mar. Freshw. Res.* 30, 265–294.

Paxton, H., 1986. Generic revision and relationships of the family Onuphidae (Annelida: Polychaeta), Records of the Australian Museum. Australian Museum, Sydney 38: 1–74.

Paxton, H., 1993. Diopatra Audouin and Milne Edwards (Polychaeta: Onuphidae) from Australia, with a discussion of developmental patterns in the genus. *Beagle, Rec. North. Territ. Museum Arts Sci.* 10, 115–154.

Paxton, H., 1998. The Diopatra chiliensis confusion - Redescription of D. chiliensis (Polychaeta, onuphidae) and implicated species. *Zool. Scr.* 27, 31–48.

Paxton, H., 2016. A new species of Diopatra (Annelida: Onuphidae) from Namibia, southwestern Africa. *Mar. Biodivers.* 46, 889–895.

Pentinsaari, M., Salmela, H., Mutanen, M., Roslin, T., 2016. Molecular evolution of a widely-adopted taxonomic marker (COI) across the animal tree of life. *Sci. Rep.* 6.

Pillay, D., Branch, G.M., Dawson, J., Henry, D., 2011. Contrasting effects of ecosystem engineering by the cordgrass Spartina maritima and the sandprawn Callianassa kraussi in a marine-dominated lagoon. *Estuar. Coast. Shelf Sci.* 91, 169–176.

Pillay, D., Branch, G.M., Forbes, A.T., 2007. The influence of bioturbation by the sandprawn Callianassa kraussi on feeding and survival of the bivalve Eumarcia paupercula and the gastropod Nassarius kraussianus. *J. Exp. Mar. Bio. Ecol.* 344, 1–9.

Pillay, D., Branch, G.M., Griffiths, C.L., Williams, C., Prinsloo, A., 2010. Ecosystem change in a South African marine reserve (1960-2009): Role of seagrass loss and anthropogenic disturbance. *Mar. Ecol. Prog. Ser.* 415, 35–48.

Pillay, D., Williams, C., Whitfield, A.K., 2012. Indirect effects of bioturbation by the burrowing sandprawn Callichirus kraussi on a benthic foraging fish , Liza richardsonii. *Mar. Ecol. Prog. Ser.* 453, 151–158.

Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* 52, 273–288.

Pires, A., Figueira, E., Moreira, A., Soares, A.M.V.M., Freitas, R., 2015. The effects of water acidification, temperature and salinity on the regenerative capacity of the polychaete Diopatra neapolitana. *Mar. Environ. Res.* 106, 30–41.

Pires, A., Paxton, H., Quintino, V., Rodrigues, A.M., 2010. Diopatra (Annelida: Onuphidae) diversity in European waters with the description of Diopatra micrura, new species. *Zootaxa* 2395, 17–33.

Pires, A., Quintino, V., Gentil, F., Freitas, R., Rodrigues, A.M., 2012. Reproductive biology of a brooding Diopatra species: Diopatra marocensis Paxton et al., 1995. *Estuar. Coast. Shelf Sci.* 110, 85–92.

Robinson, T.B., Griffiths, C.L., McQuaid, C.D., Rius, M., 2005. Marine alien species of South Africa - Status and impacts. *African J. Mar. Sci.* 27, 297–306.

Rodrigues, A.M., Pires, A., Mendo, S., Quintino, V., 2009. Diopatra neapolitana and Diopatra marocensis from the Portuguese coast: Morphological and genetic comparison. *Estuar. Coast. Shelf Sci.* 85, 609–617.

Rodriguez, L.F., 2006. Can invasive species facilitate native species? Evidence of how, when, and why these impacts occur. *Biol. Invasions* 8, 927–939.

Roozen, F.C.J.M., Lürling, M., Vlek, H., Van Der Pouw Kraan, E.A.J., Ibelings, B.W., Scheffer, M., 2007. Resuspension of algal cells by benthivorous fish boosts phytoplankton biomass and alters community structure in shallow lakes. *Freshw. Biol.* 52, 977–987.

Russell, I.A., 1996. Water quality in the Knysna estuary. *Koedoe 39(1): 1–8*.

Schaefer, N., 1970. The functional morphology of the fore-gut of three species of decapod Crustacea: Cyclograpsus punctatus Milne-Edwards, Diogenes brevirostris Stimpson and Upogebia africana (Ortmann). *Zool. Africana* 5, 309–326.

Schmeller, D.S., Henry, P., Julliard, R., Gruber, B., Clobert, J., Dziock, F., Lengyel, S., Nowicki, P., 2008. Advantages of Volunteer-Based Biodiversity Monitoring in Europe. *Conserv. Biol.* 23, 307–316.

Schumann, E.H., 2000. Oceanic exchanges and temperature variability in the Knysna Estuary. *Trans. R. Soc. South Africa* 55, 123–128.

Schwindt, E., Iribarne, O.O., 2000. Settlement sites, survival and effects on benthos of an introduced reef-building polychaete in a SW Atlantic coastal lagoon. *Bull. Mar. Sci.* 67, 73–82.

Shepherd, P.C.F., Boates, J.S., 1999. Effects Bay of of a Conunercial Baitworm and Harvest on in the Semipaliated Sandpipers Fundy Hemispheric Their Prey Reserve Shorebird. *Conserv. Biol.* 13, 347–356.

Siebert, T., Branch, G.M., 2005. Interactions between Zostera capensis, Callianassa kraussi and Upogebia africana: deductions from field surveys in Langebaan Lagoon , South Africa. *African J.*

*Mar. Sci.* 27, 345–356.

Siebert, T., Branch, G.M., 2006. Ecosystem engineers: Interactions between eelgrass Zostera capensis and the sandprawn Callianassa kraussi and their indirect effects on the mudprawn Upogebia africana. *J. Exp. Mar. Bio. Ecol.* 338, 253–270.

Simberloff, D., 2002. Today Tiritiri Matangi, tomorrow the world! Are we aiming too low in invasives control? In: Veitch, C.R., Clout, M.N. (Eds.), Turning the Tide: The Eradication of Invasive Species. IUCN SSC Invasive Species Specialist Group, Gland, Switzerland and Cambridge, UK, pp. 4–12.

Simberloff, D., Claudi, R., Hendrickson, O., Ottens, H., 2002. Managing established populations of introduced species. *Alien Invasive Species A Threat to Can. Biodiversity. Ottawa Nat. Resour. Canada, Can. For. Serv.* 269–278.

Simon, C.A., du Toit, A.N., Smith, M.K.S., Claassens, L., Smith, F., Smith, P., 2019a. Bait collecting by subsistence and recreational fishers in Knysna Estuary may impact management and conservation. *African Zool.*

Simon, C.A., Sato-Okoshi, W., Abe, H., 2019b. Hidden diversity within the cosmopolitan species Pseudopolydora antennata (Claparède, 1869) (Spionidae: Annelida). *Mar. Biodivers.* 49, 25–42.

Simon, C.A., Williams, L., Henninger, T., 2018. A new species of Rhynchospio (Annelida: Spionidae) in South Africa. *Mar. Biodivers.* 49, 663–672.

Smith, F., Smith, P., 2012. A brief field guide to the common shore creatures of the Knysna Estuary, 1st ed. The Knysna Basin Project, Knysna.

Smith, G.F., Buys, M., Herbert, D., Hamer, M., 2008. Taxonomic research in South Africa: the state of the discipline. *S. Afr. J. Sci.* 104, 254–259.

Stoeckle, M.Y., 2003. The Bar Code of Life. *Bioscience* 53, 796–797.

Stoeckle, M.Y., Thaler, D.S., 2018. Why should mitochondria define species? *Hum. Evol.* 33, 1–30.

Struck, T., Purschke, G., Halanych, K., 2006. Phylogeny of Eunicida (Annelida) and exploring data congruence using a partition addition bootstrap alteration (PABA) approach. *Syst. Biol.* 55, 1–20.

Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J., Rambaut, A., 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1 . 10 4, 1–5.

Sypitkowski, E., Ambrose, W.G., Bohlen, C., Warren, J., 2009. Catch statistics in the bloodworm fishery in Maine. *Fish. Res.* 96, 303–307.

Teske, P.R., Cherry, M.I., Matthee, C.A., 2003. Population genetics of the endangered Knysna seahorse, hippocampus capensis. *Mol. Ecol.* 12, 1703–1715.

Thompson, L.C., Pritchard, A.W., 1969. Osmoregulatory Capacities of Callianassa and Upogebia( Crustacea : Thalassinidea). *Biol. Bull.* 136, 114–129.

Thomsen, M.S., McGlathery, K., 2005. Facilitation of macroalgae by the sedimentary tube forming polychaete Diopatra cuprea. *Estuar. Coast. Shelf Sci.* 62, 63–73.

Thomsen, M.S., Muth, M.F., McGlathery, K.J., 2011. Tube-forming polychaetes enhance invertebrate diversity and abundance in sandy sediments of Mozambique , Africa. *African J. Mar. Sci.* 33, 327–332.

Thomsen, P.F., Willerslev, E., 2015. Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity. *Biol. Conserv.* 183, 4–18.

Treadwell, A.L., 1943. Polychaetous annelids. *Biol. results last cruise Carnegie (Scientific results cruise VII Carnegie Dur. 1928–1929). Carniege Inst. Washingt. Monogr. Ser.* 555, 31–59.

Treadwell, A.L., Oesch, G.R., Gibbs, W., 1943. Polychaetous annelids from the Philippine Islands in the collections of the American Museum of Natural History. *Bull. Am. Mus. Nat. Hist.*

Turpie, J.K., Clark, B., 2007. Development of a Conservation Plan for Temperate South African Estuaries on the Basis of Biodiversity Importance , Ecosystem Health and Economic Costs and Benefits, Ecosystem Health and Economic Costs and Benefits-Final Report (CAPE Regional Estuarine Management Programme).

Turpie, J.K., Heydenrych, B.J., Lamberth, S.J., 2003. Economic value of terrestrial and marine biodiversity in the Cape Floristic Region: Implications for defining effective and socially optimal conservation strategies. *Biol. Conserv.* 112, 233–251.

van der Walt, P., Geldenhuys, N., Joubert, P., Lawson, B., Nel, J., Randall, R., Retief, P., Russell, I., Yssel, S., 1990. Knysna National Lake Area: Master Plan Conservation Objectives.

van der Westhuizen, H.C., Marais, J.F.K., 1977. Stomach Content Analyses of Pomadasys Commersonni from the Swartkops Estuary (Pisces: Pomadasyidae). *Zool. Africana* 12, 500–504.

van der Zanden, M.J., Hansen, G.J.A., Higgins, S.N., Kornis, M.S., 2010. A pound of prevention , plus a pound of cure : Early detection and eradication of invasive species in the Laurentian Great Lakes. *J. Great Lakes Res.* 36, 199–205.

van Herwerden, L., 1989. Collection of mussel worms pseudonereis variegata for bait — A legislative anachronism. *South African J. Mar. Sci.* 8, 363–366.

Veitch, C.R., Clout, M.N., 2002. Turning the tide: the eradication of invasive species. *Proc. Int. Conf. Erad. Isl. Invasives* 414.

Vences, M., Thomas, M., Van Der Meijden, A., Chiari, Y., Vieites, D.R., 2005. Comparative performance of the 16S rRNA gene in DNA barcoding of amphibians. *Front. Zool.* 2.

Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y., Pyšek, P., 2011. Ecological impacts of invasive alien plants: A meta-analysis of their effects on species, communities and ecosystems. *Ecol. Lett.* 14, 702–708.

Volkenborn, N., Robertson, D.M., Reise, K., 2009. Sediment destabilizing and stabilizing bio-engineers on tidal flats: Cascading effects of experimental exclusion. *Helgol. Mar. Res.* 63, 27–35.

Watson, G.J., Farrell, P., Stanton, S., Skidmore, L.C., 2007. Effects of bait collection on Nereis virens populations and macrofaunal communities in the Solent, UK. *J. Mar. Biol. Assoc. United Kingdom* 87, 703–716.

Watson, G.J., Murray, J.M., Schaefer, M., Bonner, A., 2016. Bait worms: a valuable and important fishery with implications for fisheries and conservation management. *Fish Fish.* 18, 374–388.

Wehe, T., Fiege, D., 2002. Annotated checklist of the polychaete species of the seas surrounding the Arabian Peninsula : Red Sea , Gulf of Aden , Arabian Sea , Gulf of Oman , Arabian Gulf. *Fauna Arab.* 19, 7–238.

Wheater, C.P., Bell, J.R., Cook, P.A., 2011. Calculating population and density estimates from counts of static organisms. In: Practical Field Ecology: A Project Guide. Wiley-Blackwell, Oxford, pp. 69–70.

Whibley, I., 2003. The South African fisherman. Struik, Cape Town.

Woodin, S.A., 1981. Disturbance and Community Structure in a Shallow Water Sand Flat. *Ecology* 62, 1052–1066.

Wooldridge, T.H., Loubser, H., 1996. Larval release rhythms and tidal exchange in the estuarine mudprawn, Upogebia africana. *Hydrobiologia* 337, 113–121.

Wynberg, R.P., Branch, G.M., 1991. An assessment of bait-collecting for callianassa kraussi stebbing in langebaan lagoon, western cape, and of associated avian predation. *South African J. Mar. Sci.* 11, 141–152.

Wynberg, R.P., Branch, G.M., 1994. Disturbance associated with bait-collection for sandprawns (Callianassa kraussi) and mudprawns (Upogebia africana): Long-term effects on the biota of intertidal sandflats. *J. Mar. Res.* 52, 523–558.

Wynberg, R.P., Branch, G.M., 1997. Trampling associated with bait-collection for sandprawns Callianassa kraussi Stebbing: Effects on the biota of an intertidal sandflat. *Environ. Conserv.* 24, 139–148.

Yearsley, R.D., Jones, C.L.W., Britz, P.J., Vine, N.G., 2011. Integrated culture of silver kob Argyrosomus inodorus and bloodworm Arenicola loveni loveni in abalone farm effluent. *African J. Mar. Sci.* 33, 223–228.

Younsi, M., Daas, T., Daas, O., Scaps, P., 2010. Polychaetes of commercial interest from the Mediterranean East Coast of Algeria. *Mediterr. Mar. Sci.* 11, 185–187.

Zanol, J., Halanych, K.M., Struck, T.H., Fauchald, K., 2010. Phylogeny of the bristle worm family Eunicidae (Eunicida, Annelida) and the phylogenetic utility of noncongruent 16S, COI and 18S in combined analyses. *Mol. Phylogenet. Evol.* 55, 660–676.

Zanol, J., Silva, S.C., Hutchings, P., 2016. Marphysa ( Eunicidae , polychaete , Annelida ) species of the Sanguinea group from Australia , with comments on pseudo-cryptic species. *Invertebr. Biol.* 135, 328–344.

Zavaleta, E.S., Hobbs, R.J., Mooney, H.A., 2001. Viewing invasive species removal in a whole-ecosystem context. *Trends Ecol. Evol.* 16, 454–459.

Zenetos, A., Gofas, S., Morri, C., Rosso, A., Violanti, D., García Raso, J.E., Çinar, M.E., Almogi Labin, A., Ates, A., Azzuro, E., Ballesteros, E., Bianchi, C.N., Bilecenoglu, M., C, G.M., Giangrande, A., Gravili, C., Hyams-Kaphzan, O., Karachle, V., Katsanevakis, S., Lipej, L., Mastrototaro, F., Mineur, F., Pancucci-Papadopoulou, M. a, Ramos Esplá, A., Salas, C., San Martín, G., Sfriso, A., Streftaris, N., Verlaque, M., 2012. Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union's Marine Strategy Framework Directive ( MSFD ). Part 2 . Introduction trends and pathways. *Mediteranean Mar. Sci.* 13, 328–352.

Zhou, H., Zhang, Z., Chen, H., Sun, R., Wang, H., Guo, L., Pan, H., 2010. Integrating a DNA barcoding project with an ecological survey: A case study on temperate intertidal polychaete communities in Qingdao, China. *Chinese J. Oceanol. Limnol.* 28, 899–910.

# Appendix

## PCR conditions

| **Marker** | **Primer Name** | **Direction** | **Primer Sequence** | **Cycle Conditions** |
|---|---|---|---|---|
| **COI** | LCO1490 | Forward | GGT CAA CAA ATC ATA AAG ATA TTG G | 1 cycle: 94 °C/3 min<br>40 cycles: 94°C/1min , 40°C/1min, 72°C/90 s<br>1 cycle: 72°C/7 min |
| | HCO2198 | Reverse | TAA AAC TTC AGG GTG ACC AAA AAA TCA | |
| **16S** | 16SarL | Forward | CGC CTG TTT ATC AAA AAC AT | 1 cycle: 94 °C/3 min<br>40 cycles: 94°C/30 s, 51°C/30 s, 72°C/70 s<br>1 cycle: 72°C/7 min |
| | 16S-OnuR | Reverse | GTC TGA ACT CAG CTC ACG TAG G | |

# COI sequence information

| **Ascension ID** | **Species name** | **Species authority** | **Sequence origin** | **Reference** |
|---|---|---|---|---|
| AY838867 | *Diopatra aciculata* | Knox & Cameron, 1971 | Australia | Struck et al., 2006 |
| GQ497522 | *Diopatra dentata* | Kinberg, 1865 | Australia | Zanol et al., 2010 |
| EU878539 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Portugal | Rodrigues et al., 2009 |
| JQ950321 | *Diopatra neapolitana* | Delle Chiaje, 1841 | France | Pires et al., 2012 |
| KF815718 | *Diopatra neapolitana* | Delle Chiaje, 1841 | India | Genbank entry (Unpublished) |
| GQ456164 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Portugal | Rodrigues et al., 2009 |
| KT992099 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Spain | Arias et al., 2016 |
| KT992100 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Spain | Arias et al., 2016 |
| FJ428832 - FJ428835 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Spain, France, Italy | Berke et al., 2010 |
| FJ428846 - FJ428866 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Spain, France, Italy | Berke et al., 2010 |
| FJ428877 - FJ428889 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Spain, France, Italy | Berke et al., 2010 |
| FJ428895 - FJ428913 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Spain, France, Italy | Berke et al., 2010 |
| FJ428926 - FJ428951 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Spain, France, Italy | Berke et al., 2010 |
| KR916810 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Portugal | Lobo et al., 2016 |
| FJ428890 - FJ428894 | *Diopatra cuprea* | Bosc, 1802 | Spain, France, Italy | Berke et al., 2010 |
| FJ428914 - FJ428925 | *Diopatra marocensis* | Paxton, Fadlaoui & Lechapt, 1995 | Spain, France, Italy | Berke et al., 2010 |
| FJ646632 | *Diopatra marocensis* | Paxton, Fadlaoui & Lechapt, 1995 | Portugal | Rodrigues et al., 2009 |
| GQ456165 | *Diopatra marocensis* | Paxton, Fadlaoui & Lechapt, 1995 | Portugal | Rodrigues et al., 2009 |
| GQ456161 | *Diopatra micrura* | Pires, Paxton, Quintino & Rodrigues, 2010 | Portugal | Pires et al., 2010 |
| GQ456162 | *Diopatra micrura* | Pires, Paxton, Quintino & Rodrigues, 2010 | Portugal | Pires et al., 2010 |
| MBI-SCCWRP-00172 (BOLD ID) | *Diopatra ornata* | Moore, 1911 | USA - California | Direct Submission |

| | | | | |
|---|---|---|---|---|
| ECOSUR-OH-P0040 (BOLD ID) | *Eunice chicasi* | Leon-Gonzalez, Rivera & Romero, 2004 | Mexico | Direct Submission |
| ECOSUR-OH-P0042 (BOLD ID) | *Eunice chicasi* | Leon-Gonzalez, Rivera & Romero, 2004 | Mexico | Direct Submission |
| ECOSUR-OH-P0043 (BOLD ID) | *Eunice chicasi* | Leon-Gonzalez, Rivera & Romero, 2004 | Mexico | Direct Submission |
| ECOSUR-OH-P0046 (BOLD ID) | *Eunice chicasi* | Leon-Gonzalez, Rivera & Romero, 2004 | Mexico | Direct Submission |
| ECOSUR-OH-P0050 (BOLD ID) | *Eunice chicasi* | Leon-Gonzalez, Rivera & Romero, 2004 | Mexico | Direct Submission |

## 16S sequence information

| Genbank ID | Species name | Species authority | Sequence origin | Reference |
|---|---|---|---|---|
| AY838826.1 | *Diopatra aciculata* | Knox & Cameron, 1971 | Australia | Struck et al., 2006 |
| GQ478129.1 | *Diopatra dentata* | Kinberg, 1865 | Australia | Zanol et al., 2010 |
| KT992097.1 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Spain | Arias et al., 2016 |
| KT992096.1 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Spain | Arias et al., 2016 |
| KT992095.1 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Spain | Arias et al., 2016 |
| EU878538.1 | *Diopatra neapolitana* | Delle Chiaje, 1841 | Portugal | Rodrigues et al., 2009 |
| JQ950322.1 | *Diopatra neapolitana* | Delle Chiaje, 1841 | France | Pires et al., 2012 |
| GQ456163.1 | *Diopatra micrura* | Pires, Paxton, Quintino & Rodrigues, 2010 | Portugal | Pires et al., 2010 |
| FJ473306.1 | *Diopatra marocensis* | Paxton, Fadlaoui & Lechapt, 1995 | Portugal | Rodrigues et al., 2009 |
| KJ027327.1 | *Diopatra sugokai* | Izuka, 1907 | Japan | Budaeva et al., 2016 |
| KJ027326.1 | *Diopatra sugokai* | Izuka, 1907 | Japan | Budaeva et al., 2016 |
| KJ027324.1 | *Diopatra ornata* | Moore, 1911 | USA | Budaeva et al., 2016 |
| KJ027328.1 | *Diopatra tuberculantennata* | Budaeva & Fauchald, 2008 | Belize | Budaeva et al., 2016 |
| KJ027329.1 | *Diopatra tuberculantennata* | Budaeva & Fauchald, 2008 | Belize | Budaeva et al., 2016 |
| GQ478147.1 | *Eunice norvegica* | Linnaeus, 1767 | Stockholm | Zanol et al., 2010 |