A systematic review of the so-called cosmopolitan polydorid *Polydora hoplura* Claparède, 1869 (Polychaeta: Spionidae) on the South African coast

Thesis presented in fulfilment of the requirements for the degree of Master of Science in the Faculty of Science at Stellenbosch University

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Declaration

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Abstract

Introduction: *Polydora hoplura* is considered a cosmopolitan or alien species on the South African coast and is an important pest affecting farmed abalone and oysters in the region. The first description of *P. hoplura* by Day (1954) on the South African coast is not comprehensive, and the documentation provides no relevant species-specific morphological information. Regional sampling conducted for the present study revealed that *P. hoplura* varied intraspecifically and consisted of at least four distinct morphotypes that may potentially represent different species that may warrant redescription. By combining traditional taxonomic methods with molecular techniques, the following questions were addressed: 1) Do the morphotypes of *P. hoplura* represent a single species on the South African coast? and 2) Are the South African representatives of *P. hoplura* conspecific to those collected globally? In addition, the study included a literature review with the aim to provide a brief history of the species to assess its cosmopolitanism.

Materials and methods: Newly sampled specimens used in the morphological observations were compared with specimens from private collections and museum material. Traditional taxonomic characteristics were used to distinguish the four morphotypes, and these included morphological features, pigmentation patterns, aspects of reproduction and habitat preference. A cluster analysis was performed to assess the validity of the morphotypes. Furthermore, these morphotypes were also tested for potential genetic differentiation using both mitochondrial (Cytochrome b) and nuclear (28S) gene fragments. To gain preliminary insights into the level of global geographic genetic variation, South African specimens used in the molecular analysis were compared to a few specimens collected in New Zealand.

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Results: Genetic data obtained from mitochondrial DNA and nuclear DNA failed to differentiate the four morphotypes, suggesting that *P. hoplura* represented a single morphologically polymorphic species on the South African coast.

Morphology: Morphotypes 1–3 were recognised as adult forms of the species, while morphotype 4 represented the first record of a juvenile form of the species.

Pigmentation patterns: Morphotype 1 was characterised by the presence of dark pigmentation in the anterior region and morphotypes 2 and 3 by the absence of pigmentation. Morphotype 4 had distinct pigmentation that resembled that of late-stage larvae.

Aspects of reproduction: *P. hoplura* is poecilogenous, producing both planktotrophic and adelphophagic larvae. Late-stage adelphophagic larvae are morphologically similar to larvae at the same stage from a previous study conducted by Wilson (1928).

Habitat preferences: The cluster analysis and genetic investigation both showed that the species was not strictly host specific since individuals collected from abalone, oysters, scallops and sand showed genetic 'panmixia'.

Conclusions: Different *P. hoplura* morphotypes collected along the South African coastline represent the same gene pool when compared at the molecular level. Furthermore, the South African specimens are molecularly similar to specimens collected in New Zealand. The cosmopolitanism of the species could not be fully assessed as specimens from the Northern Hemisphere, particularly from the type locality, were not included in the study. It is concluded that the species has been introduced into South Africa, in accordance with a previous study (Mead *et al.*, 2011).

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Opsomming

Inleiding: *Polydora hoplura* word as ’n kosmopolitaans of indringerspesie aan die Suid-Afrikaanse beskou en is een van die vernaamste plae wat perlemoen- en oesterboerdery in die streek raak. Die eerste beskrywing van *P. hoplura* aan die Suid-Afrikaanse kus deur Day (1954) is nie omvattend nie en die dokumentasie bied geen relevante spesiespesifieke morfologiese inligting nie. Streeksgebonde monsters wat vir die huidige studie geneem is, toon aan dat *P. hoplura* varieer op intraspesievlak en bestaan uit minstens vier verskillende morfotipes wat potensieel verskillende spesies verteenwoordig wat moontlik herbeskrywing sou regverdig. Deur tradisionele taksonomiese metodes met molekulêre tegnieke te kombineer, is die volgende vraagstukke aangepak: 1) Verteenwoordig die morfotipes van *P. hoplura* ’n enkele spesie langs die Suid-Afrikaanse kus? en 2) Is die Suid-Afrikaanse verteenwoordigers van *P. hoplura* konspesifiek met die wat wêreldwyd versamel is? Daarbenewens sluit die studie ’n literatuuroorsig in waarmee gepoog word om ’n kort uiteensetting van die spesie se geskiedenis te bied sodat die kosmopolitanisme daarvan beoordeel kan word.

Materiaal en metodologie: Eksemplare gemonster vir die doeleindes van hierdie studie is gebruik in die morfologiese waarnemings en is vergelyk met eksemplare uit privaat versamelings en museummateriaal. Tradisionele taksonomiese eienskappe is gebruik om die vier morfotipes te onderskei en dit het morfologiese kenmerke, pigmentasiepatrone, voortplantingsaspekte en habitatvoorkeur ingesluit. ’n Trosanalise is uitgevoer om die geldigheid van die morfotipes te bepaal. Verder is die morfotipes ook getoets vir moontlike genetiese differensiasie deur sowel mitochondriale (Sitokroom b) as nuklière (28S) geenfragmente te gebruik. Om voorlopige insig te verkry in die vlakke van globale geografiese genetiese variasie, is Suid-Afrikaanse eksemplare wat in die molekulêre ontleding gebruik is, vergelyk met ’n aantal eksemplare wat in Nieu-Seeland versamel is.

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Resultate: Genetiese data afkomstig uit mitochondriale DNS en nukliêre DNS het nie daarin geslaag om die vier morfotipes te differensieer nie, wat aandui dat *P. hoplura* 'n enkele polimorfiese (morfologies gesproke) spesie aan die Suid-Afrikaanse kus verteenwoordig.

Morfologie: Morfotipes 1 tot 3 word beskou as volwasse vorme van die spesie, terwyl morfotipe 4 die eerste aantekening van 'n jong vorm van die spesie verteenwoordig.

Pigmentasiepatrone: Morfotipe 1 word gekenmerk deur die teenwoordigheid van donker pigmentasie aan die voorkant en morfotipes 2 en 3 deur die afwesigheid van pigmentasie. Morfotipe 4 het kenmerkende pigmentasie wat ooreenkomstig is met die van laatfaselarwes.

Voortplantingsaspekte: *P. hoplura* is poekilogeen en produseer sowel planktotrofiese as adelfofagiese larwes. Laatfase- adelfofagiese larwes stem morfologies ooreen met larwes in dieselfde fase uit 'n vorige studie deur Wilson (1928).

Habitatvoorkeur: Die trosanalise en genetiese ondersoek toon beide aan dat die spesie nie noodwendig gasheerspesifiek is nie, aangesien individue wat versamel is uit perlemoen, oesters, kammossels en sand genetiese 'panmixia' (lukrake paring) vertoon.

Gevolgtrekkings: Verskillende *P. hoplura* morfotipes wat langs die Suid-Afrikaanse kus versamel is, verteenwoordig dieselfde genepoel wanneer dit op 'n molekulêre vlak vergelyk word. Daarbenewens stem die Suid-Afrikaanse eksemplare molekulêre ooreen met eksemplare wat in Nieu-Seeland versamel is. Die kosmopolitiese aard van die spesie is nie volledig geassesseer nie aangesien eksemplare uit die Noordelike Halfrond, veral dié uit die tipeligging, nie by die studie ingesluit is nie. Daar word tot die gevolgtrekking gekom dat die spesie in Suid-Afrika ingebring is, in ooreenstemming met 'n vorige studie.

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A great deal of gratitude is owed to my two supervisors, Dr C.A. Simon and Prof C.A. Matthee, for their continuous support and guidance during my study. I would also like to thank the following people: S. de Lange, M. Boonzaaier and D. Jantjies for help with sampling; L. Williams for help with the molecular analysis and guidance on the subject; A. David for help with the larval development and the four larval sequences; M. Frazerburg at the Central Analytical Facility (Stellenbosch University) for guidance with regard to scanning electron microscopy; and Dr G. Read from the National Institute of Water and Atmospheric Research in New Zealand for kindly collecting specimens and donating them to the study. I am very grateful to my family for their continuous support and especially Ethan Newman for all the cups of tea and discussion. I am furthermore very grateful for the funding from the National Research Foundation through Dr Carol Simon.

This thesis is dedicated to my father, Eugene Stephen van Niekerk, who never got to see the final product. But whose memory continues to inspire and guide me.

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branchiae (b) from chaetiger 7 onwards; (B) lateral view showing lateral organs (lo) on chaetigers 2 and 3; (C) dorsal view of modified spines (ms) with subterminal flange (f) and spear-shaped companion chaetae (cc) on chaetiger 5; (D) dorsal view of geniculate dorsal superior chaetae (ds) on chaetiger 5. Scale bars: (A) 200 µm; (B and E–F) 20 µm; (C and D) 100 µm.

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List of abbreviations

Collector/s

CAS	Dr C.A. Simon
JHD	Prof J.H. Day
NS	N. Steffani
SvN	S. van Niekerk
SvN/SdL	S. van Niekerk and S. de Lange

Museums

AM	Australian Museum
BMNH	British Museum of Natural History
SAMC Iziko	South African Museum (Cape Town)
USNM	United States National Museum
ZMH	Zoological Museum of Hamburg

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Chapter 1: Introduction

Polychaete taxonomy is not a novel field of study in South Africa; many of the species occurring on the coastline were recorded and identified by Prof J.H. Day over 30 years, from the mid 1930s to the late 1960s (Brown, 2003). His work culminated in the publication of a two-volume monograph on the polychaetes of southern Africa (Day, 1967) that is still widely used as a guide for the identification of polychaete species in the region (e.g. Simon, 2011) and globally (e.g. Borda et al., 2012; Carr et al., 2012). Since the publication of the monograph, there has been a 30-year gap in taxonomic knowledge for this group of organisms locally. The newer records and updated information have mostly relied on ecological studies such as those on species abundance around offshore mining structures (Clarke, 2005; Clarke et al., 2010), the impact of polychaetes on the aquaculture industry (Schleyer, 1991; Nel et al., 1996; Simon et al., 2006; Simon & Booth, 2007) and more recently, an interest in alien invasives/settlement (Robinson et al., 2005; Griffiths et al., 2009a; Griffiths et al., 2009b; Haupt et al., 2010a; Mead et al., 2011). The use of the monograph as a single source of identification for most of the local South African polychaete species is not ideal since the similarities with Northern Hemisphere species that are apparent from the monograph can have two serious consequences for interpretation: 1) exaggerated apparent cosmopolitanism (species occurring over wide geographic ranges based on morphological and molecular investigations (Spellerberg & Sawyer, 1999)) of species and/or 2) the documentation of alien species (species found outside of their natural range) that may be native.

The first consequence of naming species according to similar-looking Northern Hemisphere species is that the idea of cosmopolitanism can be inflated. For example, within the *Polydora* complex in the family Spionidae Grube, 1850, 12 of the species recorded in the monograph on the South African coast are considered to be cosmopolitan (Table 1.1).

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Table 2.1: List of polydorids with type locality, status of the type material (Lost or Deposited at Iziko South Africa Museum, Cape Town (SAMC), the United States National Museum (USNM), the Australian Museum (AM) or the British Museum of Natural History (BMNH)) and habitat preference. Polydorids are classified as endemic, cosmopolitan (Cos) or alien. If alien, an acclimatisation status is assigned as casual (Cas), questionable (Que), cryptic (Cryp), established (Est) or invasive (Inv), according to Zenetos et al. (2010).

Species	Type locality	Type specimens	Boring/ non-boring	Questionable identification	Endemic	Acclimatisation status					Cos
						Cas	Que	Cryp	Est	Inv	
Boccardia polybranchia	New South Wales, Australia	Lost	Boring					+			+
Boccardia proboscidea	Caspar, California, USA	USNM	Boring							+	
Boccardia pseudonatrix	Knysna, South Africa	SAMC?	Boring		+						
Dipolydora armata	Madeira Island, Portugal	ZMH	Boring	+					+		+
Dipolydora caeca	Oresund, Denmark	Lost	Boring				+				+
Dipolydora capensis	Simons Town, South Africa	SAMC?	Boring		+						
Dipolydora flava	Gulf of Naples, Italy	Lost	Boring			+					+
Dipolydora giardi	Gulf of Naples, Italy	Lost	Boring	+			+				+
Dipolydora keulderae	Port Alfred, South Africa	SAMC	Boring		+						
Dipolydora normalis	Inhaca Island, Mozambique	BMNH	Non-boring		+						
Polydora dinthwanyana	Haga Haga, South Africa	SAMC	Boring		+						
Polydora ciliata	Berwick, England	Lost	Boring			+					+
Polydora colonia	Vineyard Haven, Massachusetts, USA	Lost	Boring			+					+
Polydora hoplura	Gulf of Naples, Italy	Lost	Boring						+		+
Polydora maculata	East London, South Africa	BMNH	Boring		+						
Polydora cf. *haswelli*	Sydney Harbour, New South Wales, Australia	AM	Boring	+		+					+
Polydora cf. *websteri*	Connecticut, USA		Boring	+			+				+
Pseudopolydora antennata	Gulf of Naples, Italy	Lost	Boring					+			+
Pseudopolydora kempi	Chilka Lake, India	Lost	Non-boring				+				

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The second consequence of naming species according to similar-looking Northern Hemisphere species is the inference of alien species. Unfortunately, very little is known about how many species from the monograph represent true alien species. In the last decade, three studies have listed the known introduced marine species present on the South African coast (Robinson et al., 2005; Griffiths et al., 2009a; Griffiths et al., 2009b) and at present, eight polychaetes are recognised (Mead et al., 2011). The fact that only eight are listed as possible introductions may be a serious underestimate of the real number of invasives. For example, according to the monograph, 28 of the 36 recorded species in the family Spionidae alone have type localities outside of South Africa, while only eight are native (Day, 1967). However, given that the quality of the descriptions of local representatives, many of these cosmopolitan species may in fact be local members of sibling or species complexes.

The accurate taxonomic description of lineages along the South African coastline is important since many species are known to become pests on commercially grown abalone and oysters (Schleyer, 1991; Nel et al., 1996; Schleyer, 1991). Pest polychaetes damage the shell of the molluscs, which leads to the host expending more energy on repairing shell damage than on flesh growth. The latter could result in a less marketable product, which would then lead to financial loss for the facilities affected (Simon & Booth, 2007). Internationally, the *Polydora* complex or polydorids of the family Spionidae are recognised as the most notorious pest species (Sato-Okoshi & Abe, 2012). In this group, *Polydora hoplura* Claparède, 1869 (Nel et al., 1996; Simon et al., 2006; Simon & Booth, 2007) and *Boccardia proboscidea* Hartman, 1940 (Simon et al., 2009; Simon et al., 2010) are considered the most common pest species affecting cultured molluscs on the South African coast. Neither species is native to South Africa, and the status of *B. proboscidea* as an invasive has been confirmed molecularly (Simon et al., 2009). However, the genetic identity of South African *P. hoplura* has not been confirmed conclusively.

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In addition to genetics, morphologically identified species of polydorids are also identified through the use of reproductive patterns, pigmentation patterns and habitat preferences (Blake, 1996; Radashevsky & Pankova, 2013). However, it is important to realise that traditional methods of species identification can be complicated by the presence of cryptic or sibling species (Knowlton 1993) and that this can also lead to the incorrect conclusion that species are cosmopolitan or introduced. This is supported by recent literature suggesting that many of these so-called cosmopolitan species are actually the consequence of three important factors: firstly, the misidentification of local species, secondly, the occurrence of alien species and, thirdly, the presence of sibling species (Walker, 2011).

Misidentifications often occur since type material is not available (Walker, 2011). Furthermore, type specimens may be damaged and/or lost, making it increasingly difficult to confirm subsequent records of species (Walker, 2011). This was exemplified in *Boccardia polybranchia*; the type material from Australia has been lost, and the original description is so generic that our current understanding of this species is based on a later description by Carazzi (1893) from Italian specimens. It is therefore impossible to confirm the identity of the species outside of Australia, especially since this species has never been found at its type locality since its original description (see Blake & Kudenov, 1978 and Simon et al., 2010 for discussions). This also applies to South African species, as no type material is deposited for any of the 12 apparently cosmopolitan polydorid species, which include *Polydora hoplura* (Table 1.1; Simon et al., 2010; Walker, 2011).

The second factor contributing to the apparent cosmopolitan distribution of polydorids is the spread of alien species (e.g. Bailey-Brock 2000; Simon et al., 2009). Polydorid introductions outside their natural distribution can be attributed to four major vectors: the building of canals in major water bodies, the transportation of larvae in ballast water of ships, hull fouling as well as aquaculture activities (Knowlton, 1993; Naylor et al., 2001; Cinar,

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2013). The larvae of spionids are especially well adapted for long-distance travel in ballast water, which can harbour up to 2 x 10^2 larvae m^{-3} water (Carlton & Geller, 1993). As a consequence, the Spionidae comprises up to 18% (53 species) of invasive marine taxa (Cinar, 2013). Furthermore, the genus *Polydora* makes up 5.4% of the species (16 species) and is considered one of the most speciose alien genera (Cinar, 2013). Confirmed extralimital introductions of polydorids include *Boccardia proboscidea* to Hawai'i on cultured oysters (*Crassostrea gigas*) and *Polydora uncinata* Sato-Okoshi, 1998 to Chile on abalone (*Haliotis discus hannai*) (Bailey-Brock, 2000; Radashevsky & Olivares, 2005).

The third factor contributing to the record of apparently cosmopolitan species is the misidentification of sibling species (Walker, 2011). *Polydora hoplura* is considered cosmopolitan (Day, 1967) or locally introduced (Mead et al., 2011) and is best known in South Africa as a pest affecting cultured abalone and oysters (Nel et al., 1996; Simon et al., 2006; Simon & Booth, 2007). It was first recorded in South Africa by Day (1954), who provided a vague description of the species and stated that specimens resembled a previous description of specimens from the Gulf of Naples by Fauvel (1927). Furthermore, the description provided by Day (1967) was almost identical to that by Fauvel (1927). The South African *P. hoplura* is thus in need of a proper taxonomic review. This set the basis for the study presented herein, and after an initial assessment of morphological variation present in the species, it was noted that the sampled specimens varied considerably and consistently with regard to certain morphological characteristics. Three adult morphotypes (morphotype 1-3) and one juvenile morphotype (morphotype 4) was detected. The morphotypes differed with respect to combinations of pigmentation patterns, body size and prostomium shape: In morphotype 1, the prostomium is bilobed and has faint to intense pigmentation from chaetiger 1 to chaetiger 4 and in some individuals pigmentation is present on the posterior; in morphotype 2, the prostomium is bilobed and pigmentation is absent at the anterior; in

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morphotype 3, the prostomium is rounded and lacks pigmentation on the peristomium; and morphotype 4 is consistently smaller than morphotypes 1–3 and has distinct pigmentation spots at the base of each pair of branchiae from chaetiger 8 to chaetiger 15.

The observed variation led to the origin of this MSc dissertation, and the following two questions were posed: 1) Do specimens of *P. hoplura* on the South African coast represent a single species? and 2) Are South African specimens the same species as those found along other continental margins? In order to answer these questions, it has been suggested that a combination of techniques be employed, including scanning electron microscopy, morphometric measurements and genetic analyses (see discussion in Knowlton, 1993). Scanning electron microscopy has been used to successfully show that there are sufficient morphological differences in palp morphology to support the genus level difference between *Polydora* and *Dipolydora* (Worsaae, 2001). In species identification, the technique is used to add more morphological characters to differentiate among species (see Simon et al., 2010 on *Boccardia proboscidea*). In the present study, a combination of data sources was used with specific focus on morphology, aspects of reproduction, pigmentation, habitat preference and genetics, and it was hoped that the outcome of the study would provide new insights into the cosmopolitan and introduction status of the species.

1.1 Morphology

Polydorids are identified by a modified fifth chaetiger with specialised spines (Blake, 1996; Sato-Okoshi & Okoshi, 2000). This is not exclusive to the polydorid complex and is shared by another genus, *Atherospio* Mackie and Duff, 1986. All polydorids have modified notopodial spines on the fifth chaetiger and are different from *Atherospio*, which has modified neuropodial spines (Mackie & Duff, 1986; Blake, 1996). The distinction between polydorids and *Atherospio* is sufficient to separate the latter from the former (Mackie & Duff,

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1986; Blake, 1996). The polydorids are also distinguished according to the shape of the prostomium, the length of the caruncle, the position where the branchiae start and end and the shape of the pygidium (Blake, 1996). Furthermore, Radashevsky and Fauchald (2000) suggested that finer detail such as the location of various chaetae in relation to each other should be included to aid species identification.

In the *Polydora* complex, many of these characteristics are not species specific and are shared across species. It is therefore the variation and combination of these characteristics that make it possible to assign individuals to a certain species. For example, *Boccardia proboscidea* and *Boccardia polybranchia* Haswell, 1885 have similar types of modified spine on chaetiger 5 and dark pigmentation along the margin of the caruncle and prostomium. However, they differ with respect to the shape of the prostomium, branchiae and pigmentation pattern on the posterior end of the body (Simon et al., 2010). Differences among species can be even more subtle, as has been observed within sibling species *Polydora calcarea* Templeton, 1836 and *Polydora manchenkoi* Radashevsky and Pankova, 2006 (see below).

Species identification can also be confounded by intraspecific variation. For example, Bick (2001) found that *Dipolydora armata* (Langerhans, 1880) from the western Mediterranean Sea consisted of at least three morphotypes based on variation in the shape of the bundles of posterior modified spines. A subsequent revision of the species by Radashevsky and Nogueira (2003) could not consistently identify different morphological characters to separate these morphotypes into different species, and it was concluded that all specimens collected globally still belonged to the same species.

The correct identification can further be complicated by differences in the preservation and orientation of the morphological character being investigated. For instance,

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the shape of the prostomium of *D. armata* seemed to vary from rounded to incised, but Bick (2001) showed that this variation in shape was actually induced by the method by which the specimens were preserved. Variation in morphological characters may also be a result of the orientation of the character being observed. For example, Read (2010) showed that by changing the orientation of the modified spines of chaetiger 5 in *Polydora haswelli* Blake and Kudenov, 1978, the accessory structure on the spine could appear tooth-like when it was in fact a flange.

1.2 Aspects of reproduction

The mode of reproduction in polydorids is considered fixed within a species (Blake & Arnofsky, 1999). In most polydorids, such as *Polydora websteri* Hartman in Loosanoff and Engle, 1943, males produce spermatophores that are deposited outside the female's burrow; females then collect the spermatophores with their palps to fertilise the eggs (Rice, 1981; Blake & Arnofsky, 1999); a few polydorids, such as *Polydora colonia* Moore, 1907, reproduce asexually (David & Williams, 2012). Even though the reproductive mode is fixed within a species, aspects of reproduction such as the ultrastructure of sperm and larval development can vary and lead to species level differences. For instance, differences in the aggregation of spermatids have separated the morphologically indistinguishable species that are now known as *Polydora calcarea* and *P. manchenkoi* (Radashevsky & Pankova, 2006).

Polydorids exhibit three types of larval developmental mode: planktotrophy, endolecitotrophy and ectolecitotrophy (Blake & Arnofsky, 1999). In planktotrophy, larvae are usually released at the chaetiger 3–4 stage and spend up to 45 days in the water column before metamorphosis (Blake & Arnofsky, 1999). In endolecithotrophy, larvae hatch from large eggs, are not supplied with nurse eggs and are released at chaetiger 20 (Radashevsky, 1994). In ectolecithotrophy, larvae are hatched from small eggs, are provided with nurse eggs, are released at chaetigers 9–14 and metamorphose soon after release from the capsule

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(Blake & Arnofsky, 1999). Species level differences due to larval developmental mode are exemplified by the planktotrophic *Pseudopolydora paucibranchiata* Okuda, 1937 and the ectolecitotrophic *Pseudopolydora kempi* Southern, 1921 (Blake & Woodwick, 1975). Polydorid species usually produce larvae via one of the above-mentioned larval developmental modes but can also be poecilogenous (Hoagland & Robertson, 1988; Blake & Arnofsky, 1999). On the North American coastline, *Boccardia proboscidea* exhibits three modes of larval development with morphological and molecular analysis confirming that the species is truly poecilogenous (Gibson, 1997; Gibson et al., 1999; David et al., 2014).

1.3 Pigmentation

In species identification, pigmentation patterns are generally thought to be consistent; examples are routinely included on morphological plates as in *Pseudopolydora dayii* Simon, 2009 with its distinctive triangular pigmentation marking on the fifth chaetiger (Simon, 2009). Similarly, *Pseudopolydora achaeta* Radashevsky and Hsieh 2000 is characterised by the distinctive lateral bands of pigmentation in the anterior region (Radashevsky & Hsieh, 2000). In contrast, pigmentation patterns can be very similar between species, as in *Polydora narica* Light, 1969 and *P. haswelli*, where pigmentation bands are present in both species on the first four anterior chaetigers (Light, 1969; Sato-Okoshi & Abe, 2012).

Despite these examples, intraspecific variation has also been recorded in several members of the genus *Polydora* from China, Australia and Japan (Sato-Okoshi et al., 2013; Teramoto et al., 2013). For example, the black pigmentation on the posterior of *Polydora brevipalpa* Zachs, 1933 and the anterior of *Polydora onagawaensis* Teramoto, Sato-Okoshi, Abe, Nishitani and Endo, 2013 can vary from absent to intense, while the largest variation in pigmentation was noted on the posterior and anterior of *P. haswelli* (Sato-Okoshi & Abe, 2012; Sato-Okoshi & Abe, 2013). To date, only a few studies have genetically assessed whether differently pigmented members of the same species really belong to the same

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species. Studies investigating the status of *P. haswelli*, *P. brevipalpa*, *P. onagawaensis*, *P. calcarea* and *P. websteri*, however, concluded that these taxa were still valid species and that the intraspecific pigmentation merely reflected considerable intraspecific variation (Sato-Okoshi & Abe, 2013; Teramoto et al., 2013).

1.4 Habitat preference

In recent years, habitat preference has been recognised as an important diagnostic characteristic in the identification of species (Knowlton, 1993; Radashevsky et al., 2006; Bastrop & Blank, 2006; Blank et al., 2008; Pleijel et al., 2009; Radashevsky & Pankova, 2013) and has become particularly important when distinguishing among sibling species (Knowlton, 1993; Pleijel et al., 2009). For example, *P. calcarea* and *P. manchenkoi* can be distinguished by the former being a generalist borer of calcareous substrates while the latter bores exclusively into gastropod shells inhabited by hermit crabs (Radashevsky & Pankova, 2006). Recently, Radashevsky and Pankova (2013) highlighted, through the use of genetic markers, that differences in habitat preferences of shell-boring and tube-dwelling individuals were not always indications of species status since individuals from *Dipolydora carunculata* Radashevsky 1993 showed both shell-boring and tube-dwelling habitat preferences but *Polydora triglanda* Radashevsky & Hsieh 2000 showed the converse. This indicates that both a genetic and morphological approach should be employed to accurately determine whether specimens inhabiting different substrates belong to the same species or not.

1.5 Genetic approaches

Relatively little molecular work has been done on the polydorids. Only 12 of the more than 100 polydorid species have been sequenced with data available on Genbank. These sequences represent three of the nine genera (*Polydora*, *Dipolydora* and *Boccardia*) and include mostly the common pest species (Simon et al., 2009; Sato-Okoshi & Abe, 2012; Sato-Okoshi et al.,

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2013; Teramoto et al., 2013; Radashevsky & Pankova, 2013) *Polydora cornuta* (Rice et al., 2008).

Genetic markers used include the mitochondrial gene fragments Cytochrome Oxidase I (COI), 16S rRNA and Cytochrome b and nuclear gene fragments 18S rDNA (Rice et al., 2008; Simon et al., 2009; Sato-Okoshi & Abe, 2012; Sato-Okoshi & Abe, 2013; Teramoto et al., 2013; Radashevsky & Pankova, 2013). These studies have thus far aided in 1) separation of individual species into sibling species, 2) confirmation of the identity of species in disparate locations and 3) identification of source populations of alien species. For example, while *Polydora cornuta* Bosc, 1802 specimens collected throughout its known range were considered a single species based on morphological characteristics (Radashevsky, 2005), a subsequent investigation by Rice et al. (2008) showed that the species actually consisted of at least three sibling species on the North American coastline. Results concurring with those of Rice et al. (2008) were shown in the *Streblospio benedicti* species complex (Schulze et al., 2000). Genetic studies have also aided in the confirmation of the identity of species found over large geographic areas, as in the case of orbinid *Proscoloplos* (Meyer et al., 2008) in which *Proscoloplos cygnochaetus* Day 1954, *P. bondi* Kelaher and Rouse, 2003 and *P. confusus* Hartman-Schroëder, 1962, collected in South Africa, Australia and Chile, actually constitute a single species, *P. cygnochaetus* (Meyer et al., 2008).

1.6 *Polydora hoplura* species account

Polydora hoplura was first recorded in the Gulf of Naples in Italy (Claparède, 1869). Either no type material for *P. hoplura* has ever been deposited at a museum or it has been lost. Thus, subsequent records of the species can only be compared with published descriptions of the species. Additional specimens collected from the Gulf of Naples were recorded by Carazzi (1893) and later by Fauvel (1927). Later records of the species came from the United Kingdom, South Africa, New Zealand and Australia (Wilson, 1928; Day,

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1954; Read, 1975; Blake & Kudenov, 1978; Hutchings & Turvey, 1984). More recent records of the species are from the Netherlands (Haydar & Wolff, 2011), Portugal (Freitas et al., 2011) and the Canary Islands (Bilbao et al., 2011).

The initial morphological description of *P. hoplura* from the Gulf of Naples was rigorous, and the species was described as having a bilobed prostomium, pigmentation on the peristomium, two or four eyes, a caruncle that extended to the middle of chaetiger 3, branchiae and bidentate hooded hooks that started on chaetiger 7, posterior modified spines on the last 15 chaetigers and a sucker-shaped pygidium (Claparède, 1869). Unfortunately, the morphological plate accompanying the description only included the posterior region of the species (Claparède, 1869). The description by Fauvel (1927) mentioned some variation in the shape of the prostomium, and the morphological plates were much more detailed than those of the original description of the species. In the Southern Hemisphere, the only detailed description of the species is that by Read (1975); the other descriptions are brief and their morphological plates only highlight certain characters (Day, 1954; Blake & Kudenov, 1978; Hutchings & Turvey, 1984). The morphological descriptions of international specimens of *P. hoplura* are congruent with respect to length and total number of chaetigers, the shape of the spines on chaetiger 5, the distribution of branchiae and number of hooded hooks from chaetiger 7, the presence of posterior modified hooks and the shape of the pygidium (Claparéde, 1869; Carazzi, 1893; Read, 1975; Blake & Kudenov, 1978; Hutchings & Turvey, 1984).

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1.7 Aims and hypotheses

Firstly, the present study aimed to use an integrated approach to determine the taxonomic status of the four morphotypes of *P. hoplura* recorded on the South African coast. The following hypotheses were formulated:

H_A The South African representatives of *Polydora hoplura* constitute a single species.

H_0 The South African representatives of *P. hoplura* do not constitute a single species.

Secondly, to obtain a preliminary perspective on geographic genetic variation, the present study also aimed to assess the genetic relationship between individuals of *P. hoplura* collected on the South African coast and morphologically similar individuals collected in New Zealand. Morphotype was not assigned (see Chapter 2: Materials and methods), and the following hypotheses were formulated:

H_A Representatives of *P. hoplura* collected on the South African coast are genetically similar to those collected on the coastline of New Zealand.

H_0 Representatives of *P. hoplura* collected on the South African coast are not genetically similar to those collected on the coastline of New Zealand.

Lastly, by making use of distribution data, a brief history of *P. hoplura* on the South African coast was compiled in order to assess the validity of the cosmopolitan status of the species.

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Chapter 2: Materials and methods

2.1 Specimen collection

Type material of *Polydora hoplura* collected by Claparède (1869) was never deposited at a museum or is lost (Walker, 2011). Thus, specimens included in this study were collected from farmed and wild molluscs, and these were compared to specimens from private collections and museum material. The latter were obtained from the Iziko Museum of Cape Town (South Africa), the Australian Museum and the National Museum of Victoria (Australia).

Fresh samples were collected from farmed *Crassostrea gigas* and also from farmed and wild *Pecten sulcicostatus* in 2011 (Fig. 2.1). Some of these specimens were fixed in formalin and used for morphological observations, and five specimens of each morphotype were fixed in ethanol and used for molecular analysis (Table 2.1).

Formalin-fixed specimens collected from 2005 to 2010 came from the private collection of Dr C.A. Simon and were exclusively used for morphological observations. Additional ethanol-fixed specimens were collected by Dr C.A. Simon in November 2012 from farmed *Haliotis midae*. Four larval sequences were donated to the study by A.A. David in November 2012.

Specimens were donated by Dr G. Read at the National Institute of Water and Atmospheric Research in New Zealand (February 2012). The morphotype of these specimens could not be assigned (discussed later).

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Figure 2.1: Map of South Africa with all collection sites along the coast Abbreviations: W: wild and F: farmed sites

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Table 2.1: Specimens collected and used in this study from the South African coastline. Abbreviations: SvN – S. van Niekerk; Sdl – S. de Lange; CAS – C.A. Simon; ELN – E. Newman; Um – unidentified morphotype; Ps – *Pecten sulcicostatus*; Hm – *Haliotis midae*; Ts – *Turbo sarmaticus*; Sl – *Scutellastra longicosta*; Pp – *Perna perna*; Cs – *Crassostrea gigas*; S – sand; Ul – unidentified limpets ; Dash (-): absent from host species/substrate; Plus (+): present on host species/substrate; Bolded plus (+): specimens used for genetic study.

Locality	Collector (collection date)	*Polydora hoplura* morphotype	Host species/Substrate										Number of specimens used		Museum accession number	Genbank accession number
			Wild							Farmed			Morphological analysis	Molecular analysis		
			Ps	Hm	Ts	Sl	Pp	S	Ul	Hm	Ps	Cs				
Saldanha Bay	SvN/SdL (2011)	1	-	-	-	-	-	-	-	-	+	+	5	5	SAM A65602	
		2	-	-	-	-	-	-	-	-	+	+	7	5	SAM A65603	
		3	-	-	-	-	-	-	-	-	+	+	1	5	SAM A65604	
	SvN/ELN (2010)	4	-	-	-	-	-	++	-	+	-	+	0	4		
	CAS (2012)	4	-	-	-	-	-	-	-	+	-	-	5	0	SAM A65605	
		Um	-	-	-	-	-	-	-	+	-	-	0	7		
False Bay	SvN/SdL (2011)	2	+	-	-	-	-	-	-	-	-	-	1			
Struisbaai	CAS (2005)	1	-	+	-	-	-	-	+	-	-	-	14			
		2	-	+	+	-	-	-	-	-	-	-	8			
		3	-	+	-	-	-	-	+	-	-	-	2			
Mossel Bay	CAS (2005)	1	-	+	+	-	-	-	-	-	-	-	5			
		2	-	-	+	-	-	-	-	+	-	-	6			
		3	-	-	+	-	+	-	-	-	-	-	2			
	CAS (2005)	1	-	+	-	-	-	-	-	-	-	-	3			
		3	-	-	+	+	-	-	-	-	-	-	11			
Grootbank	CAS (2005)	1	-	-	+	-	-	-	-	-	-	-	1			
		3	-	-	+	-	-	-	-	-	-	-	1			
Port Elizabeth	SvN/SdL (2011)	4	-	-	-	-	-	-	-	-	-	+	0	1		
Haga Haga	CAS (2005)	1	-	-	+	-	-	-	-	-	-	-	2	0		
		2	-	-	-	-	-	-	-	+	-	-	1			

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2.2 Extraction of fresh specimens

Shells of oysters and/or scallops were placed in bowls and covered with a vermifuge (0.05% phenol in seawater solution) (Handley, 1995; Nel et al., 1996; Lleonart et al., 2003; Simon et al., 2010; Bilbao et al., 2011; see Appendix A) for 15–20 min. Samples were heated through the illumination of 60 W desk lamps to hasten the emergence of worms from their burrows (Handley, 1995). Emerging worms were gently pulled from their burrows with fine forceps to prevent them from breaking and placed in fine-filtered seawater. Shells were then transferred to fine-filtered seawater, and the remaining worms were removed by cutting the shells with small cutting pliers (Lexie M. Walker, University of Queensland, Australia, personal communication). A preliminary study showed that combining both these methods (phenol in seawater and cutting with pliers) ensured that more worms were removed from the shells than using either method separately. Freshly collected specimens were relaxed in 7% $MgCl_2$ in tap water (Appendix A) and were 1) fixed in formalin for two days and stored in 70% ethanol for morphological observations or 2) directly fixed in 96% ethanol and stored at 4 °C for molecular investigation.

2.3 Morphological observations

2.3.1 Whole-specimen observation, line drawings and permanently prepared material

All specimens could be assigned to a particular morphotype (Table 2.1). However, specimens collected by Dr C.A. Simon and Dr G. Read in 2012 could not be assigned due to their being preserved directly for molecular analysis, a process that makes the specimens fragile. The formalin-fixed specimens were stained with methyl green and examined on a Leica L2 stereo microscope (Leica, Wetzlar, Germany). The characters used to describe whole specimens are listed in Appendix B. Permanent slides of chaetigers 5 and 7 and posterior chaetigers with modified spines were prepared by mounting sections in Aquatex®, which were dried for two

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days and sealed with clear nail varnish. All prepared sections were viewed in oil under 100 x magnification. Line drawings of whole specimens and permanent slides were created using a drawing tube attached to a Leica DM 1000 light microscope (Leica, Wetzlar, Germany).

2.3.2 Scanning electron microscopy

Specimens were prepared for critical point drying by dehydrating them in alcohol through a series of increasing alcohol concentrations (90%, 95% and 99%); each specimen was submerged for 60 min at a given concentration (adapted from Cross, 2001). Specimens were critically point dried over CO_2 and mounted on a microscope stub with double-sided tape in one of three orientations (dorsal, ventral or lateral [Appendix B]) and sputter coated for 2–4 min with gold palladium. Specimens were viewed under a Zeiss EVO MA 15 scanning electron microscope at the Central Analytical Facility (CAF) at Stellenbosch University. Mounted specimens were stored in a desiccator for future reference. Table 2.2 lists the morphological characters evaluated.

2.4 Statistical analysis

2.4.1 Gross morphological differences among morphotypes

To test whether the different morphotypes could be separated into morphospecies, the morphological characters (adapted from Blake & Arnofsky, 1999) from the descriptions were transformed to presence/absence data (Table 2.2). Characters where scored (Appendix D) and analysed using a Bray-Curtis analysis of similarity in Primer 5.0 (Plymouth, UK). Cluster analysis was conducted to determine whether different morphotypes grouped together. Host species or substrate was overlaid onto the resulting dendogram to test for host specificity. The Bray-Curtis analysis and cluster dendrogram was used to best illustrate that different morphotypes did not cluster together.

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Table 2.2: Morphological characters evaluated for descriptions of coding from cluster analysis

Characteristic coded		
1. Anterior margin of prostomium	Bilobed Rounded	1 0
2. Number of eyes	Four Less than four	1 0
3. Eyes	Present Absent	1 0
4. Occipital antenna	Present Absent	1 0
5. Caruncle length	End of chaetiger 3 Shorter than chaetiger 3	1 0
6. Pigmentation on prostomium and peristomium	Present Absent	1 0
7. Notochaetae on chaetiger 5	Present Absent	1 0
8. Number of notochaetae on chaetiger 5	More than or equal to three Less than three	1 0
9. Number of modified spines on chaetiger 5	More than or equal to five Less than five	1 0
10. Ventral chaetae on chaetiger 5	Present Absent	1 0
11. Number of ventral chaetae on chaetiger 5	More than or equal to three Less than three	1 0
12. Number of hooded hooks per neuropodium from chaetiger 10 onwards	More than or equal to 10 Less than 10	1 0
13. Branchiae full size on chaetiger	Before chaetiger 9 After chaetiger 9	1 0
14. Modified spines start on segment after branchiae stops	First segment after Second or more after	1 0
15. Modified spines	Present Absent	1 0
16. Number and distribution of modified spines in posterior chaetigers	One spine per series More than one spine per series	1 0
17. Pigmentation posterior in posterior chaetigers	Present Absent	1 0
18. First segment with methyl green staining	Before segment 7 After segment 7	1 0
19. Methyl green staining and occurrence of posterior modified spines	Present with modified spines Absent	1 0
20. Total body length	More than 15 mm Less than 15 mm	1 0
21. Width at chaetiger 5	Less than 0.2 mm More than 0.2 mm	1 0
22. Total number of chaetigers	More than 80 Less than 80	1 0

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2.4.2 Fine-scale differences among posterior modified spines

Posterior modified spines of five individuals of each morphotype (including specimens collected by Day, 1954) were excised. Spines were submerged in 4% peroxide solution to remove excess flesh before they were permanently mounted on a slide with Aquatex® (Bolte, 1996). Spines were viewed under 100 x magnification on a Leica Dm 1000 light microscope (Leica, Wetzlar, Germany) and photographed with a camera attachment (Leica, Wetzlar, Germany).

Excised spines were measured flat in order to define the precise angle of the hook of the spine. Measurements taken from each spine (adapted from Christison et al., 2005) included the total length of the spine (AB), the tip of the spine to the base of the spine (CD), the tip of the spine to the tangent of the spine (ED), the widest section of the spine (FG), the angle of the curvature of the spine (Θ) and the angle between the tangent and the spine (Φ) (see Fig. 2.2 for illustrations). Lines AB, CD, ED and FG and angles Θ and Φ of morphotypes 1–3 were statistically compared using a Kruskal-Wallis analysis of variance (ANOVA).

Furthermore, specimens from Day (1954) were identified as morphotype 1 and were compared with morphotype 1 of this study; this was analysed using a Mann-Whitney U-test. All statistical analyses were performed using SPSS 20 (IBM, Chicago, USA).

Due to the published drawing by Day (1967) not having a scale, only angles Θ and Φ were compared with morphotype 1 from this study (Fig. 2.2). The angles of both the drawing and specimens from morphotype 1 were compared and described.

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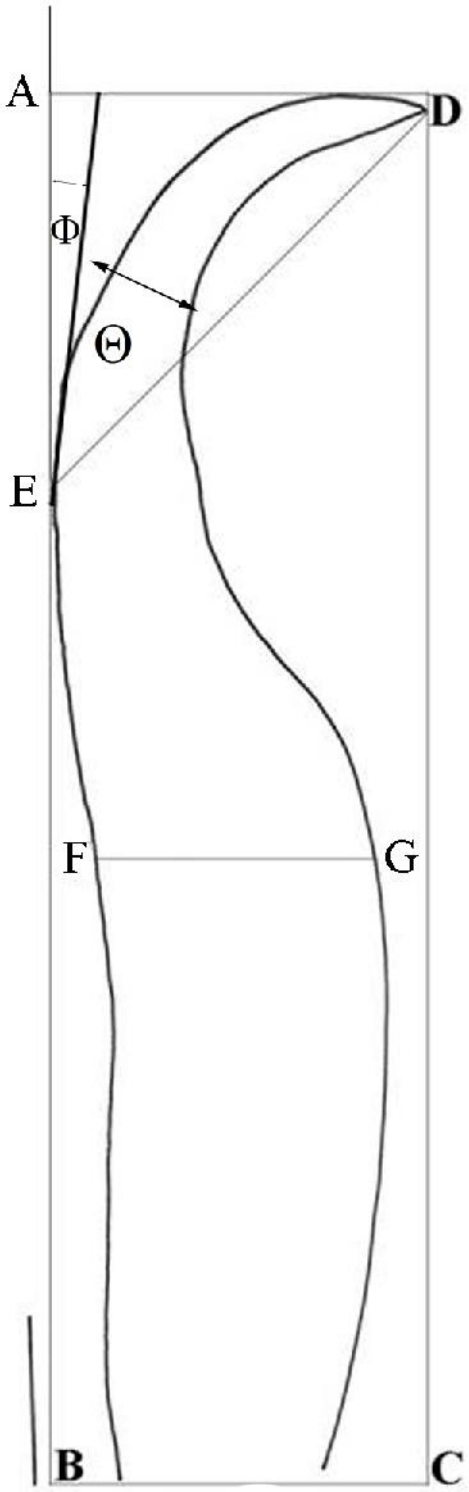

Figure 2.2: Measurements for statistical comparison of posterior modified spines of *Polydora hoplura* (A–G). Refer to table below for descriptions. Scale bar: 0.02 mm.

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2.5 Molecular methodology

2.5.1 Specimen collection

Specimens used in the molecular analysis were collected from Saldanha Bay with the exception of one specimen of morphotype 4 collected from Port Elizabeth. With the exception of the latter, the sampling was restricted to a specific site to eliminate the variance associated with interlocality variation (Table 2.1).

2.5.2 Outgroup selection

Boccardia proboscidea Hartman, 1940 and *Boccardia pseudonatrix* Day, 1961 were selected as outgroups in the study, following Sigvaldadottir et al. (1997). Both the mitochondrial DNA Cytochrome b and nuclear 28S gene fragments were amplified for *B. proboscidea*. Due to difficulties amplifying the 28S gene, only the Cytochrome b gene fragment was amplified for *B. pseudonatrix*.

2.5.3 Protocol

Whole genomic DNA was extracted using a Nucleospin™ extraction kit (Machery & Nagel, Duren, Germany) according to the manufacturer's instructions. The mitochondrial gene fragment was amplified using the 424F (5'-GGWTAYGTWYTWCCWTGRGGWCARAT-3'; Boore & Brown, 2000) and 876R (5'-RAAWARRAAGTATCAYTCAGG-3'; Oyarzun et al., 2011) primers. The nuclear gene fragment 28S was amplified using 4.8a rDNA (5'-ACCTATTCTCAAACTTTAAATGG) and 28S rD7b1 (5'-GACTTCCCTTACCTACAT; Whiting, 2002) primers. Mitochondrial DNA and nuclear DNA were amplified using a modified polymerase chain reaction (PCR). Amplification was achieved in a 25 µl reaction containing 2.5 µl of a 10 X PCR buffer; 2.5 µl DNA (1:20 DNA:H_2O dilution of extracted DNA); 1.25 µl Cytochrome b or 28S reverse primer; 2.0 µl of MgCl (25 mM); 2.5 µl dNTPs

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(10 mM); 1 unit of TAQ; and 12.9 µl ddH_2O. The reactions were run under these settings: initial denaturing at 95 °C for 5 min and 40 PCR cycles at 95 °C for 30 s, 45 °C for 30 s, 72 °C for 1 min and 72 °C for 10 min and kept at 15 °C till further analysis. Positive amplification was confirmed through gel electrophoresis (1% Agarose gel) with ethidium bromide staining viewed under ultraviolet light. Positive amplicons were excised from the gel and DNA was purified with a BioSpin gel purification kit (Qiagen). The purified DNA was sequenced at the CAF using the 424F Cytochrome b and 4.8a rDNA primers.

2.5.4 Sequence analysis

Sequences were manually edited and aligned in BioEdit (Hall, 1999). To test whether different morphotypes clustered separately; a Bayesian inference was conducted in Mr. Bayes v4.0 (Huelsenbeck et al., 2001). A hierarchical likelihood ratio test in MrModeltest v2.3 suggested the use of HKY+G and GTR+I models of substitution for Cytochrome b and 28S respectively (Naylander, 2004). For both Cytochrome b and 28S, two runs of four chains (three hot and one cold) were run for 1.0 x 10^6 generations that were sampled every 500 generations. Burn in was calculated at 25% of the cold run. Haplotype networks were created in TCS (Clement et al., 2000) at 90% connectivity and redrawn in PowerPoint 2010 (Microsoft 2007).

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Chapter 3: Results

3.1 Descriptions of morphotypes

Systematics

Family SPIONIDAE Grube, 1850

Genus *Polydora* Bosc, 1802

Polydora hoplura Claparéde, 1869

Polydora hoplura morphotype 1

(Figs 3.1– 3.7)

Best descriptions from literature conforming to morphotype 1

Polydora hoplura: Claparède, 1869, p. 58, Plate XXII, Fig. 2; Fauvel, 1927, p. 50, Fig. 17 a–g; Day, 1954, p. 415; Day, 1967, p. 468, Fig. 18.2 k–m; Read, 1975, p. 410

Material examined

South Africa

Saldanha Bay: One specimen was collected by JHD in 1953 from a sandy tube (SAMC, LB 378B); five specimens were collected by SvN/SdL in 2011 from shells of farmed scallops *Pecten sulcicostatus* Sowerby, 1842.

Struisbaai: Fourteen specimens were collected by CAS in 2005 from an unidentified limpet and wild *Haliotis midae* Linnaeus, 1758.

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Mossel Bay: Eight specimens were collected by CAS in 2005 from shells of *H. midae*, *Turbo samaticus* Linnaeus, 1758 and *Scutellastra longicosta* Lamarck, 1819.

Grootbank: One specimen was collected by CAS in 2005 from shells of *T. sarmaticus.*

Haga Haga: Two specimens were collected by CAS in 2005 from shells of *T. sarmaticus.*

Description

Large specimens, up to 28 mm for 140 chaetigers and 0.34 mm wide at chaetiger 5. Dorsal anterior pigmentation varies from faint (Fig. 3.1A) to intense (figs 3.2A & 3.3A–F). Pigmentation on peristomium may continue along prostomium and caruncle (Fig. 3.1A) to end of chaetiger 3 (figs 3.2A and 3.3A–F). Sporadic pigmentation along posterior of body present in a few individuals. Pygidial pigmentation may be absent or present as dark ring on the pygidium around the anal opening (Fig. 3.1B). Ventral pigmentation may be absent (Fig. 3.4A) or present as a faint collar between peristomium and chaetiger 1 (figs 3.2B, 3.4B and 3.4C) or intensely coloured collar with more pigment between chaetigers 1 and 2, and 2 and chaetiger 3 (Fig. 3.4D). Prostomial shape varies from weakly notched (figs 3.2A and 3.3A) to rounded bilobed (Fig. 3.5A). Eyes may be absent (Fig. 3.3A and B), one (figs 3.1A and 3.2A) or two pairs arranged in trapezoidal arrangement (Fig. 3.3C–F);. Caruncle extends posteriorly to middle or end of chaetiger 3 (figs 3.1A, 3.2A and 3.5A). Occipital antenna present behind eyes (figs 3.1A, 3.2A and 3.5A and B). Nuchal organ present as paired ciliated grooves along margin of caruncle (Fig. 3.5A). Lateral organs present on chaetigers 2 and 3 (Fig. 3.5B), and on chaetigers 7 and 8. Dorsal ciliary bands between branchiae present from chaetiger 7-10 (Fig. 3.5 A).

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Notochaetae: Absent on chaetiger 1. On chaetigers 2, 3 and 4 arranged in two rows; first row unilimbate, second row lanceolate. Two to five dorsal geniculate chaetae present on chaetiger 5 (Fig. 3.5D). Single row of unilimbate chaetae present on chaetiger 6 onwards. One or two yellow recurved posterior modified spines accompanied by three needle-like notochaetae present on 11% of chaetigers (figs 3.1C and I; 3.6A and C; 3.7A).

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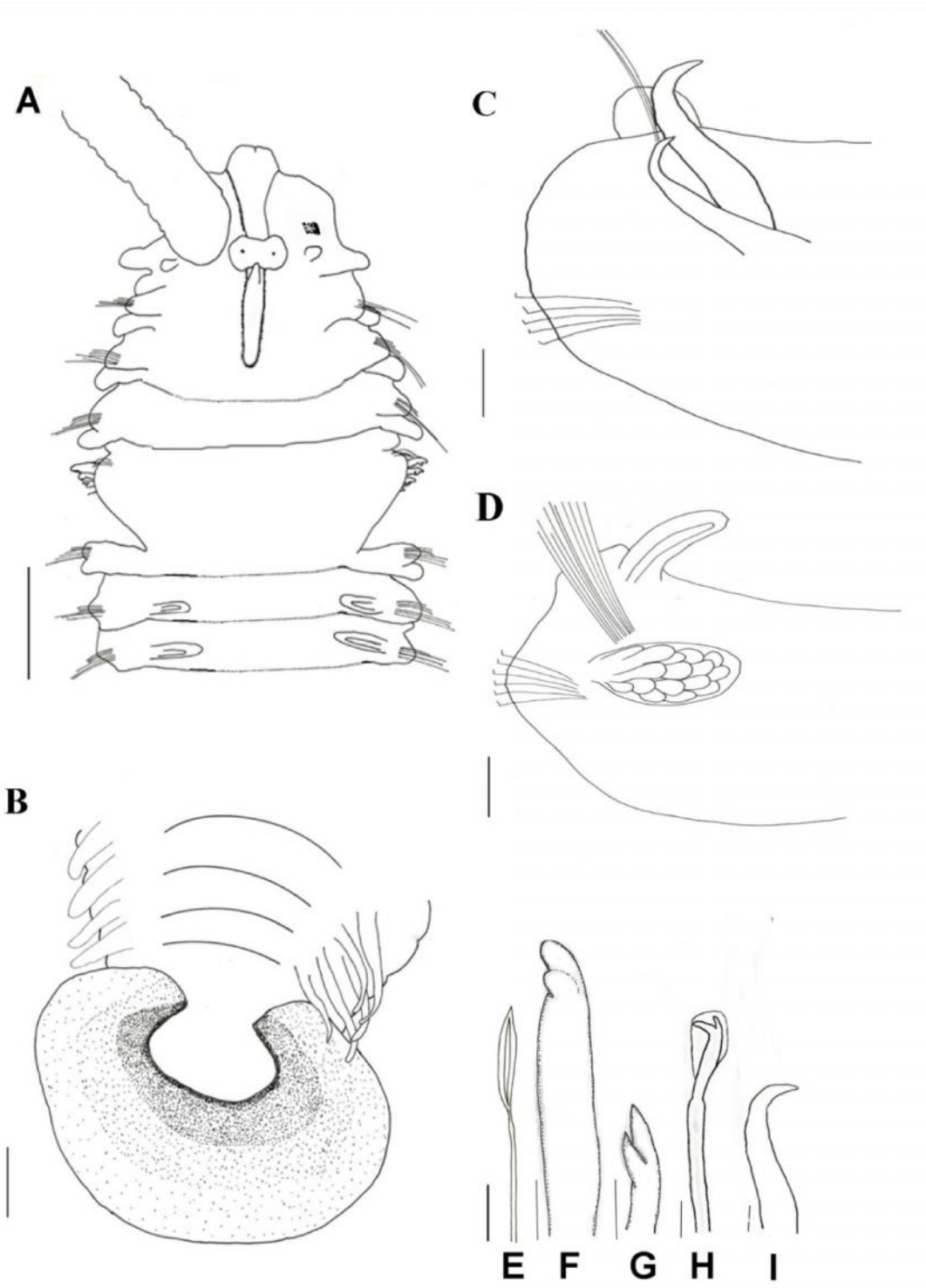

Figure 3.1: *Polydora hoplura* (Claparède, 1869) morphotype 1 adult morphology: (A) dorsal view of anterior region, faint colouration on peristomium and along prostomium and caruncle; (B) dorsal view of posterior region, spines and notopodia omitted on left and right side of chaetigers respectively, intense colouration on pygidium; (C) cross-section of a posterior chaetiger with hook-shaped modified spines and needle-like companion chaetae; (D) cross-section and structure of glandular pouch in relation to chaetiger 7; (E) spear-shaped companion chaetae of chaetiger 5; (F) most worn modified spine of chaetiger 5; (G) youngest modified spine of chaetiger 5; (H) hooded hook with constriction on shaft; (I) posterior modified spine. Scale bars: (A and B) 0.1 mm; (C and D) 0.05 mm; (E–I) 0.02 mm.

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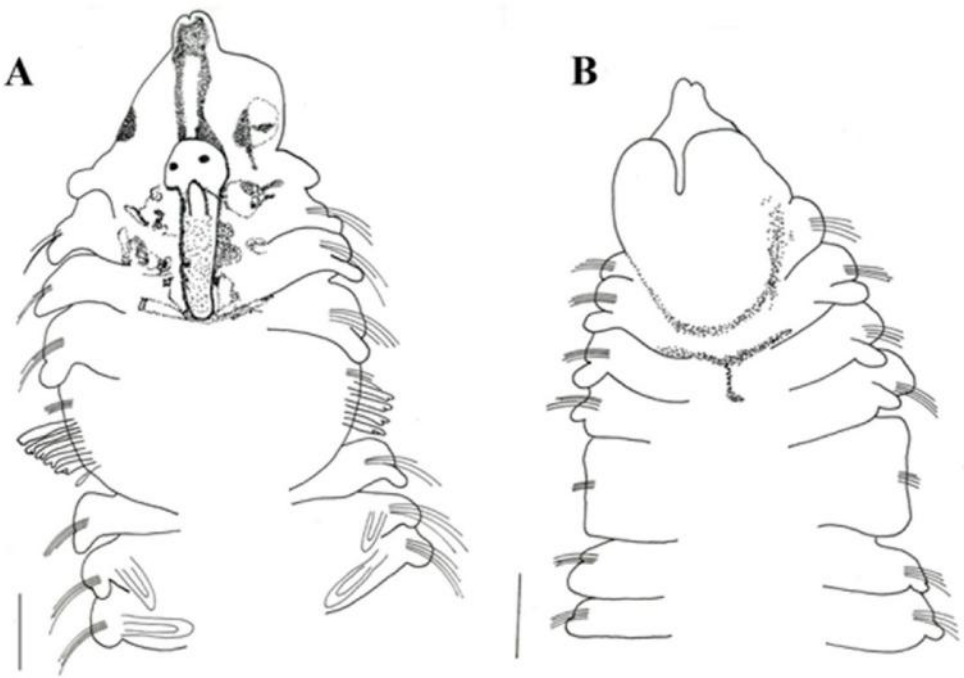

Figure 3.2: *Polydora hoplura* morphotype 1 adult morphology: (A) dorsal view of anterior region, intense colouration on the peristomium, along the edges of the caruncle and prostomium and on chaetigers 1–3; (B) ventral view of anterior region – note colouration on chaetigers 1–3. Scale bars: (A and B) 0.1 mm.

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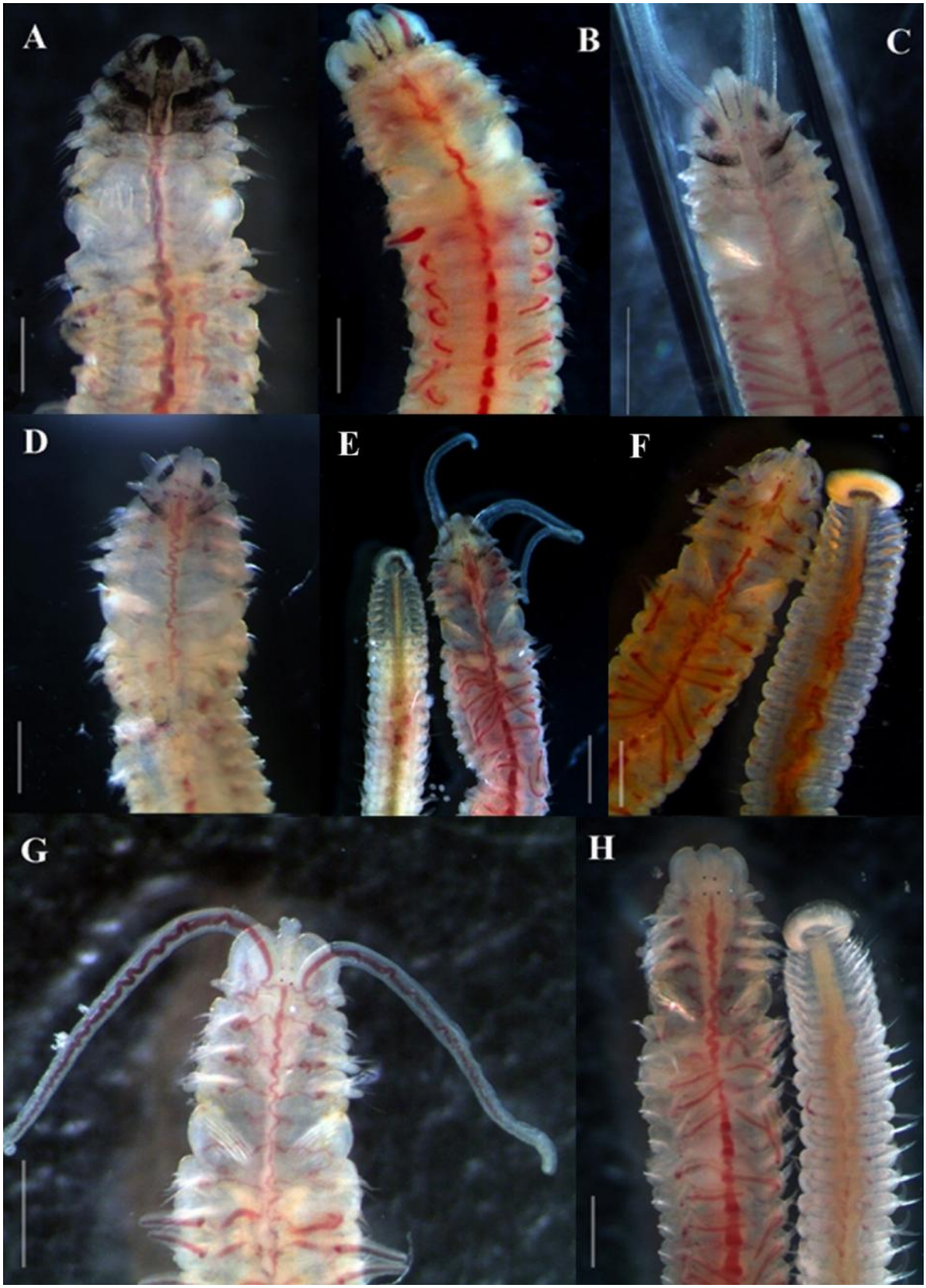

Figure 3.3: *Polydora hoplura* adult morphology: (A–F) *P. hoplura* morphotype 1, rounded bilobed prostomium – note the intense pigmentation in (A) becoming fainter by (F). Pigmentation present along edge of prostomium and continues along the caruncle and ends on chaetiger 3; (G) *P. hoplura* morphotype 2, rounded bilobed prostomium with no pigmentation anteriorly; (H) *P. hoplura* morphotype 3, rounded prostomium with no pigmentation. Scale bars: (A, B, D–F and H) 0.5 mm; (C and G) 1.0 mm.

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Figure 3.4: *Polydora hoplura* adult morphology: Ventral view of *P. hoplura* morphotype 1 with rounded bilobed prostomium and (A) no pigmentation, (B) faint collar on chaetiger 1, (C) complete collar and (D) complete collar and pigmentation on chaetigers 2 and 3.

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Neurochaetae: One row of unilimbate neurochaetae on chaetigers 2–4. Three to five inferior geniculate neurochaetae present on chaetiger 5. Single row of neurochaetae on chaetiger 6; absent thereafter.

Hooded hooks: Bidentate hooded hooks from chaetiger 7 onwards; 90° angle between main tooth and shaft (Fig. 3.1D and H); constriction present on shaft. Hooks remain bidentate throughout. Hooded hooks increase in number from 8 on chaetiger 7 to 11 per series from chaetiger 10 onwards, then decrease to two per ramus on last chaetiger.

Chaetiger 5: Four to six distally falcate modified spines with a subterminal flange on shaft (Fig. 3.5C). Anterior flange worn and tooth-like (figs 3.1F and 3.5C). Youngest modified spines are not as worn and flange clearly visible (Fig. 3.5C). Modified spines alternate with spear-shaped companion chaetae (figs 3.1E and 3.5C).

Filiform branchiae start on chaetiger 7 (figs 3.1A, 3.2A and 3.5A), initially ¼ of body width, increasing to full size (½ of body width) by chaetigers 8–11; separate from the notopodial lobe and touch mid-dorsum (Fig. 3.3C). Branchiae present on approximately 86% of chaetigers. Branchiae never overlap with posterior modified spines. Number of branchiae increase with number of chaetigers (Fig. 3.7A). Pygidium is wider than posterior-most chaetigers, saucer shaped with dorsal notch (figs 3.1B and 3.6A).

Paired glandular pouches present from chaetiger 7-20; and absent thereafter. Glandular pouch with 13 glandular secretory cells (Fig. 3.1D). External openings of glandular secretory cells club shaped on chaetigers 7–9 (Fig. 3.6B).

Methyl green staining pattern present from chaetiger 6 onwards. On first few chaetigers pattern present as large individually stained spots on side of chaetiger. Number of stained spots increase on chaetigers that follow. Spots initially present on side of chaetigers,

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posteriorly extending to the middle of each chaetiger. Number of stained spots decreases on last few chaetigers with single spot present on last chaetiger.

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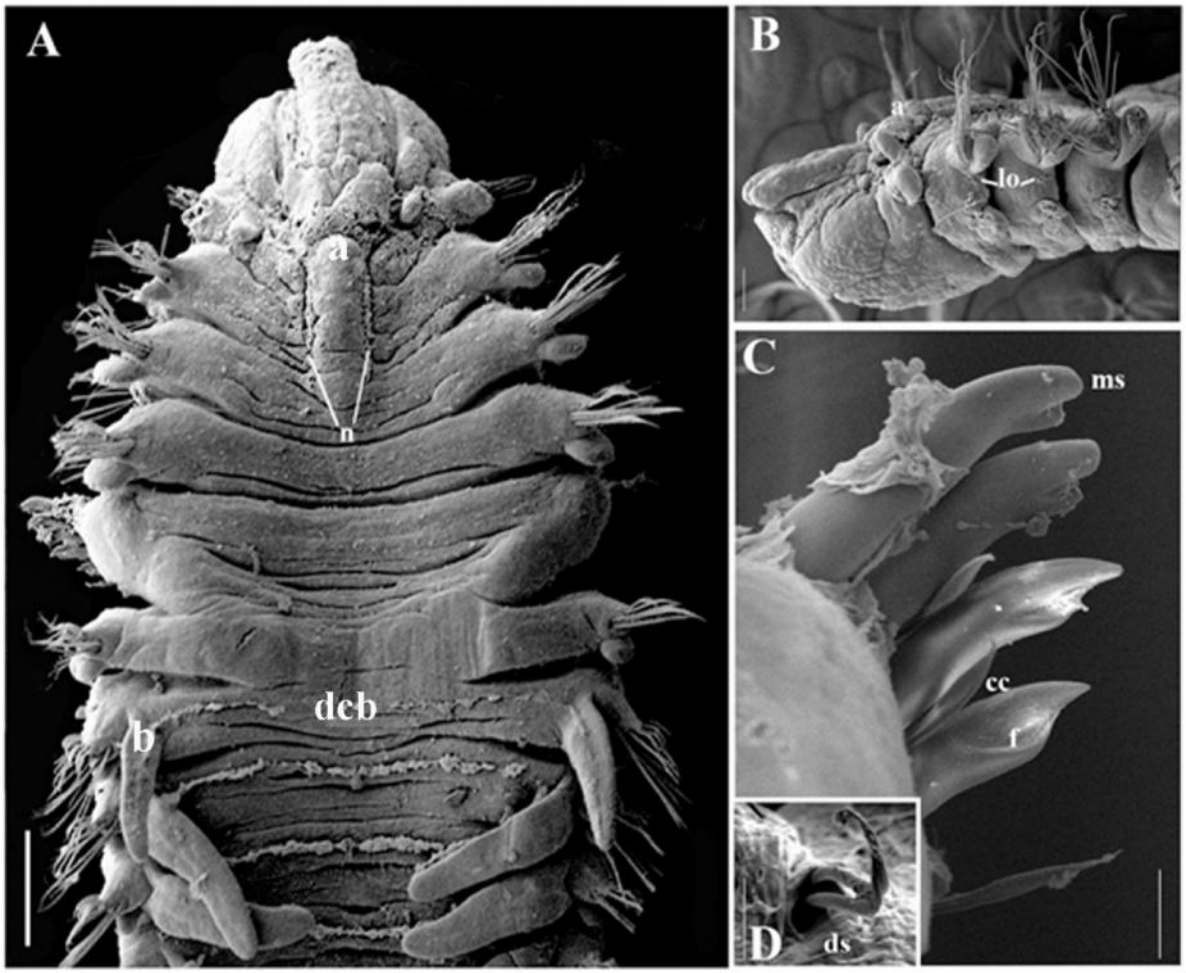

Figure 3.5: *Polydora hoplura* morphotype 1 adult morphology: (A) dorsal view of anterior region with bilobed prostomium, occipital antenna (a), nuchal organ (n) along margin of the caruncle, dorsal ciliary bands (dcb) on chaetigers 7–9 and branchiae from chaetiger 7 onwards (b); (B) lateral view of lateral organs (lo) on chaetigers 2 and 3 – note the occipital antenna (a); (C) dorsal view of modified spines on chaetiger 5 (ms) with subterminal flange (f) and spear-shaped companion chaetae (cc); (D) dorsal view of dorsal superior chaetae (ds) on chaetiger 5. Scale bars: (A) 200 µm; (B) 100 µm; (C and D) 20 µm.

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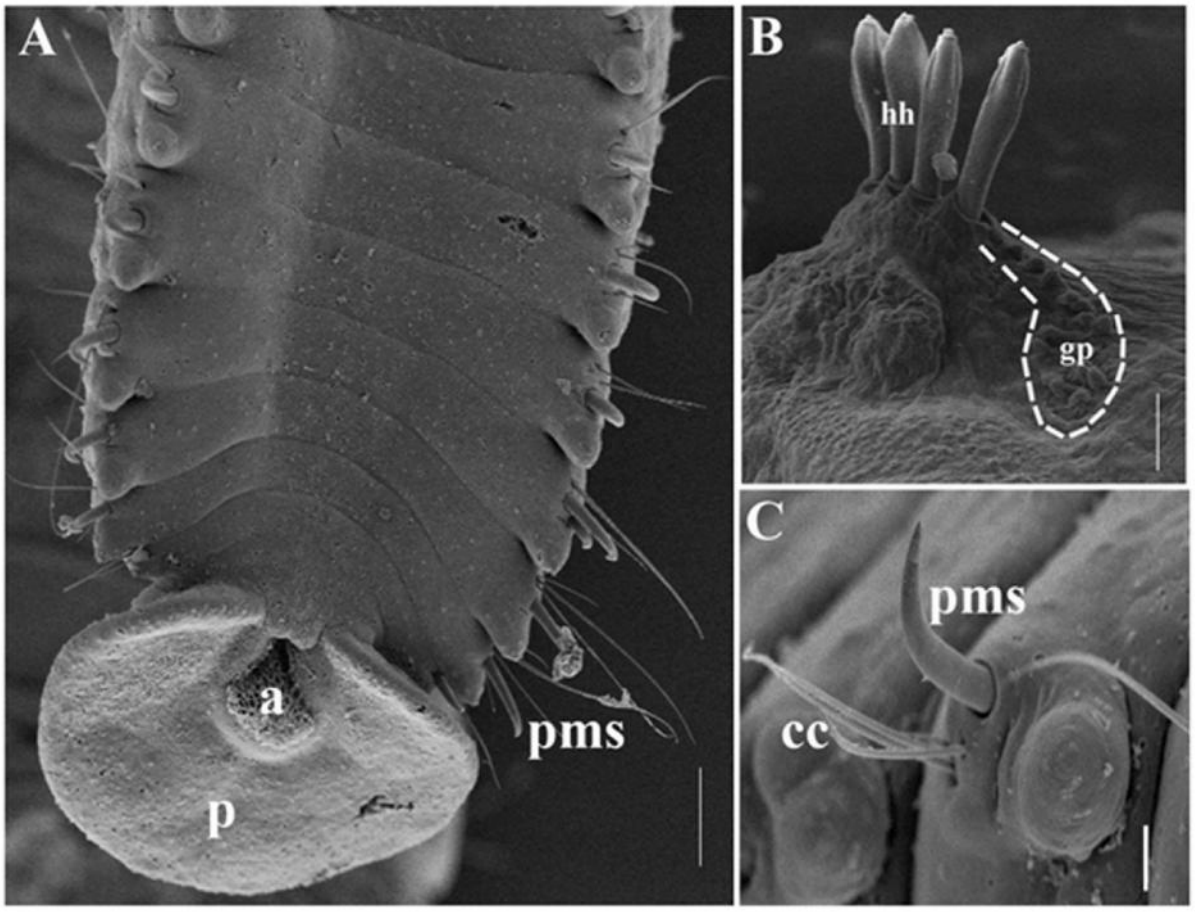

Figure 3.6: *Polydora hoplura* morphotype 1 adult morphology: (A) dorsal view of posterior region with saucer-shaped pygidium (p), posterior modified spines (pms) and anus (a); (B) hooded hooks of chaetiger 8, external openings of glandular pouch (gp) club shaped (enclosed by dashed line); (C) dorsal view of posterior modified spine (pms) and needle-shaped companion chaetae (cc). Scale bars: (A) 100 µm; (B) 10 µm; (C) 20 µm.

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Host and distribution

Live specimens of morphotype 1 were found boring into the shells of farmed *Pecten sulcicostatus* collected from Saldanha Bay. Twenty-five specimens collected by CAS in 2005 (see Simon, 2011) were classified as morphotype 1; they were present at all sites sampled (from Struisbaai to Haga Haga) and were collected from *Haliotis midae*, *Turbo sarmaticus* and *Scutellastra longicosta*. The specimen collected by Day (1954) was classified as morphotype 1 and was collected from dredgings from Saldanha Bay, representing the only specimen collected from sand.

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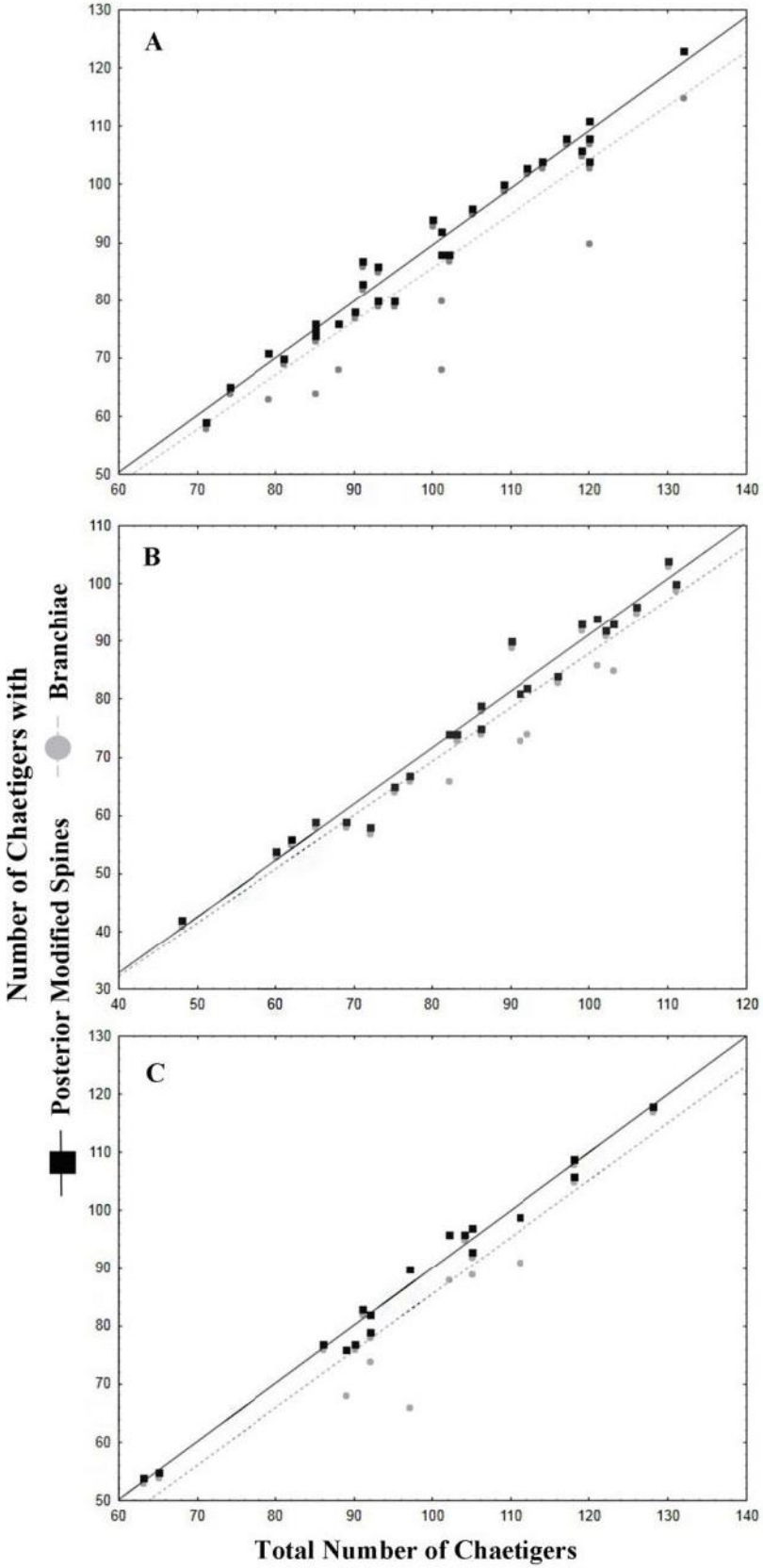

Figure 3.7: *Polydora hoplura* relationship between number of chaetigers with branchiae (black squares) or posterior modified spines (grey circles) and the total number of chaetigers: (A) *P. hoplura* morphotype 1, (B) *P. hoplura* morphotype 2 and (C) *P. hoplura* morphotype 3.

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Polydora hoplura morphotype 2

(Figs 3.3 and 3.8–3.10)

Best descriptions from the literature conforming to morphotype 2

Carazzi 1893, p. 20

Material examined

South Africa

Saldanha Bay:	Five specimens were collected by NS in 2001 from an unknown substrate (SAMC A 21489); seven specimens were collected by SvN/SdL in 2011 from farmed *P. sulcicostatus.*
False Bay:	One specimen was collected by SvN/SdL in 2011 from wild *Pecten sulcicostatus.*
Struisbaai:	Eight specimens were collected by CAS in 2005 from wild *Haliotis midae* and *Turbo sarmaticus.*
Mossel Bay:	Six specimens were collected by CAS in 2005 from *T. sarmaticus* and farmed *H. midae.*
Haga Haga:	One specimen was collected by CAS in 2005 from cultured H. *midae.*

Australia

Rapid Bay:	One specimen was collected by Hutchings and Turvey in 1977 from among sessile organisms on a jetty (AM W19298).
New South Wales:	One specimen was collected by C. Pregenzer in 1975 from an oyster (AM F43060).
Tasmania:	One specimen was collected by M. Skeel in 1977 from *Crassostrea gigas* (AM W26121).

Description

Specimens up to 19 mm long for 104 chaetigers and 0.28 mm wide at chaetiger 5. Specimens lack pigmentation (figs 3.3G and 3.8A). Prostomium weakly notched to rounded bilobed (figs. 3.3G, 3.8A and 3.9A). Eyes may be absent or two pairs present in trapezoidal arrangement (figs 3.3G and 3.8A). Caruncle extends to the end of chaetiger 2 (figs 3.8A and 3.9A). Occipital antenna present on caruncle behind eyes (figs 3.8A and 3.9A).

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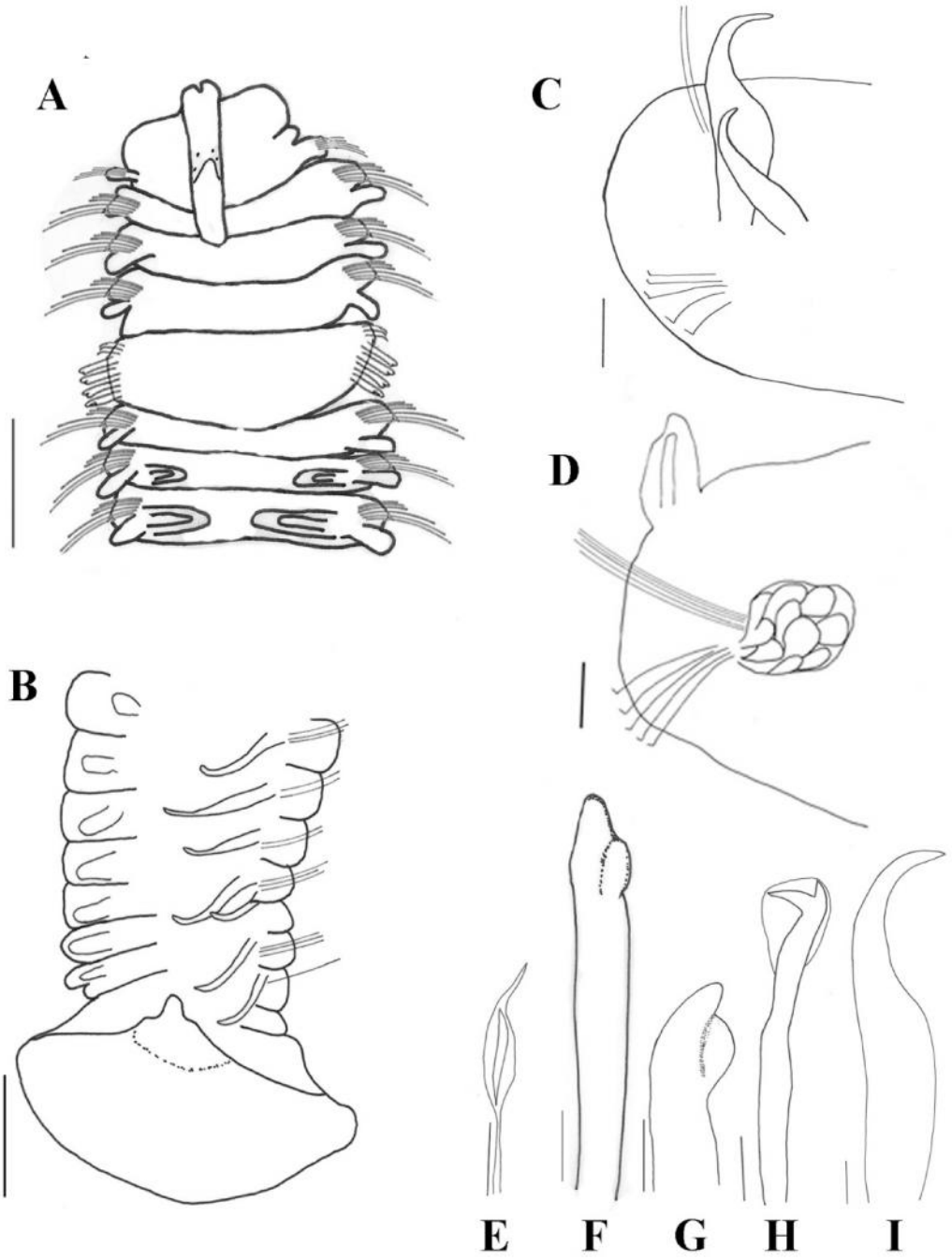

Figure 3.8: *Polydora hoplura* (Claparède, 1869) morphotype 2 adult morphology: (A) dorsal view of anterior region – note the absence of colouration on peristomium; (B) dorsal view of posterior region, spines and notopodia omitted on left and right side of chaetigers respectively – note the absence of pigmentation on the pygidium; (C) cross-section of a posterior chaetiger with gross structure of modified spines and companion chaetae; (D) cross-section of chaetiger 7 with gross morphology of glandular pouch; (E) spear-shaped companion chaetae of chaetiger 5; (F) most worn modified spine of chaetiger 5; (G) youngest modified spine of chaetiger 5; (H) hooded hook with constriction on shaft; (I) posterior modified spine. Scale bars: (A and B) 0.1 mm; (B and C) 0.05 mm; (E–H) 0.01 mm; (I) 0.02 mm.

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Nuchal organ present as ciliated grooves along margin of caruncle (Fig. 3.9A).

Lateral organs on chaetigers 1–3 (Fig. 3.9B) then on chaetigers 7 and 8. Dorsal ciliary bands present from chaetiger 7 onwards (Fig. 3.9A).

Notochaetae: Absent on chaetiger 1. Two rows of chaetae on chaetiger 2–4, first row unilimbate second row lanceolate. Two to five geniculate dorsal superior chaetae on chaetiger 5 (Fig. 3.9D). Chaetae of chaetigers 6–9, single row of unilimbate chaetae. Yellow recurved modified spines present for posterior 11% of chaetigers (figs 3.7B, 3.8C and I; 3.10A and C). One or two spines per series. Spines accompanied by up to three needle-like companion chaetae (Fig. 3.10C). Hooks only present on posterior abranchiate chaetigers.

Neurochaetae: Single row of unilimbate chaetae on chaetiger 2–4. Up to five geniculate ventral inferior chaetae on chaetiger 5. Unilimbate neurochaetae present on chaetiger 6 and then absent.

Hooded hooks: Eight bidentate hooks present on chaetiger 7; 90° angle between main tooth and shaft and constriction on shaft of spine (Fig. 3.8H). Up to 11 hooks per series from chaetiger 10 onwards. Never becoming unidentate.

Chaetiger 5: Six distally falcate spines with subterminal flange (Fig. 3.9C). Flange appears tooth-like on worn anterior spines (figs 3.8F and 3.9C). Flange more pronounced on younger spines (figs 3.8G and 3.9C). Spines alternate with spear-shaped companion chaetae (Fig. 3.8E).

Filiform branchiae present from chaetiger 7 onwards (figs 3.8A and 3.9A). Initially ¼ of body width on chaetiger 7; increase to ½ of body width from chaetiger 8-11. Touch mid-dorsum (Fig. 3.9A).Decrease to ¼ of body width for last 3 branchiate chaetigers. Separate

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from notopodial lobe. Branchiae on 79% to 93% of chaetigers, and number is proportional to total number of chaetigers (Fig. 3.7B).

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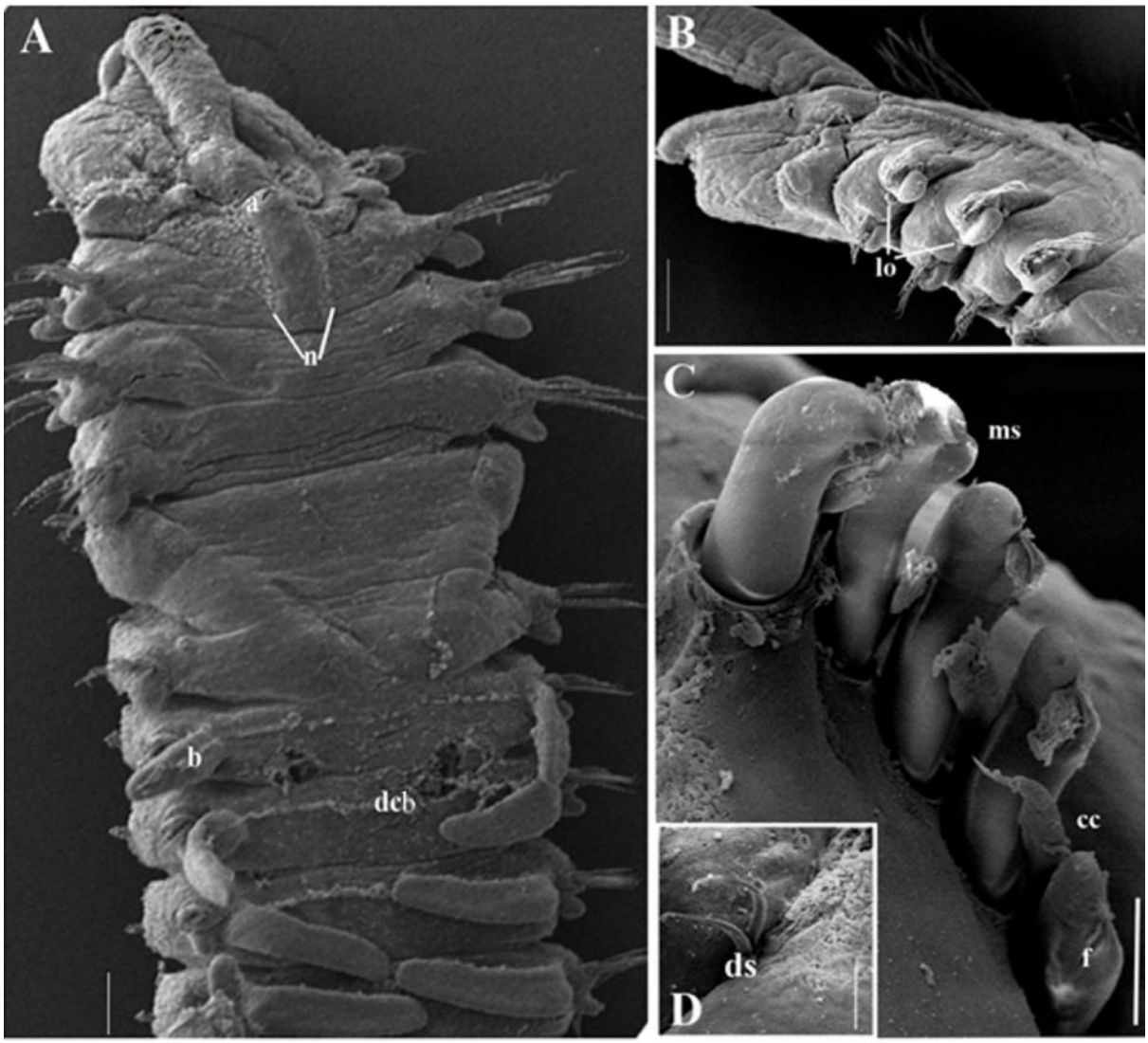

Figure 3.9: *Polydora hoplura* morphotype 2 adult morphology: (A) dorsal view of anterior region with bilobed prostomium, occipital antenna (a), nuchal organ (n), dorsal ciliary bands (dcb) from chaetiger 7 to chaetiger 9 and branchiae (b) from chaetiger 7 onwards; (B) lateral view showing lateral organs (lo) on chaetigers 2 and 3; (C) dorsal view showing modified spines (ms) with subterminal flange (f) and spear-shaped companion chaetae (cc) on chaetiger 5; (D) dorsal view of geniculate dorsal superior notochaetae (ds) on chaetiger 5. Scale bars: (A) 100 µm; (B) 200 µm; (C) 20 µm; (D) 10 µm.

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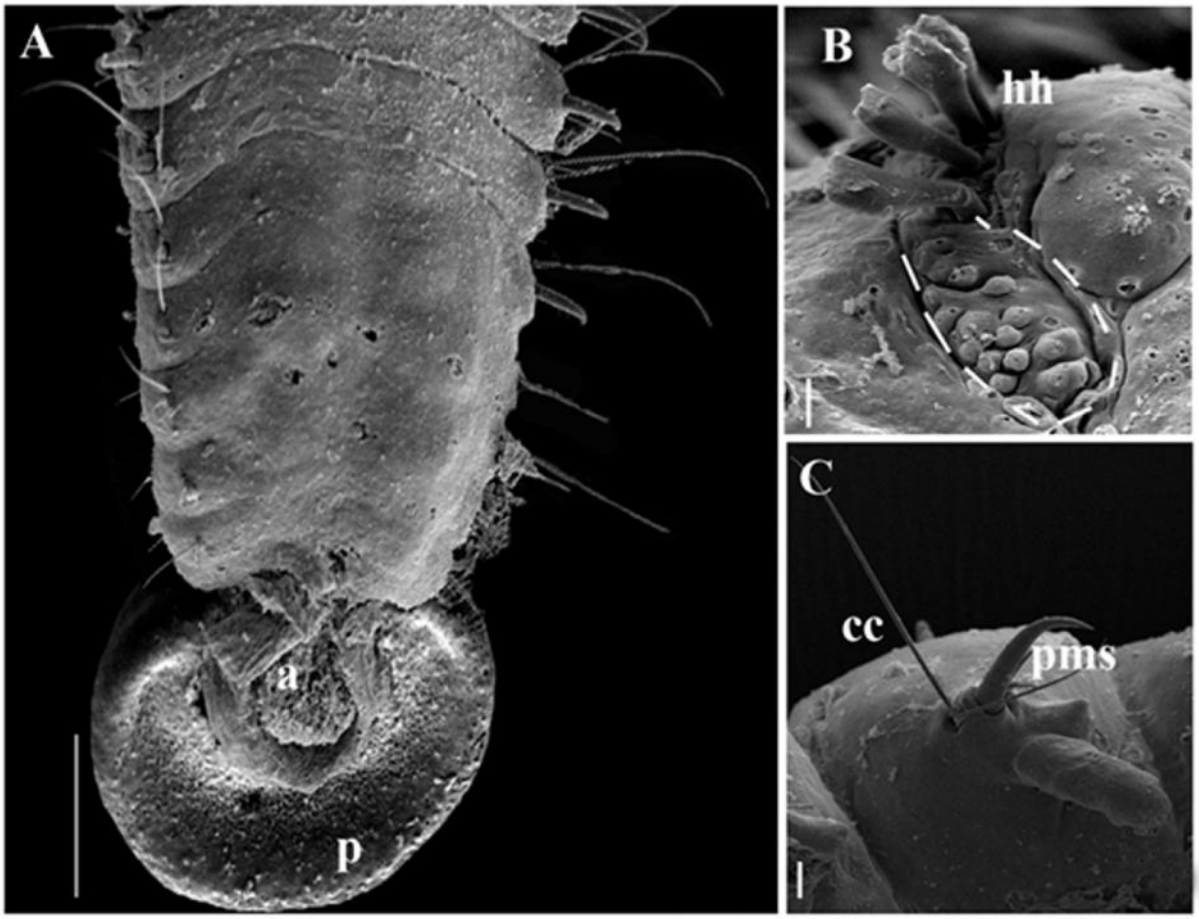

Figure 3.10: *Polydora hoplura* morphotype 2 adult morphology: (A) dorsal view of posterior region showing pygidium (p) and anus (a); (B) hooded hooks on chaetiger 8 – note the oval-shaped external openings of glandular pouches (enclosed by dashed line); (C) dorsal view of posterior modified spines (pms) and needle-shaped companion chaetae (cc). Scale bars: (A) 100 µm; (B) 10 µm; (C) 20 µm.

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Glandular pouches present from chaetiger 7, continuing to chaetiger 11 or 12; not visible on middle of the animal. Oval pouch with up to 10 smaller glandular secretory cells (Fig. 3.8D); external openings of glandular secretory cells on chaetigers 7–9 oval shaped (Fig. 3.10B). Pygidium a large flared disk with dorsal notch (figs 3.8B and 3.10A).

Methyl green staining pattern present on chaetiger 7 onwards. Spots present along the sides of chaetigers initially, then extending toward middle of chaetigers. Number of spots decreasing towards the posterior. Staining pattern stops on first chaetiger with posterior modified spines or continues to the third last chaetiger.

Host and distribution

Specimens were found boring into *Pecten sulcicostatus* collected from Saldanha Bay and False Bay in 2011, and farmed *Haliotis midae* and wild *Turbo sarmaticus* collected from Struisbaai, Mossel Bay and Haga Haga.

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Polydora hoplura morphotype 3

(Figs 3.3, 3.11–3.13)

Material examined

South Africa

Saldanha Bay: Two specimens were collected by NS in 2001 from an unknown substrate (SAMC A 21489). One specimen was collected by SvN/SdL in 2011 from shells of farmed *Pecten sulcicostatus.*

Struisbaai: Two specimens were collected by CAS in 2005 from an unidentified limpet and from wild *Haliotis midae.*

Mossel Bay: Thirteen specimens were collected by CAS in 2005 from *Perna perna* Linneaus, 1758, *Turbo sarmaticus* and *Scutellastra longicosta.*

Grootbank: One specimen was collected by CAS in 2005 from shells of wild *T. sarmaticus.*

Description

Specimens up to 27 mm long for 118 chaetigers and 0.34 mm wide at chaetiger 5. No pigmentation (figs 3.3H and 3.11A). Prostomium rounded (figs 3.11A and 3.12A). Eyes absent or up to four in trapezoidal arrangement (Fig. 3.3H). Caruncle extends to middle or end of chaetiger 3 (Fig. 3.11A). Occipital antenna present behind eyes on caruncle (figs 3.11A and 3.12A). Nuchal organ extends along the margin of caruncle (Fig. 3.12A). Lateral organs present on chaetiger 1 and chaetiger 7 (Fig. 3.12B). Dorsal ciliary bands present from chaetiger 7 onwards (Fig. 3.12A).

Notochaetae: Absent on chaetiger 1. Two rows present on chaetigers 2–4, first row unilimbate, second row lanceolate. Up to five geniculate superior chaetae present on chaetiger 5 (Fig. 3.12D). Single row of unilimbate notochaeta present from chaetiger 6 onwards. One or two yellow recurved spines present per series; present for last 11% of chaetigers (figs 3.7C; 3.11C and I; 3.13A and C). Two or three needle-like chaetae present (Fig. 3.13C).

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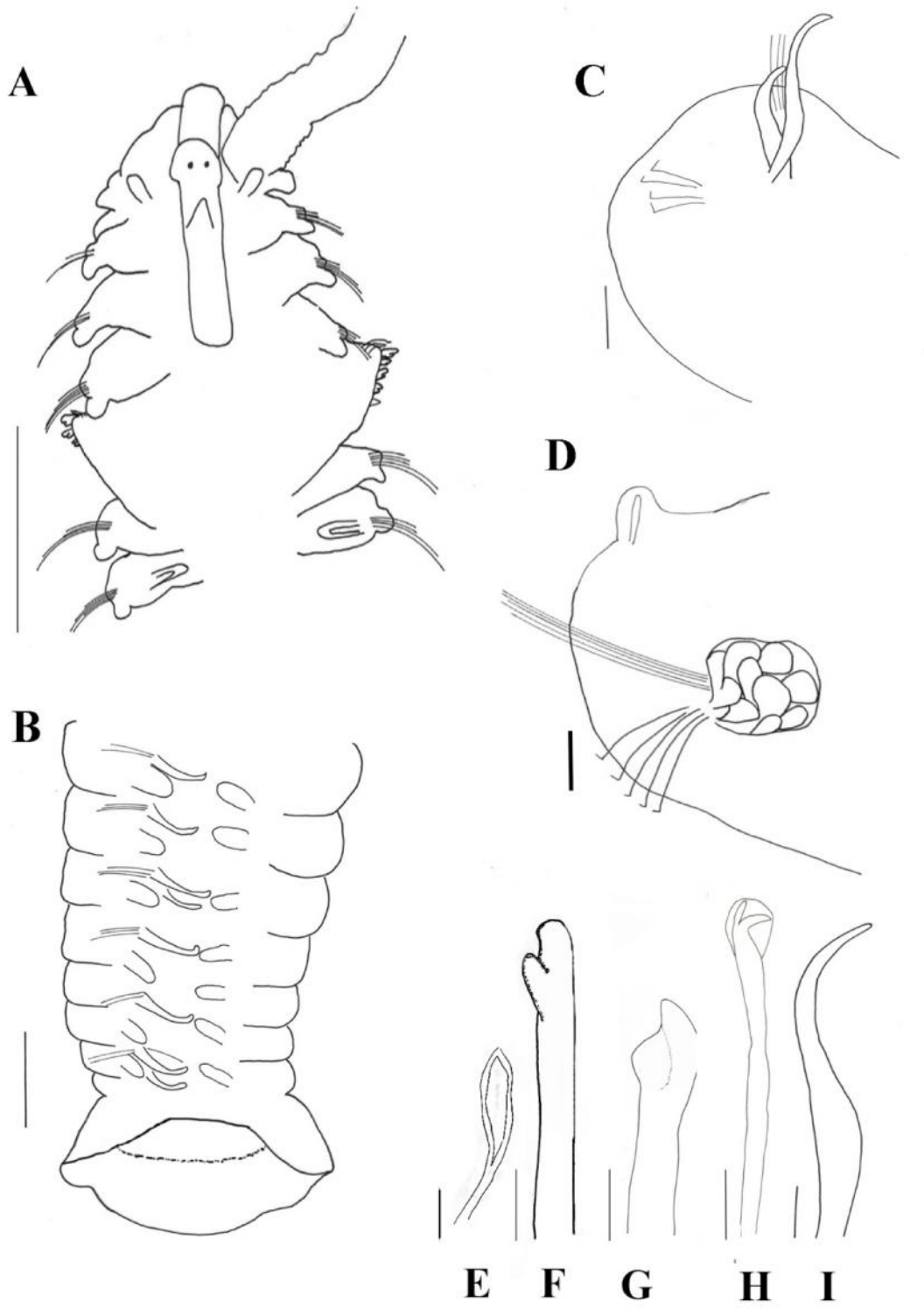

Figure 3.11: *Polydora hoplura* (Claparède, 1869) morphotype 3 adult morphology: (A) dorsal view showing anterior region, lack of pigmentation on the peristomium; (B) dorsal view of posterior region – note the absence of pigmentation on the pygidium; (C) cross-section of a posterior chaetiger with posterior modified spines and needle-like companion chaetae; (D) cross-section of chaetiger 7 with gross structure of glandular pouch; (E) spear-shaped companion chaetae of chaetiger 5; (F) most worn modified spine of chaetiger 5; (G) most embedded modified spine of chaetiger 5; (H) hooded hook with constriction on shaft; (I) posterior modified spine. Scale bars: (A and B) 0.1 mm; (C and D) 0.05 mm; (E–H) 0.01 mm; (I) 0.02 mm.

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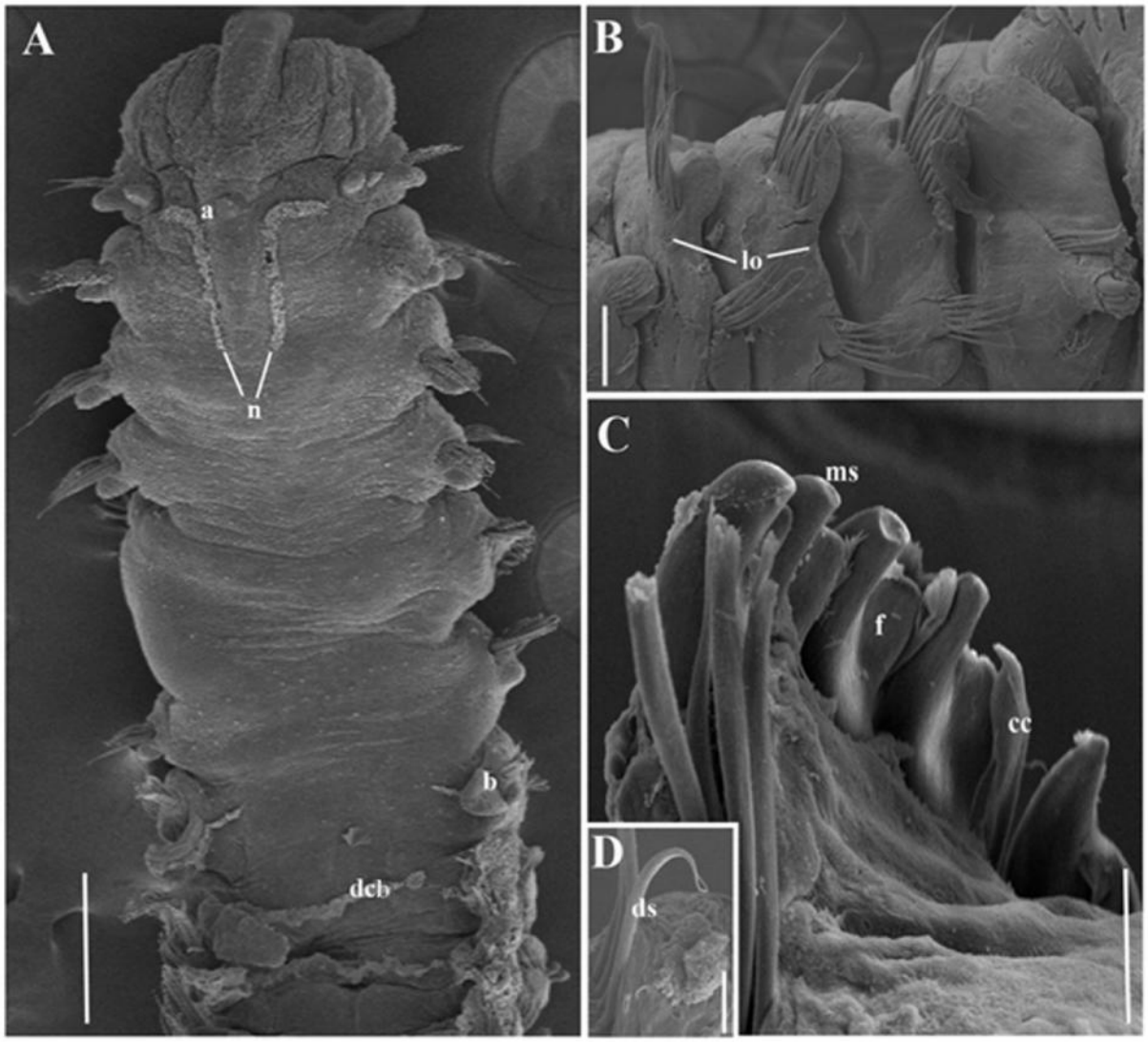

Figure 3.12: *Polydora hoplura* morphotype 3 adult morphology: (A) dorsal view of anterior region showing rounded prostomium, antenna (a), nuchal organ along the ridge of the caruncle (n), dorsal ciliary bands (dcb) on chaetigers 7–9 and branchiae (b) from chaetiger 7 onwards; (B) lateral view showing lateral organs (lo) on chaetigers 2 and 3; (C) dorsal view of modified spines (ms) with subterminal flange (f) and spear-shaped companion chaetae (cc) on chaetiger 5; (D) dorsal view of geniculate dorsal superior chaetae (ds) on chaetiger 5. Scale bars: (A) 200 µm; (B and E–F) 20 µm; (C and D) 100 µm.

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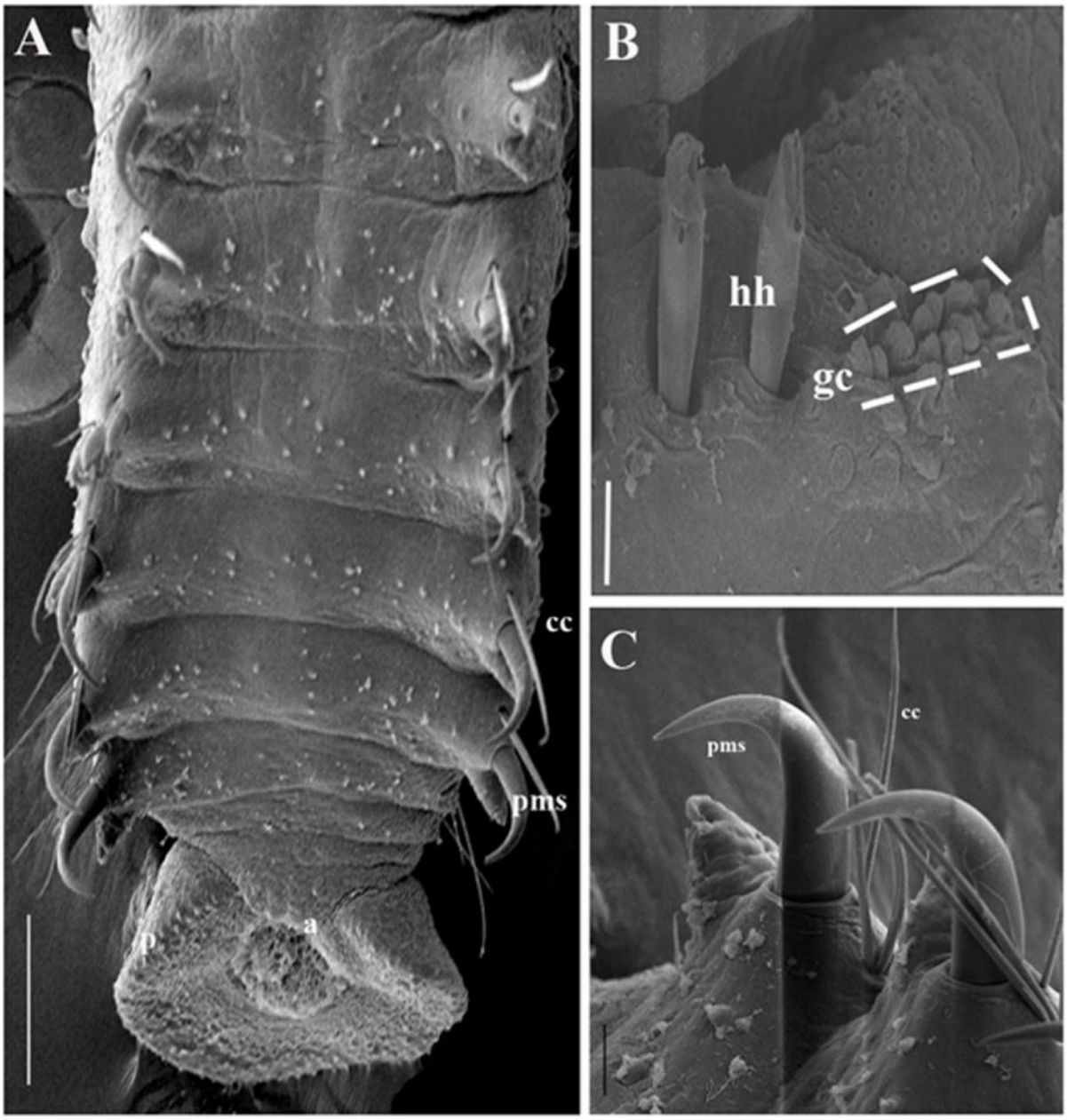

Figure 3.13: *Polydora hoplura* morphotype 3 adult morphology: (A) dorsal view of posterior region showing pygidium (p), anus (a), posterior modified spines (pms) and needle-like companion chaetae (cc); (B) hooded hooks on chaetiger 8 – note the oval-shaped external openings of glandular pouches (enclosed by dashed line); (C) dorsal view of posterior modified spines (pms) and needle-shaped companion chaetae (cc). Scale bars: (A) 100 µm; (B and C) 20 µm.

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Neurochaetae: Single row of unilimbate chaetae on chaetigers 2–4. Two to five geniculate inferior ventral neurochaetae present on chaetiger 5. Then single row of unilimbate chaetae present on chaetiger 6, absent thereafter.

Hooded hooks: Eight bidentate hooded hooks present from chaetiger 7-9 (Fig. 3.11H). Up to 11 hooks present from chaetiger 10 onwards, but decreasing posteriorly to two on last chaetiger. Constriction present on shaft with 90 ° angle between main tooth and shaft of hook. Hooks never become unidentate.

Chaetiger 5: Up to six distally falcate modified spines with subterminal flange present (Fig. 3.12C). Flange worn and tooth-like on anterior modified spines (Fig. 3.11F). Youngest modified spine with prominent flange (Fig. 3.11G). Spines alternate with spear-shaped companion chaetae (Fig. 3.11E).

Filiform branchiae from chaetiger 7 onwards (figs 3.11A and 3.12A). Branchiae are initially ¼ of body width on chaetiger 7, increasing to ½ of body width on chaetigers 8–11. Touch mid dorsum (Fig. 3.3H). Number of branchiate chaetigers increases with increasing number of chaetigers (Fig. 3.7C).

Glandular pouches present in chaetigers 7–11 or 12, absent thereafter (Fig. 3.11D). Large pouch filled with up to 10 smaller glandular secretory cells on chaetiger 7 (Fig. 3.11D). External openings of glandular secretory cells are oval shaped on chaetigers 7–9 (Fig. 3.13B). Size gradually decreases after chaetiger 9, not visible after chaetiger 20. Pygidium is a broad saucer-shaped disk with dorsal notch (figs 3.11B and 3.13A).

Methyl green staining pattern present from chaetiger 6 onwards. Single spot stained on sides of chaetiger present.

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Host and distribution

First recorded in 2011 from shells of *Pecten sulcicostatus* from Saldanha Bay. Specimens were also collected from *Haliotis midae* and *Perna perna* from Struisbaai and Mossel Bay and *Turbo sarmaticus* and *Scutellastra longicosta* from Grootbank.

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Polydora hoplura morphotype 4

(Figs 3.14 and 3.15)

Material examined

South Africa

Saldanha Bay: Four specimens were collected by CAS from farmed *Haliotis midae* in 2012.

Description

Up to 5 mm long for 41 chaetigers and 0.16 mm wide at chaetiger 5. Prostomium shape varies from bilobed (figs 3.14A and 3.15A) to rounded (Fig. 3.15B). One pair of eyes (Fig. 3.14A) or two pairs in trapezoid arrangement (Fig. 3.15A and B). Caruncle extends to end of chaetiger 2 (figs 3.14A and 3.15A). Diffuse pigmentation on first 4 chaetigers (Fig. 3.14A) or may be absent. Chaetiger 1 pigmented ventrally. Single black spot present at the base of each branchia from chaetiger 8 to 15 (figs 3.14A and 3.15A). Posterior region lacks pigmentation.

Notochaetae: Absent on chaetiger 1. Chaetae of chaetigers 2–4 arranged in two tiers, first tier unilimbate, second tier lanceolate. Up to five superior geniculate chaetae on chaetiger 5. Single row of unilimbate notochaetae on chaetiger 6 onwards. One or two yellow recurved modified hooks per ramus present on last four chaetigers.

Neurochaetae: Single row of unilimbate chaetae on chaetigers 1–4. Chaetiger 5 with up to five geniculate inferior ventral chaetae. Single row of unilimbate chaetae on chaetiger 6 only. Bidentate hooded hooks present on chaetiger 7, 90° angle between main tooth and shaft of hook (Fig. 3.14E). Six hooded hooks present per series. Hooks never become unidentate.

Chaetiger 5: Three distally falcate modified spines (Fig. 3.14 B & C). Spear-shaped companion chaetae on chaetiger 5 (Fig. 3.14 D). Spines are worn anteriorly (Fig. 3.14B), with the flange of most anterior spines appearing tooth-like (Fig. 3.14 C).

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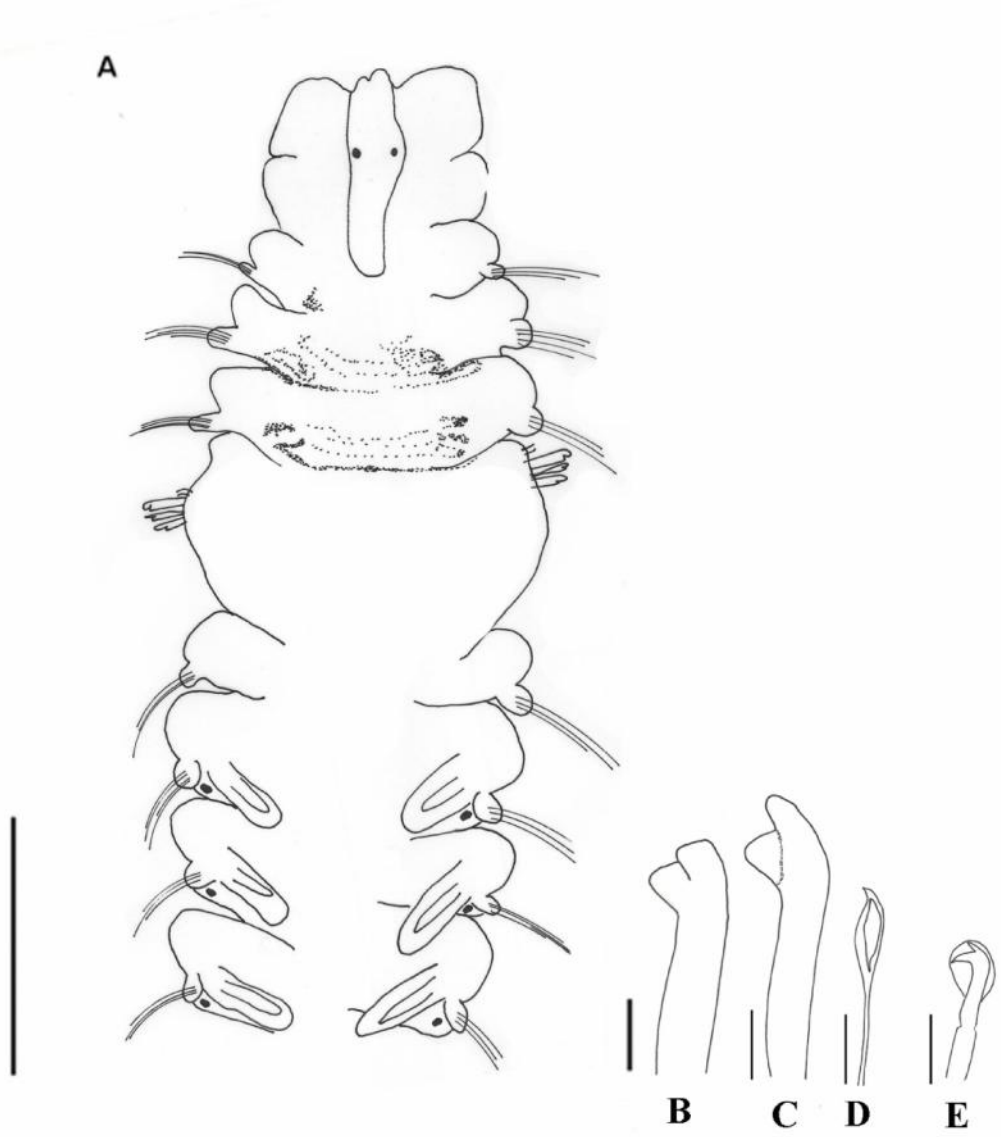

Figure 3.14: *Polydora hoplura* (Claparède, 1869) morphotype 4: (A) dorsal view – note the bilobed prostomium and pigmentation to chaetiger 4; (B) most anterior modified spine and (C) youngest spine on chaetiger 5; (D) spear-shaped companion chaetae on chaetiger 5; (E) bidentate hooded hook. Scale bars: (A–E) 0.1 mm.

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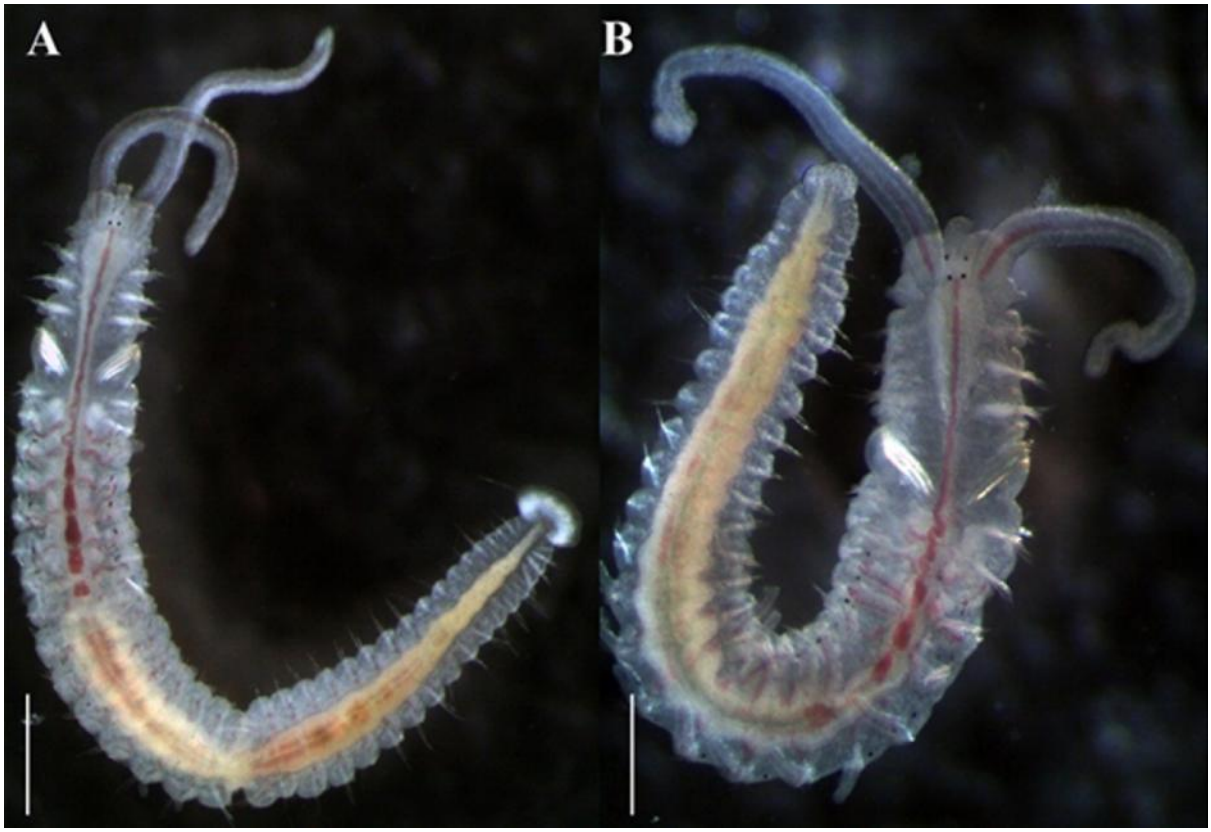

Figure 3.15: *Polydora hoplura* morphotype 4: (A) with rounded-bilobed prostomium, pigmentation present at the base of each branchiae from chaetiger 8 onwards; (B) with rounded prostomium – note the pigmentation at the base of each pair of branchiae from chaetiger 8 onwards. Scale bars: (A and B) 0.5 mm.

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Filiform branchiae present from chaetiger 7 onwards (figs 3.14A and 3.15A and B). Separate from notopodial lobe. First pair is small (¼ of body width), increasing to ½ body width from chaetiger 8 onwards. Branchiae present for up to 68% of total body length. Pygidium is saucer shaped with large dorsal notch (Fig. 3.15A).

Methyl green staining pattern from chaetiger 9 continues to last chaetiger. Few spots stained on chaetiger 9. Number of stained spots increase towards the posterior, but single stained spot present at the side of last chaetiger.

Host and distribution

Specimens of morphotype 4 were first discovered in sand tubes from Saldanha Bay in 2010 and were subsequently recorded as a surface fouler on *Haliotis midae* and *Crassostrea gigas.* Specimens were also collected from Saldanha Bay in late 2012. Morphotype 4 has only been collected from Kleinzee, Saldanha Bay and Port Elizabeth thus far.

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Larval development

Polydora hoplura morphotype 2

(Fig. 3.16)

Material examined

Adelphophagic 13-chaetiger and 17-chaetiger larvae were collected by CAS in 2008 from farmed *Haliotis midae* and preserved on slides.

Description

Thirteen-chaetiger larvae: Palps present at this stage and extend to the fourth chaetiger (Fig. 3.16A). Four eyes present (Fig. 3.16A). Caruncle extends to the end of chaetiger 1. Older melanophores present on chaetigers 1–6 are not continuous across the chaetigers (Fig. 3.16A). Ramified (irregularly shaped) melanophores are younger and are present from chaetiger 7 onwards (Fig. 3.16A). Pygidial chaetiger is pigmented.

Chaetiger 5: Two distally falcate modified spines present. Bidentate hooded hooks present on chaetiger 7 continuing to last chaetiger, up to three per ramus. Constriction present on shaft of hook. Hooks never become unidentate. Filiform branchiae from chaetiger 7-9 (Fig. 3.16A). Separate from notopodial lobe. Branchiae ¼ of body width throughout. Telotroch extends around pygidial chaetiger. Pygidial chaetiger is cuff shaped with dorsal cleft.

Seventeen-chaetiger larvae: Four eyes present (Fig. 3.16B). Caruncle extends to end of chaetiger 1. Shape of the melanophores changes as chaetigers are added; older melanophores present on chaetigers 1–9, and younger melanophores appear ramified from chaetiger 10 (Fig. 3.16B). Pigmentation present on posterior margin of pygidium.

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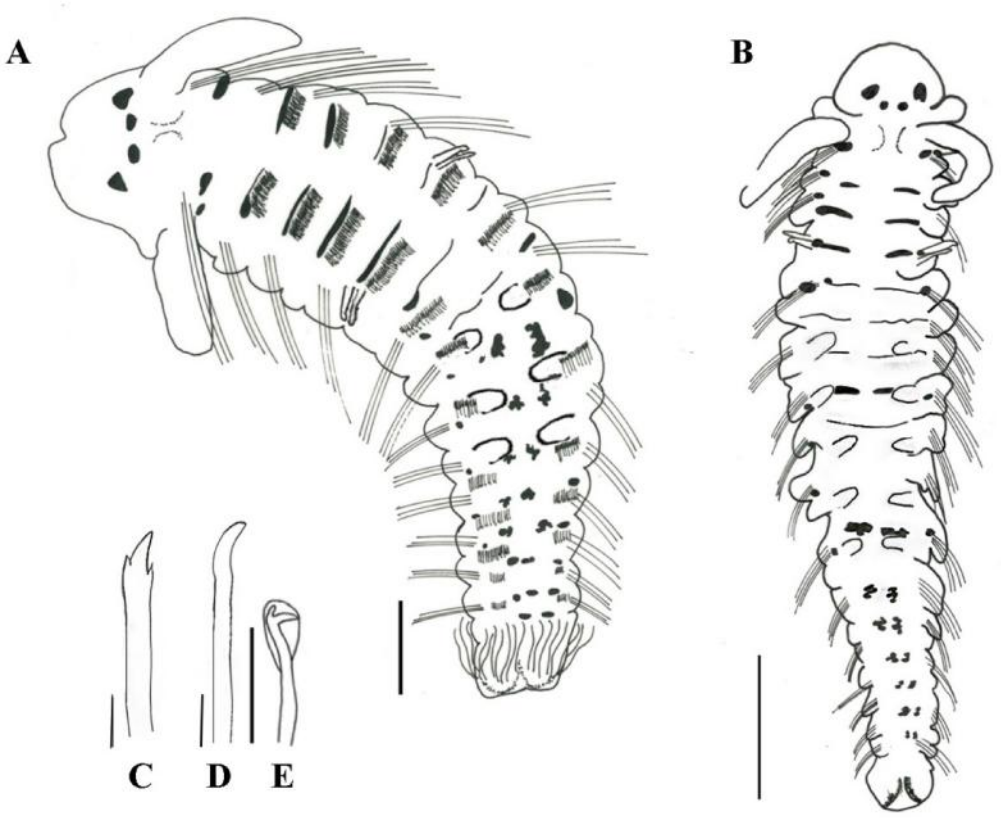

Figure 3.16: *Polydora hoplura* Claparède, 1869 larval morphology. Dorsal views of (A) 13-chaetiger larvae and (B) 17-chaetiger larvae. (C) Modified spine with lateral spurs; (D) modified spine; (E) bidentate hooded hook of 17-chaetiger larva. Scale bars: (A) 0.05 mm; (B) 0.02 mm; (C–E) 0.1 mm.

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Single row of notochaetae on chaetigers 2–4, then on 6 onwards. Chaetiger 5 with two types of modified spine, first with two lateral spurs (Fig. 3.16C) and second blunt and distally falcate (Fig. 3.16D). Unable to observe neurochaetae. Hooded hooks are bidentate and up to three in a series (Fig. 3.16E). Hooks never become unidentate and have constriction on shaft. Present from chaetiger 7, continue to the last chaetiger. No posterior modified spines present.

Branchiae filiform ¼ of the body width (Fig. 3.16B), separate from notopodial lobe. Pairs of branchiae never touch mid dorsum. Telotroch present around pygidial chaetiger. Cuff-shaped pygidial chaetiger with dorsal notch (Fig. 3.16B).

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3.2 Statistical analysis

3.2.1 Comparison of morphotypes with cluster analysis

The cluster analysis revealed very few differences within and among morphotypes 1–4 (n = 98) (Fig. 3.17A). Instead, high levels of similarity are present among individuals of the same morphotype, as indicated by the red asterisks (80%–95%). Similarly, individuals of different morphotypes share high levels of similarity, as indicated by the blue asterisks (73%–91%). The lowest level of similarity recorded between morphotypes was 64% (Fig. 317A). From the overlay of host species/substrate onto the dendrogram, it is clear that morphotypes do not prefer any particular host (Fig. 3.17B).

3.2.2 Fine-scale comparison of posterior modified spines among different morphotypes

Morphotype 1 versus morphotype 2 versus morphotype 3

Table 3.1 shows the results of the Kruskal-Wallis ANOVA. There was no significant difference among morphotypes in the total length, the height to the tip of the spine the widest section of each spine and the tip of the spine to the shaft of the spine. The same analysis showed no significant difference between the angle between the tangent and the spine (Fig. 2.2Φ), and the angle of the curve of the spine among morphotypes (Fig. 2.2θ).

Morphotype 1 versus Day (1954)

Table 3.1 summarises the results from the Mann-Whitney U-test. No significant difference was found when total length (Fig. 2.2 line AB), height of the tip of the spine (Fig. 2.2 line CD) and widest section of the spine (Fig. 2.2 EF) were compared (Table 3.1). The angle between the tangent and the spine (Fig. 2.2Φ) and the curve of the spine (Fig. 2.2θ) also revealed no significant differences among the groups (Table 3.1).

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Morphotype 1 versus Day (1967)

The published drawing of Day (1967) of *P. hoplura* spine does not include a scale, thus only the angles could be determined. Both the angle of the tangent and the spine (22°) (Fig. 2.2Φ) and the angle of the curve of the drawing (46°) (Fig. 2.2θ) are larger than those of morphotype 1, which ranged from 13° to 17° and 21° to 43° respectively.

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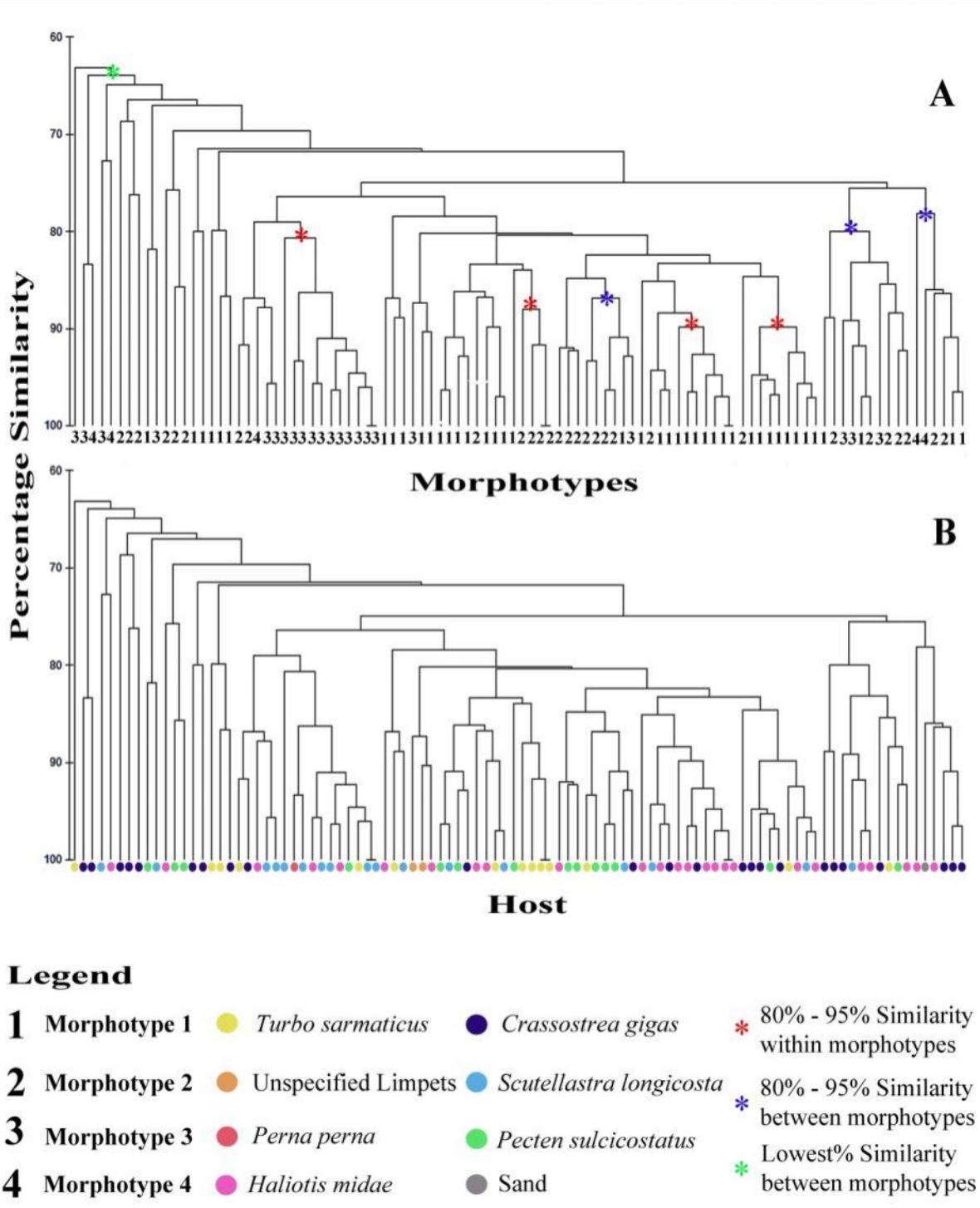

Figure 3.17: (A) Cluster analyses of *Polydora hoplura* according to morphotype and (B) when host species/substrate is overlaid onto dendogram

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Table 3.1: Comparisons of the posterior modified spines among morphotypes 1–3 from this study and between posterior modified spines of morphotype 1 of the current study and specimens collected by Day (1954)

	AB	**CD**	**FG**	**DE**	**θ**	**Φ**
Morphotype 1 ***x*** **± SD** **(min_{mm} & max_{mm})**	0.13 ± 0.035 (0.07 & 0.16)	0.19 ± 0.038 (0.13 & 0.23)	0.029 ± 0.006 (0.022 & 0.033)	0.069 ± 0.011 (0.06 & 0.08)	14 ± 1.9 (12 & 17)	38 ± 8.8 (24 & 47)
Morphotype 2 ***x*** **± SD** **(min_{mm} & max_{mm})**	0.11 ± 0.026 (0.07 & 0.14)	0.16 ± 0.037 (0.14 & 0.2)	0.023 ± 0.004 (0.018 & 0.028)	0.061 ± 0.013 (0.04 & 0.07)	17 ± 5.9 (8 & 23)	40 ± 4.7 (34 & 45)
Morphotype 3 ***x*** **± SD** **(min_{mm} & max_{mm})**	0.14 ± 0.028 (0.03 & 0.13)	0.14 ± 0.069 (0.03 & 0.21)	0.023 ± 0.01 (0.013 & 0.037)	0.06 ± 0.013 (0.01 & 0.04)	16 ± 6.2 (8 & 24)	43 ± 3.6 (39 & 47)
Morphotype 1 vs. morphotype 2 vs. morphotype 3 **Kruskal-Wallis ANOVA**	df = 2 *H* = 1.999 *p* = 0.368	df = 2 *H* = 1.267 *p* = 0.531	df = 2 *H* = 1.671 *p* = 0.434	df = 2 *H* = 0.984 *p* = 0.612	Df = 2 *H* = 1.159 *p* = 0.434	df = 2 *H* = 1.244 *p* = 0.537
Morphotype 1 vs. Day (1954) **Mann-Whitney U-test**	*U* = 12 *p* = 0.624	*U* = 9 *p* = 0.905	*U* = 6 *p* = 0.413	*U* = 6 *p* = 0.413	*U* = 16.5 *p* = 0.107	*U* = 12.5 *p* = 0.539

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3.3 Molecular analysis

Thirty-six Cytochrome b and 30 28S rRNA sequences were generated respectively. The Cytochrome b sequence consisted of 379 base pairs of which 128 were variable characters (34%) and 78 were parsimony informative (21%). The 28S sequence consisted of 947 base pairs of which 54 were variable characters (6%) and 17 were parsimony informative (2%).

3.3.1 Parsimony haplotype networks

Mitochondrial haplotype network

The mitochondrial parsimony haplotype network revealed 19 haplotypes (Fig. 3.18). Haplogroup 1 consisted of 15 haplotypes with no clear correspondence with specific morphotypes. Larvae produced by morphotype 1 females shared a haplotype with an adult morphotype 3 individual. Individuals collected from different host species also shared haplotypes. Haplogroup 2 consisted of four individuals with two haplotypes; one individual was collected in New Zealand. Three individuals from South Africa were separated by a few mutation steps from a specimen collected in New Zealand. Two singleton haplotypes that did not connect to either haplogroups 1 or 2 with more than 90% confidence were revealed: 1) an adelphophagic larva and 2) a morphotype 2 individual.

Nuclear haplotype network

The nuclear parsimony haplotype network revealed the presence of 11 haplotypes (Fig. 3.19). Sixteen individuals, representing all four morphotypes, shared a haplotype (Fig. 3.19). A specimen from New Zealand shared a haplotype with South African specimens. Two unconnected haplotypes were also revealed: 1) a morphotype 2 singleton, the same as from

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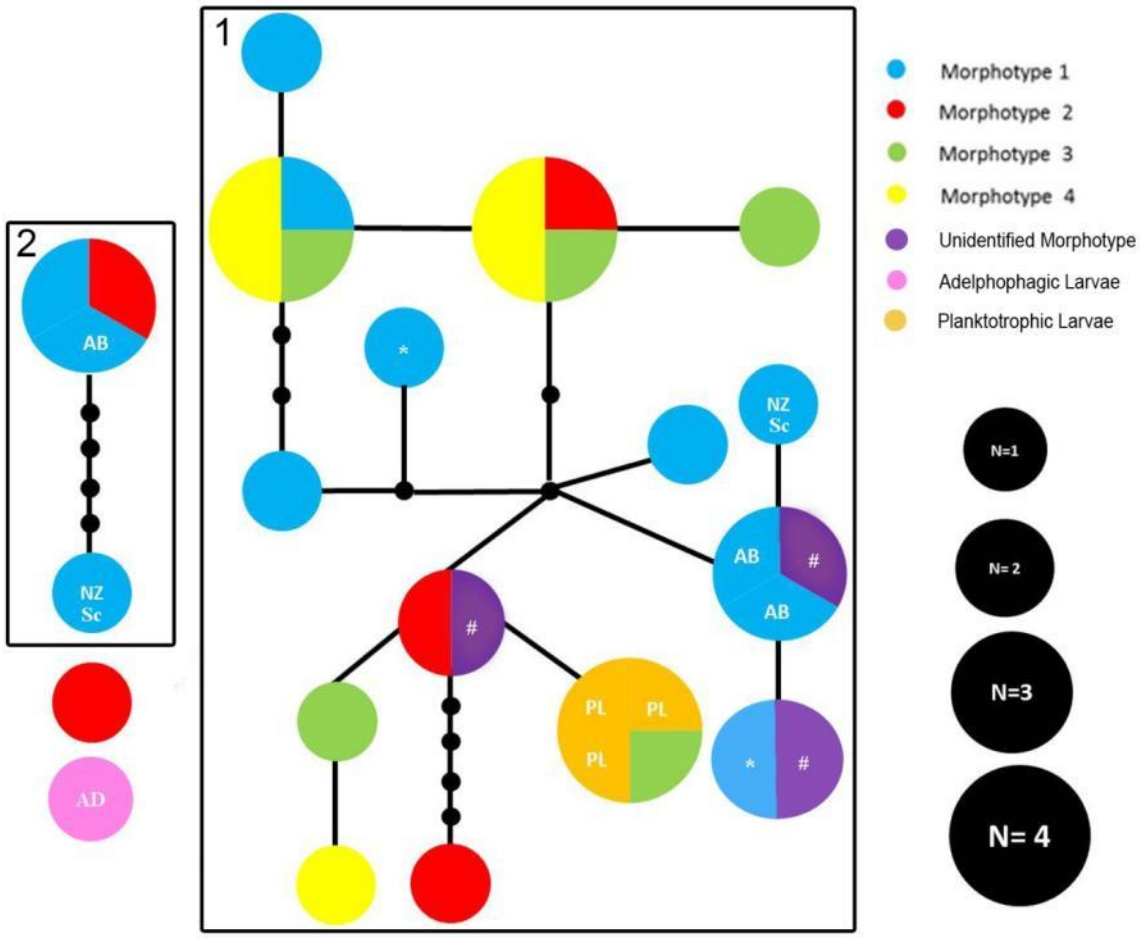

Figure 3.18: Mitochondrial parsimony haplotype network of *Polydora hoplura* at 90% connectivity. Each branch represents a single mutational step. Additional mutational steps (or missing haplotypes) are indicated as black dots on the branches. All specimens were collected from *Crassostrea gigas* unless otherwise stated. 1) Haplogroup 1; 2) Haplogroup 2. Adult females that produce planktothrophic (*) and adephophagic (#) larvae. Abbreviations: AB: abalone; Sc: scallops; NZ: New Zealand; AD: adelphophagic; PL: planktotrophic.

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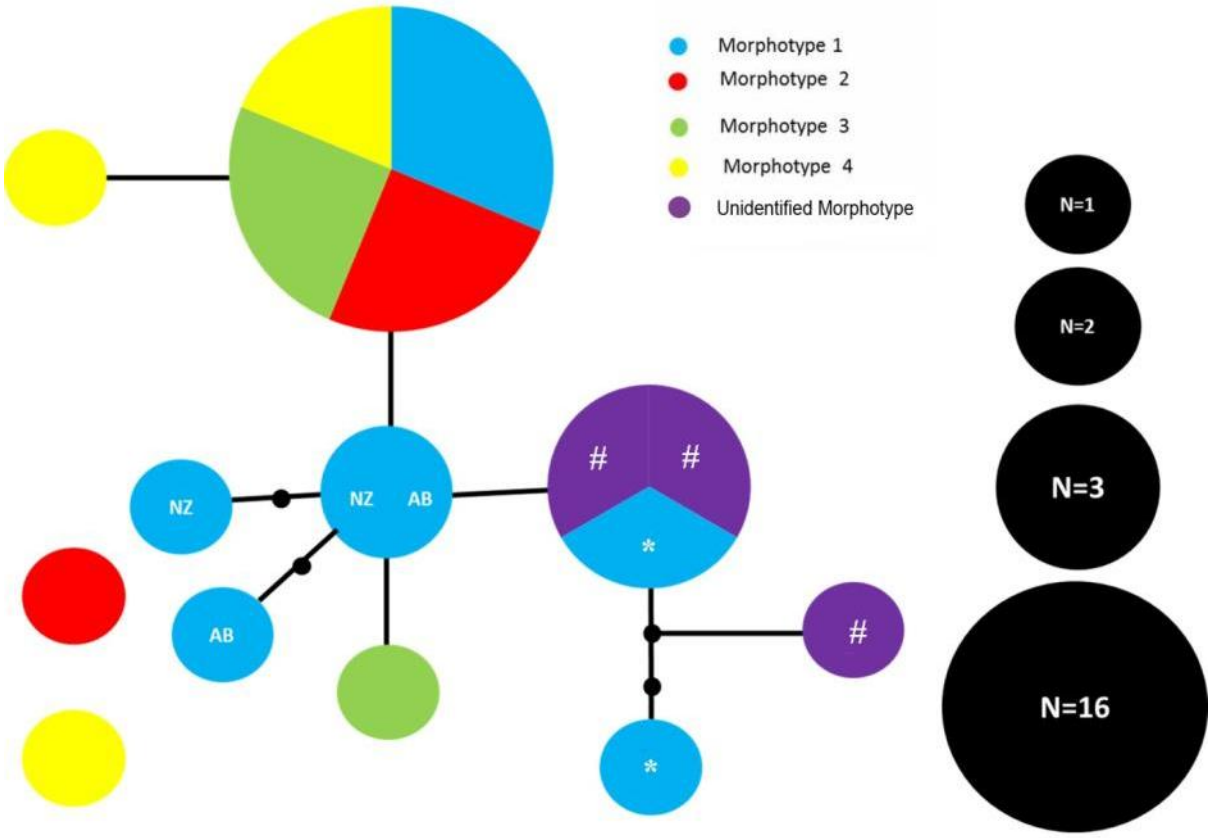

Figure 3.19: Nuclear parsimony haplotype network of *Polydora hoplura* at 90% connectivity. Each branch represents a single mutational step whereas dots on the branches indicate additional mutational steps (or missing haplotypes). Adult females that produce planktothrophic (*) and adephophagic (#) larvae. All specimens were collected from *Crassostrea gigas* unless otherwise stated. Abbreviations: AB: abalone; NZ: New Zealand.

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the Cytochrome b haplotype network and 2) a morphotype 4 singleton that represented a specimen collected in Port Elizabeth.

3.3.2 Phylogenetic investigation into the Cytochrome b and 28S partial gene fragments

Figure 3.20 shows the Bayesian inference tree for the Cytochrome b sequences. Larval development did not separate the morphotypes. Females producing planktotrophic larvae clustered with females producing adelphophagic larvae (Fig. 3.20). Furthermore, these females did not cluster with a particular morphotype. Planktotrophic and adelphophagic larvae (Fig. 3.20) did not cluster with a particular morphotype or the morphotype that the larvae were collected with. Specimens collected in New Zealand clustered with specimens collected in South Africa. The morphotypes could not be separated by host species as specimens collected from oysters, abalone and scallops clustered together on the tree.

Figure 3.21 shows the Bayesian inference tree for the 28S gene. It revealed one large clade consisting of most specimens, with the two unconnected specimens from the parsimony network (see above) falling out basally in the tree respectively. Similar to the Cytochrome b tree, the larval developmental mode did not separate the morphotypes as females displaying these modes (adelphophagy and planktotrophy) clustered together on the tree. Furthermore, host preference and site could not separate the morphotypes since one specimen collected on scallops from New Zealand clustered with specimens collected from abalone and oysters from South Africa.

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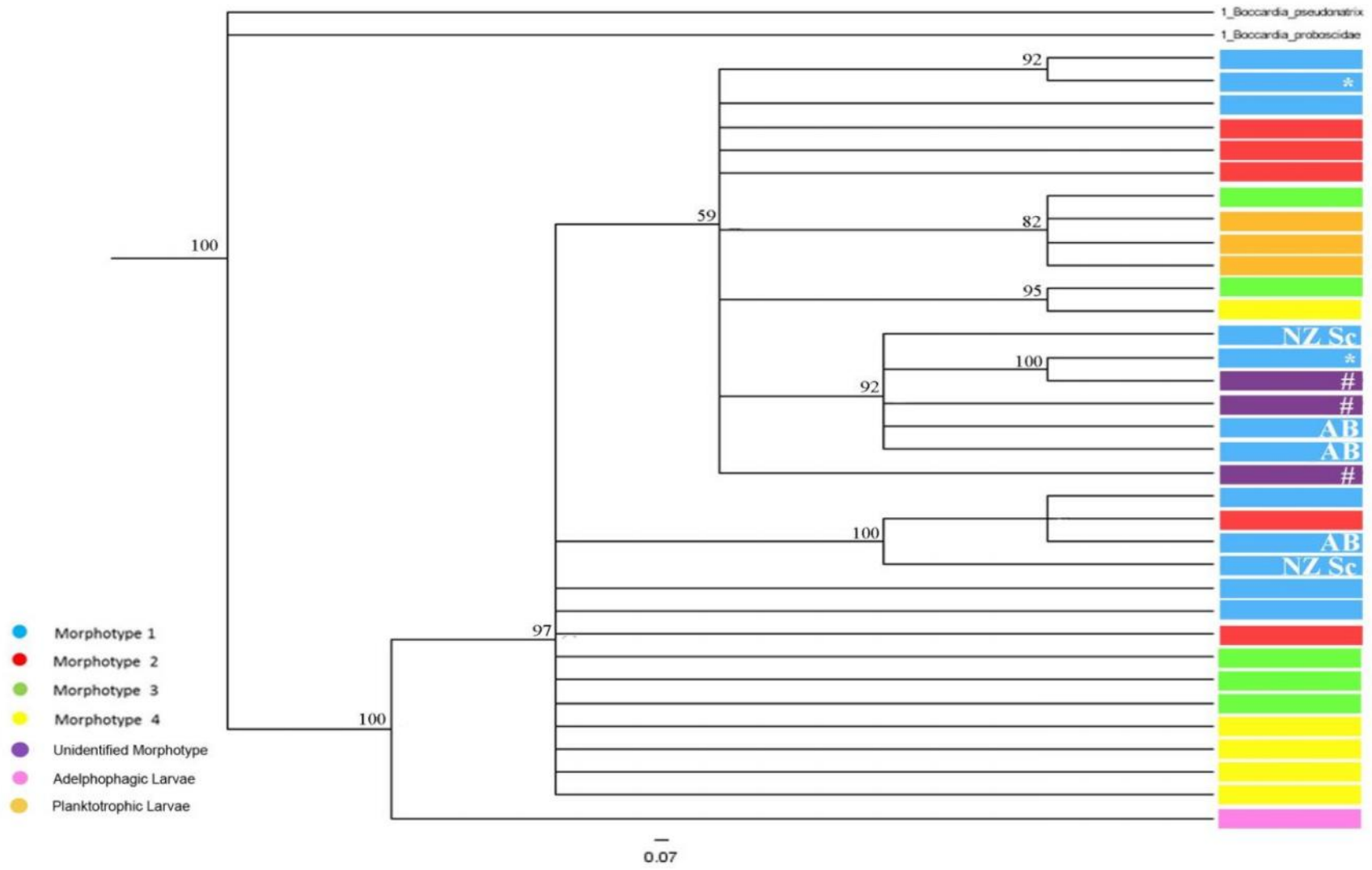

Figure 3.20: Cytochrome b Bayesian inference of *Polydora hoplura* collected from South Africa and New Zealand. The tree was rooted using *Boccardia proboscidea* and *Boccardia pseudonatrix* as outgroups. Posterior probability values are given above each branch. All specimens were collected on *Crassostrea gigas* from Saldanha Bay unless otherwise stated. Adult females that produce planktothrophic (*) and adephophagic (#) larvae. Abbreviations: PE: Port Elizabeth; NZ: New Zealand; Ab: abalone; Sc: scallops.

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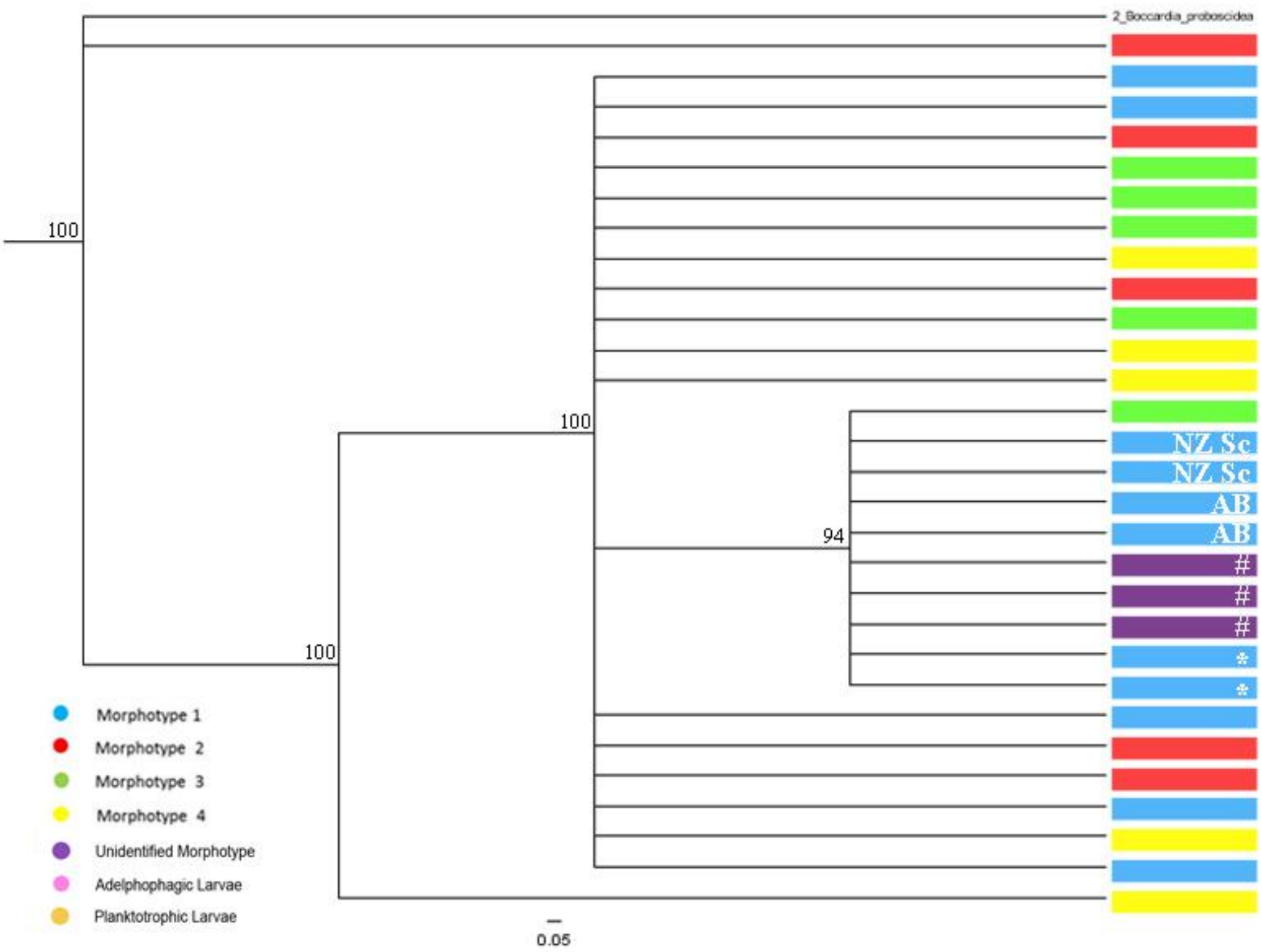

Figure 3.21: 28S rRNA Bayesian inference of *Polydora hoplura* from South Africa and New Zealand. *Boccardia proboscidea* was used to root the tree. Posterior probability values are given above each branch. All specimens were collected on *Crassostrea gigas* from Saldanha Bay unless otherwise stated. Adult females that produce planktotrophic (*) and adelphophagic (#) larvae. Abbreviations: PE: Port Elizabeth; NZ: New Zealand; AB: abalone; Sc: scallops.

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Chapter 4: Discussion

4.1 Are South African *Polydora hoplura* morphotypes a single species?

4.1.1 Morphology

All specimens collected on the South African coastline share characteristics, which include the presence of an occipital antenna, similar modified spines on chaetiger 5, hooded hooks and branchiae from chaetiger 7 and modified spines at the posterior.

An occipital antenna was present on all specimens examined in this study. The antenna was always present on the caruncle behind the eyes, if they were present. This organ has not been recorded in any of the previous records of the species in South Africa (Day, 1954; Day, 1967) but was consistently present in all re-examined museum material (Table 4.1).

The modified spines of chaetiger 5 are distally falcate and have up to five geniculate noto- and neurochaetae in all specimens examined (Table 4.1). In the specimens from this study, the modified spines of chaetiger 5 have a flange: a ridge-like structure that never separates from the shaft of the spine but may appear tooth-like in more worn spines or depending on the angle from which they are viewed. Furthermore, all museum material from South Africa also exhibited a flange (Table 4.1). This is in contrast to the lateral spur accessory structure illustrated by Day (1967).

All morphotypes have yellow recurved posterior modified spines. These spines are only present for the posterior 11% of the body in all specimens examined from South Africa. Excised spines of morphotypes 1–3 showed no statistical differences in the lengths or angles measured among the specimens used. However, spines from morphotype 1 were larger, wider and more curved than the other morphotypes. These spines were further compared with

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excised spines from morphotype 1 specimens collected by Day (1954), and the results presented herein suggest that the spines were similar in size and width.

The drawing published by Day (1967) differed from the shape of the spines observed in specimens examined in this study. The spine published by Day (1967) was sickle shaped. This shape was not observed in any of the museum specimens from Day (1954). Furthermore, the published drawing did not include a scale. Thus, statistical comparisons could not be performed. The angle of the curvature of the posterior modified spines of the published drawing by Day (1967) was compared with posterior modified spines of morphotype 1 from the current study. The results of the present study show that the curve is not very different from that of morphotype 1 in this study (Table 3.1). The distortion may be attributed to differences in microscopic equipment used between then and now. The misleading shape of the drawing, though not statically different as in the results of the current study, was used as the morphological justification for describing *P. uncinata* (see Sato-Okoshi & Okoshi, 1998).

Despite these shared characteristics, observations of South African specimens suggest the presence of four distinct morphotypes. These were diagnosed primarily on the basis of the shape of the prostomium and the presence or absence of pigmentation on the anterior region. Morphotypes 1 and 2 are characterised by the bilobed shape of the prostomium, whereas morphotype 3 has a rounded prostomium. Morphotype 4 is characterised by variation in the prostomium shape and body size.

Body length was important in differentiating among morphotypes. New material of morphotype 1 was larger than material of all other morphotypes. Day's (1954) specimen was smaller than all morphotypes, with the exception of morphotype 4. Furthermore, South Africa museum specimens of morphotypes 2 and 3 were similar to the detected morphotypes in this

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study. All morphotypes and museum material were smaller than the published size of the species described in Day (1967).

The morphotypes of *P. hoplura* also displayed variation with regard to the total number of chaetigers (Table 4.1). Morphotype 1 had more chaetigers than morphotypes 2–4, including all museum material. Specimens from morphotype 2 had fewer chaetigers than the new material of morphotypes 1 and 3 and had fewer chaetigers than South African and Australian museum material of morphotype 2. The specimen from Day (1954) had fewer chaetigers than all specimens examined in the study, excluding morphotype 4, as morphotype 4 had consistently fewer chaetigers than all other specimens observed.

Specimens from morphotype 4 were first discovered in sand from Saldanha Bay in 2010 and later from Port Elizabeth in 2011. They agreed well with the general characteristics of *P. hoplura*; the prostomium shapes of morphotype 4 were rounded or bilobed, similar to those of morphotypes 1 to 3 from this study. However, they were considerably smaller than the other three morphotypes and had pigmentation at the base of each pair of branchiae, which was absent from both morphotypes 1–3 examined in this study and from published descriptions of the species (Table 4.1).

The resulting dendogram from the cluster analysis (Fig. 18A) indicated high percentages of similarity within morphotypes (up to 100%) and among different morphotypes (64%–95%). Thus, the findings of the present study suggest that despite the large amount of variation outlined above, no consistent morphological differences could be detected among individuals belonging to different morphotypes.

The cluster analysis was supported by genetic comparisons using mitochondrial Cytochrome b and nuclear 28S gene fragments. Both gene fragments were congruent, revealing that all four morphotypes of *P. hoplura* from South Africa belonged to the same

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species and that there were no separate groupings of morphotypes. Furthermore, given that morphotype 4 was consistently smaller, had distinctly different pigmentation and was genetically similar to the other three morphotypes, this morphotype more than likely represented a juvenile stage of the species.

4.1.2 Pigmentation pattern

Colour polymorphisms have rarely been reported in polychaetes (Pleijel et al., 2009). This may be a consequence of taxonomists working with fixed material in which the pigmentation has faded (Pleijel et al., 2009). In this study, the researcher showed that *P. hoplura* displayed a high degree of intraspecific variation with regard to pigmentation pattern.

Morphotype 1 was characterised by the presence of pigmentation that varied from very faint to very dark on the peristomium and was present up to chaetiger 3. Considerable variation was also noted at the posterior region of morphotype 1, with pigmentation varying from absent to intensely blackened. Morphotypes 2 and 3 were characterised by a lack of pigmentation but were separated by the shape of the prostomium, which was bilobed and rounded respectively.

Morphotype 4 was characterised by the presence of spots of pigment at the base of each pair of branchiae. Most specimens belonging to morphotype 4 did not have any pigmentation at the anterior, with the exception of a single individual that had pigmentation on the prostomium.

The variability in pigmentation noted in the species has either not been addressed by previous authors (Carazzi, 1893; Fauvel, 1927; Blake & Kudenov, 1978; Hutchings & Turvey, 1984) or been addressed in very little detail (Claparède, 1869; Day, 1954; Read,

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1975). This study is the first to describe the variability of observed pigmentation in this species. However, other members of the polydorid group have been shown to exhibit intraspecific colour variation. A recent study by Sato-Okoshi and Abe (2012) showed that in *Polydora brevipalpa* Zachs, 1933, pigmentation on the pygidium varied from deep white to black. Importantly, they demonstrated morphologically and genetically that *P. brevipalpa* still constituted a single-colour polymorphic species. Similar results were shown in *Polydora uncinata* Sato-Okoshi, 1998 and *Polydora aura* Sato-Okoshi, 1998 (Sato-Okoshi & Abe, 2012).

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Table 4.1: Morphological characteristics of *Polydora hoplura* Claparède, 1869 specimens examined from South Africa and Australia

Locality and reference	New material	Saldanha Bay, South Africa Day, 1954	New material	Saldanha Bay, South Africa	New South Wales, Australia Blake and Kudenov, 1978	New material	Saldanha Bay, South Africa	Rapid Bay, Australia Hutchings and Turvey, 1984	Tasmania, Australia, Blake and Kudenov, 1978
Museum accession number	-	LB 378B	-	A 21489	W26121	-	A 21489	W19298	F43060
Number of specimens examined	27	1	23	5	1	17	2	1	1 (incomplete)
Morphotype	1	1	2	2	2	3	3	3	3
Shape of prostomium	Rounded bilobed	Rounded bilobed	Rounded bilobed	Rounded bilobed	Rounded	Rounded	Rounded	Bilobed	Rounded
Number of eyes	Absent, 2 or 4	Absent	Absent or 2	Absent	2	Absent, 2 or 4	Absent	2	Absent
Extent of caruncle	End of chaetiger 3 or midway chaetiger 3	End of chaetiger 3	End of chaetiger 2, midway chaetiger 3 or end of chaetiger 3	End of chaetiger 3 or midway chaetiger 3	End of chaetiger 2	End of chaetiger 2, midway chaetiger 3 or end of chaetiger 3	End of chaetiger 3	End of chaetiger 2	-
Number of modified spines on chaetiger 5	4–6	5	4–6	5 or 6	4	4–6	5	4	6

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Geniculate noto- and neurochaetae of chaetiger 5	2–5 notochaetae 3–5 neurochaetae	4 Neurochaetae broken off	2–5 notochaetae 2–5 neurochaetae	3–5 notochaetae 3–5 neurochaetae	No notochaetae 4 neurochaetae	2–5 notochaetae 2–5 neurochaetae	2 or 3 notochaetae 5 neurochaetae	Notochaetae broken off 4 neurochaetae	6 notochaetae 3 neurochaetae
Maximum number of hooded hooks	11	8	8–11	10	10	8–11	10	11	Up to 8
Percentage branchiate chaetigers	86%	82%	87%	86%	87%	85%	87%	87%	-
Percentage posterior modified spines	11%	16%	10%	11%	10%	11%	11%	10%	-
Pygidium shape	Flared disk with dorsal notch	Flared disk with dorsal notch	Saucer shaped with dorsal notch	Flared disk with dorsal notch	Flared disk with a dorsal notch	Saucer shaped with dorsal notch	Flared disk with a dorsal notch	Saucer shaped with dorsal notch	-
Methyl green staining present from	chaetigers 6–11	chaetiger 8	chaetigers 7–10	chaetigers 7–10	chaetiger 11	chaetigers 6–10	chaetiger 8	chaetiger 11	chaetiger 9
Methyl green staining continues to	2nd last chaetiger	Posterior modified spines start	3rd last chaetiger	4th last chaetiger	2nd last chaetiger	Posterior modified spines start or 3rd last chaetiger	Posterior modified spines start	Absent from last third of body	End of fragment
Pigmentation	On prostomium and peristomium	On peristomium and prostomium	Absent	Absent	Absent	Absent	Absent	Absent	Absent

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The study by Sato-Okoshi and Abe (2012) identified the black bars of pigmentation on the palps, in conjunction with a molecular analysis, as a reliable method of identifying species. To the researcher's knowledge, only two studies describe the pigmentation of palps in *P. hoplura* (Carazzi, 1893; Day, 1954) while most studies fail to show this (Claparède, 1869; Fauvel, 1927; Read, 1975; Blake & Kudenov, 1978; Hutchings & Turvey, 1984; Bilbao et al., 2011). More recently Picker and Griffiths (2011) published a photograph of the palps of this species, clearly showing black bars along the length of the palps. In this study, only five of 98 individuals had palps with black bars. However, the low sample number included in the present study did not allow the researcher to reach a definite conclusion about the reliability of this character for species identification. The researcher therefore suggests that this character be further investigated as a reliable character to identify *P. hoplura*. The data represented in this study are not enough to assess the evolution of the colour in different morphotypes or what mechanism drives the maintenance of pigmentation patterns. However, it is clear from the cluster analysis and genetic investigation that *P. hoplura* represents a single species in South Africa.

4.1.3 Aspects of reproduction: Larval developmental mode

According to Blake and Arnofsky (1999), *Polydora hoplura* is ectolecithotrophic. However, in the present study, two reproductive developmental modes were detected within the species: ectolecithotrophy and planktotrophy. This has only been recorded in *P. hoplura* once, only in a South African study (but see David et al., 2014). The larval development of the species was first described by Wilson (1928) in England and has since been reported from New Zealand and Australia (Wilson, 1928; Read, 1975; Lleonart et al., 2003). Although many specimens were utilised for morphological descriptions in the current study, few were reproductive at the time of collection. Consequently, only larvae from a morphotype 2 female (representing two stages, chaetiger 13 and 17) were recorded. A comparison with drawings of

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larvae at the same developmental stages produced by Wilson (1928) shows that the adelphophagic larvae's development is very similar with regard to size, number of eyes, branchiate distribution and the distribution of melanophores along the length of the body. The 13-chaetiger-stage larvae from the current study differ from the Wilson (1928) description by chaetiger 5 already being modified and having spines. The 17-chaetiger-stage larvae are similar to those from Wilson's (1928) study with regard to the fifth chaetiger and the presence of two types of modified spine: the first is a simple falcate spine and the second is a distally falcate spine with two lateral spurs.

Larvae from both developmental modes were genetically analysed using the Cytochrome b gene fragment, and the results showed that the larvae analysed still belonged to a monophyletic *P. hoplura*. The adelphophagic larva did not cluster with the morphotype 2 female as expected; additionally, it did not share a haplotype with any of the individuals in the study and represented a singleton. The planktotrophic larvae shared a haplotype with a morphotype 3 individual. This result is suggestive of larval development being independent of morphotype. However, this result could be confounded by low sample size.

4.1.4 Habitat preference

Individuals from *Polydora hoplura* that were examined in this study bored into various calcareous substrates and were free-living in sand. From the literature, specimens collected outside of South Africa have been recorded as shell-borers (Claparède, 1869; Carazzi, 1893; Fauvel, 1927; Read, 1975) and a single reference documents them as a fouler among sessile organisms on a jetty (Hutchings & Turvey, 1984). Habitat preference was overlaid onto the cluster analysis, and the results showed that the morphotypes of *P. hoplura* were not host specific. Juveniles of *P. hoplura* were initially found in sand tubes in Saldanha Bay, far from potential calcareous hosts; furthermore, juveniles were collected from sand

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tubes on the surface of abalone shells. The outcome of the present study supports the speculation by Williams and Radashevsky (1999) that juveniles create tubes on a softer substrate (i.e. sand) in the absence of a calcareous substrate or create a sandy tube on a harder substrate before boring into the substrate.

Although polydorids are commonly recognised as either shell-borers such as *Dipolydora bidentata* Zachs, 1933 or free-living (construct sandy tubes) such as *Dipolydora cardalia* Berkeley, 1927 (Radashevsky & Pankova, 2013), very few studies have recorded polydorids to exhibit both shell-boring and tube-building life histories. However, *Dipolydora carunculata* includes both shell-boring or free-living habits. A recent study by Radashevsky and Pankova (2013) showed that individuals with different habitat preferences were still members of the same species. Similar results can be interpreted from a study on *Boccardia proboscidea* in which specimens collected from sediment and abalone were shown to be of the same species (see Radashevsky & Pankova, 2013 on *B. proboscidea*).

The researcher has demonstrated that *Polydora hoplura* displays very high levels of intraspecific variation with regard to morphology, pigmentation patterns, larval development and habitat preferences on the South African coast. In all categories discussed, both the cluster analysis and genetic investigation were congruent, thus the researcher concludes that the four morphotypes of *P. hoplura* represent one species on the South African coastline.

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4.2 Are South African specimens of *Polydora hoplura* the same species as specimens collected elsewhere?

To establish whether South African specimens were the same as those collected from other countries, the researcher conducted a morphological comparison of South African specimens with museum material from Australia and New Zealand. In addition, she genetically compared South African specimens with specimens collected from New Zealand.

Polydora hoplura Claparède, 1869 belongs to the *P. ciliata*/*websteri* group, united by the presence of a flange on the modified spines of the fifth chaetiger (Blake, 1996). Other characteristics common to *P. hoplura* are the distally falcate modified spines with lateral flange, spear-shaped companion chaetae on the fifth chaetiger, bidentate hooded hooks starting on chaetiger 7, filiform branchiae that are present from chaetiger 7 and branchiae occurring on as much as 87% of the chaetigers. Furthermore, P. *hoplura* is characterised by one or two yellow recurved posterior modified spines for the last 11% of the body and a saucer-shaped pygidium with a dorsal notch (Tables 4.1 and 4.2).

Fauvel's (1927) description is the only one documenting variation in the shape of the prostomium within the species. He briefly states that the prostomium may vary from rounded to bilobed, but this is not shown in the plates published. However, the description does not include variation in pigmentation patterns as seen in the current study (see Fig. 17A in Fauvel, 1927). Specimens collected by Hutchings and Turvey (1984) could not be assigned as the prostomium shape was not included in the published description. Museum material collected by Blake and Kudenov (1978) was examined and could surprisingly be assigned to a different morphotype from what its description suggested (see tables 4.1 and 4.2). From the description by Blake and Kudenov (1978), specimens resembled morphotype 2. However,

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upon re-examination, the specimens from Tasmania resembled morphotype 3 (tables 4.1 and 4.2).

In South African specimens, variation in the number of eyes was observed; they were either absent or present, with two or four eyes arranged in a trapezoid manner. This variation in the number of eyes is consistent with the range from the literature. However, this character is not included in the descriptions by Carazzi (1893) and Hutchings and Turvey (1984), which suggests that in their studies, eyes were absent (Table 4.2). Blake and Arnofsky (1999) incorporated this characteristic into their study and found that the presence and number of eyes were not a strong enough characteristic for species separation.

Caruncle length has been used as an important characteristic to define species (e.g. Radashevsky, 1993). South African specimens vary in caruncle length, from the end of chaetiger 2 to the end of chaetiger 3 (Table 4.1), which covers the range from the literature (Claparède, 1869; Fauvel, 1927; Read, 1975; Blake & Kudenov, 1978). In the original description the caruncle extends to the middle of chaetiger 3 (Claparède, 1869) and in later records to the end of chaetiger 3 (Table 4.2). The length of the caruncle of morphotype 4 extends to the end of chaetiger 2. The descriptions by Carazzi (1893) and Day (1967) do not include the lengths of the caruncle. The current study demonstrates that the caruncle length of *P. hoplura* varies (i.e. it varies from chaetiger 2 to chaetiger 3) (tables 4.1 and 4.2) and that it should be used with caution as a defining character for species identification. This conclusion is consistent with earlier studies that show that caruncle size can be size dependent (Williams & Radashevsky, 1999; Sato-Okoshi & Takatsuka, 2001).

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Table 4.2: Published morphological characteristics of *Polydora hoplura* Claparède, 1869 complex from Italy, South Africa, Australia and New Zealand

Reference	Claparéde, 1869	Carazzi, 1893	Fauvel, 1927	Day, 1967	Read, 1975	Blake and Kudenov, 1978	Hutchings and Turvey, 1984
Locality	Gulf of Naples	Gulf of Naples	Gulf of Naples	South Africa	Evans Bay, New Zealand	New South Wales and Tasmania, Australia	South Australia
Morphotype classification*	1	2	1–3	1	1	2	-
Total body length (mm)	-	50	50–60	50	40	40	40
Width (mm) of chaetiger 5	-	2	1 or 2	-	2	2	-
Total number of chaetigers	-	200	200	-	180	160	160
Shape of prostomium	Bilobed	Bilobed	Rounded-incised	Bilobed	Weakly incised anteriorly	Weakly incised	Weakly incised
Number of eyes	2 or 4	-	4	2 or absent	2	-	-
Extent of caruncle	Midway chaetiger 3	-	End of chaetiger 3	-	End of chaetiger 3	End of chaetiger 3	End of chaetiger 3
Spines of chaetiger 5	-	Falcate spines	Blunt with lateral point	Hooks with lateral spur	Falcate with tooth-like flange	Falcate with tooth-like flange	Blunt-pointed with tooth-like flange
Number of spines on	-	5 or 6	5	-	8	-	-

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chaetiger 5							
Maximum number of hooded hooks	-	-	8	-	10	8 or 10	-
Distribution of branchiae and posterior modified spines overlap?	-	No	No	-	No	No	No
Percentage branchiate chaetigers	-	86%	86%	-	85%	-	-
Distribution of posterior modified spines	Last 15 chaetigers	Last 10–20 chaetigers	Last 10–20 chaetigers	-	Last 20 chaetigers	-	-
Pygidium shape	Sucker shaped	Cup shaped	Suction cup	Saucer shaped	Large flared disk with dorsal gap	Large disk with dorsal gap	Broad and flat with deep ventral notch
Host	Barnacles	Barnacles and oysters	Oysters	Limestone and sand	Oysters	Oysters	Among sessile organisms on jetty piles
Pigmentation	Black spots on the peristomium; diffuse along the body	Diffuse along the body	Reddish and yellowish at anterior and posterior	Prostomium blackened anteriorly; pygidium darkened	Dusky brown on prostomium and peristomium	Body colourless	-

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The original description does not mention the presence of an occipital antenna; however, it is mentioned in all descriptions from the 1970s onwards (Read, 1975; Blake & Kudenov, 1978; Hutchings & Turvey, 1984) and was consistently present on all specimens examined in this study. The absence of the occipital antenna from the early descriptions may be attributed to its inconspicuousness.

All published descriptions agree that the spines on chaetiger 5 are distally falcate but that they differ with regard to the accessory structure, which has been described as a lateral spur (Day, 1967; Bilbao et al., 2011) or an accessory tooth (Fauvel, 1927; Read, 1975; Blake & Kudenov, 1978; Hutchings & Turvey,1984). In all the South African specimens, the modified spine of chaetiger 5 has a flange: a ridge-like structure that never separates from the shaft of the spine but can appear tooth-like in the most anterior or worn spines. The accessory structures on museum material examined in this study all had a flange on the modified spines of chaetiger 5. The misrepresentation of the shape of the accessory structure in previous studies might be due to the age of the spine, the type of microscopy used, the angle of observation or a combination of these (see Read, 2010 on *Polydora haswelli*).

In the South African *Polydora hoplura*, glandular pouches are paired, medium-sized sacs containing up to 13 smaller sacs. Glandular pouches have only been mentioned by Fauvel (1927) and have not been included in any other published description of the species. The specimens in this study had more glandular pouches than those described by Fauvel (1927). The use of glandular pouches as an additional character for species identification has been recorded in the genus *Boccardia* (Simon et al., 2010) in which species level differences exist between two members of this genus: *B. polybranchia*, with few large sacs in a pouch, and *B. proboscidea*, with many small sacs in a pouch. The use of glandular pouches as a diagnostic characteristic has not been established and should be further investigated.

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The South African specimens from the study were genetically compared with specimens collected from New Zealand. The South African specimens were collected from oysters, abalone and sand, and the New Zealand specimens were collected from scallops. The genetic analysis revealed that these specimens still belonged to the same species. Furthermore, one specimen from New Zealand shared a haplotype with specimens collected in Saldanha Bay on the West Coast of South Africa. It is unlikely that the species was able to disperse naturally over such a vast distance. The data are suggestive of the species being moved from one country to another; however, the method of translocation may never be fully understood (reasons will be discussed later).

It is clear from this study that traditional characters such as prostomium shape, number of eyes, caruncle length and shape of the modified spines on chaetiger 5 that have previously been considered to be very reliable taxonomic characters can be plastic and that such variation should be noted very carefully. The inclusion of such variation in the description will aid in identification of specific species traits and minimise the probability of misidentification. If a very high level of variation is shown, genetic analysis should be included to confirm the identity of the species. In this study, genetic markers Cytochrome b and 28S showed that the specimens collected from South Africa and New Zealand were members of the same species.

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4.3. Current status of *Polydora hoplura* on the South African coast

Polydora hoplura was initially recorded from the Gulf of Naples (Claparède, 1869); since then it has been recorded from England (Wilson, 1927), South Africa (Day, 1954; Day, 1970), New Zealand (Read, 1975) and Australia (Blake & Kudenov, 1978; Hutchings & Turvey, 1984), with more recent records from the Netherlands (Haydar & Wolff, 2011), Portugal (Freitas et al., 2011) and the Canary Islands (Bilbao et al., 2011). This extensive geographic range has led to the species being considered cosmopolitan.

Polydora hoplura was first recorded in South Africa in the mid 1950s (Day, 1954) at a single locality near Saldhana Bay. The species has predominately been recorded from farmed *Crassostrea gigas*, *Pecten sulcicostatus* and *Haliotis midae* (Nel et al., 1996; Simon et al., 2006; Simon & Booth, 2007), from wild-caught *C. gigas, H. midae, P. sulcicostatus*, *Turbo sarmaticus*, *Perna perna* and *Scutellastra longicosta* (Simon, 2011; David et al., 2014) and from sand (personal observation). Currently the known range of the species on the South African coastline extends from Kleinzee on the West Coast (personal communication, L. Williams, Stellenbosch University) to Haga Haga on the East Coast (Simon, 2011). The movement from the West Coast to the East Coast suggests that the species' 1) range has expanded and/or that it 2) was moved via anthropogenic factors. The natural range expansion of the species seems unlikely as the biogeography and oceanic currents are not conducive to such movements (Griffiths et al., 2010), but on the other hand, many species have been shown to cross these phylogeographic breaks (see review in Teske et al., 2012). Nevertheless, the movement of the species through human-mediated methods is currently being investigated (personal communication, L. Williams, Stellenbosch University) and seems more likely. The question remains, How did this species arrive on the South African coast?

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Polydora hoplura may have been introduced by several pathways of marine invasions. On the South African coastline, Griffiths et al. (2009a) suggested five possible pathways of marine invasions, which included wood borers and introductions via dry ballast, hull fouling, aquaculture and ballast water. It is unlikely that the species could have been introduced to South Africa via wood fouling or dry ballast as it has only been recorded once from a sandy substrate and to the researcher's knowledge has never been found boring into wood.

Globally, the associated introductions of alien species via the oyster and abalone industry has been well documented, for example the introduction of *Polydora uncinata* on *Haliotis discus hannai* to Chile (Radashevsky & Olivares, 2005), *Boccardia proboscidea* infesting *Ostrea edulis* to Hawai'i (Bailey-Brock, 2000) and *B. knoxi* Rainer, 1973 on *C. gigas* to Tasmania (Sato-Okoshi et al., 2008). Another major potential means of introduction to South Africa is the commercial cultivation of marine molluscs (Cinar, 2013). Oyster cultivation was first attempted on the South African coast in 1673, but the earliest record of the importation of the hardier Japanese oyster *Crassostrea gigas* Thunberg was in 1973 to Knysna (Haupt et al., 2010b). The farming of molluscs can be a likely source of introduction of *P. hoplura*; however, this is not clear as the initial record of the species precedes the aquacultural activities in the area. Therefore, this study cannot confirm that *P. hoplura* was introduced via the oyster/abalone industry. This suggests that the species was introduced via ballast water. Records of the species in the last decade from along the South African coastline may partially be attributed to the transport of commercially important gastropods and bivalves within the borders of South Africa (Simon et al., 2006; Simon & Booth, 2007; Haupt et al, 2009; Simon, 2011).

A recent review by (Cinar 2012) suggested hull fouling as a common method of introduction by marine invertebrates. *Polydora hoplura* was initially associated with barnacles that were known to foul the hulls of ships (Claparède, 1869). The species has also

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been recorded as a fouling species among sessile organisms on a jetty in Australia (Hutchings & Turvey, 1984). To date, the species has never been found fouling ship hulls. However, this does not exclude the possibility that the species might be associated with a known hull-fouling species.

By the 1950s all ships were transporting ballast water rather than dry ballast (Griffiths et al., 2009a). Ballast water has contributed to at least a third of marine invasions internationally and continues to be one of the leading means of introduction of invasive species (Hewitt & Campbell, 2010; Cinar, 2013). Species most likely to be introduced via ballast water generally have a planktonic phase in their lifecycle in order to travel vast distances (Griffiths et al., 2009a). Carlton and Geller (1993) showed that the larvae of spionids were especially well adapted to transportation within ballast water. These animals produce planktotrophic larvae that are well-adapted swimmers and can spend up to 45 days in the water column (Blake & Arnofsky, 1999). For example, Blank et al. (2008) attributed the introduction of a spionid *Marenzelleria viridis* from the east coast of North America to the Baltic Sea to transportation within ballast water. Further introductions via ballast water include *Streblospio benedicti* to Hawai'i and *S. gynobranchiata* to the Mediterranean (Zenetos et al., 2010). Cinar (2013, Fig. 8) further showed that most polychaete introductions to Southern Africa could be attributed to introductions via ballast water. Therefore, ballast water appears to be a possible important vector of introduction for *P. hoplura* to Saldanha Bay, especially since *P. hoplura* is well suited to movement via ballast water and the species can produce planktotrophic larvae (David et al., 2014).

We will never fully understand how *P. hoplura* reached South African shores as it is difficult to separate introduction via ballast water and aquaculture; the life history traits of this species lends itself to both methods of introduction. The researcher could not confirm the origin of the introduction of the species, as specimens from Europe were not included in the

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study due to logistical issues. In order to confirm the cosmopolitan status of the species, specimens from the type locality and other habitats should be genetically analysed.

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Chapter 5: Conclusions

The study proved that the four morphotypes of *Polydora hoplura* on the South African coast represented a single species by using morphological and genetic approaches. Anterior pigmentation patterns vary intraspecifically from present in morphotype 1 to absent in morphotypes 2 and 3; morphotype 4 differs from the other three in that this morphotype has pigmentation at the base of each pair of branchiae. This pigmentation bears a distinct resemblance to retracting melanophores seen in late-stage larvae of the species (Fig. 3.16B). Adelphophagic larvae were morphologically compared with larvae at the same stage from Wilson (1928), and the researcher concluded that this was the same species.

Morphotype 4 is consistently smaller than the other three morphotypes and furthermore mostly utilises a different habitat than the other three morphotypes. Morphotype 4 first settles on a softer substrate (i.e. sand) and then burrows into a harder substrate such as shell, similar to the results from Williams and Radashevsky (1999). However, in the absence of shells, morphotype 4 will create sandy tubes. Based on the size, differences in pigmentation and habitat use, the researcher concludes that morphotype 4 represents a juvenile form of *P. hoplura*. This is the first description of a juvenile *P. hoplura*.

It is clear from the study that the species is not host specific and bores into various calcareous substrates, which include abalone, oysters, scallops and mussels, with some specimens collected from sand. This lack of host specificity complicates tracing the method of introduction to South Africa but probably enhances the ability of the species to spread throughout the country.

Although it was confirmed that *P. hoplura* from South Africa and New Zealand was the same species, the cosmopolitanism of the species could not be confirmed by this study due to lack of genetic samples from the type locality and other sites within its distribution range. Thus, further investigation is needed. The species was recorded in South Africa before

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Australia and New Zealand, which suggests that the species arrived in South Africa first (Day, 1954). The identification of polydorids received interest in 1957 in Australia with the species only being recorded in the late 1970s (Walker, 2011). Furthermore, polydorids have been identified in New Zealand from 1973 (Rainer, 1973), with *P. hoplura* first recorded in New Zealand in 1975 (Read, 1975). The current study does show that specimens from New Zealand share a haplotype with those from South Africa, and it is possible that these disparate populations share a potential source population or that the species was transported from one country to the other. The researcher cannot exclude the possibility of transportation between the two countries, and this requires further investigation. The findings in this study indicate that the species was introduced to South Africa, in congruence with Mead et al. (2011).

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Appendices

Appendix A Recipes of Chemical solutions

Phenol solution in Seawater

0.5 g Phenol crystals is dissolved in 1l of seawater and is aerated.

Mg_2Cl in tapwater

7 g of Mg_2Cl is dissolved in 100 ml of tapwater

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Appendix B – Characters examined on Scanning Electron Microscopy

Orientation of specimen	Morphological character
Dorsal	Prostomium shape
	Occipital antenna
	Nuchal organ
	Lateral organ of chaetiger 1
	Dorsal cilliary bands
	Modified spines and companion chaetae of chaetiger 5
	Dorsal superior spines of chaetiger 5
Ventral	Hooded hooks
	Glandular pouches (external openings)
Lateral	Occipital antenna
	Lateral organs of chaetiger 2-4
	Lateral organs of chaetigers 7-9

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Appendix C- Scored Characters used in Cluster Analysis

Table 1 Scored Characters of morphotype 1 of *Polydora hoplura* used in the Cluster Analysis.

Character scored	Individual number of morphotype 1																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	1	1	0	0	1	0	1	0	1	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0
3	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1
4	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	1	0	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1
6	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0
8	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	0
9	1	1	1	1	1	0	1	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	1	1	0	0	1	0	1	1
10	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
11	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
12	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	1	0	0	0	0	1	1	1	1	1	0	0	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
14	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	0	1	1	1	0	0	0	1	1	0	0	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1
17	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
18	?	0	1	?	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0
19	?	1	1	?	1	0	1	0	1	0	0	0	0	0	0	0	0	1	1	0	1	1	1	1	0	0	1	1	1	1
20	?	1	1	1	0	0	1	1	1	1	0	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0
21	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0
22	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1

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Table 2 Scored Characters of morphotype 2 of *Polydora hoplura* used in the Cluster Analysis.

Characters scored	Individual number of morphotype 2																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
3	0	0	0	0	1	1	1	1	1	1	1	1	0	1	1	1	0	1	0	1	0	0	0
4	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	0	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
8	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1	1
9	1	1	1	0	1	1	0	1	1	1	1	1	1	0	0	0	0	1	0	1	1	1	1
10	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
11	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	1	0	0	0	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0
14	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	0	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1
17	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0
19	1	0	0	0	1	0	0	0	1	0	0	0	1	0	1	1	1	1	1	0	0	1	0
20	1	0	1	1	0	1	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1
21	1	1	0	0	1	0	0	0	1	0	0	0	1	0	0	0	1	1	0	1	0	0	0
22	1	1	1	1	0	0	1	1	1	1	1	1	0	1	1	0	0	0	1	0	0	0	1

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Table 3 Scored Characters of morphotype 3 of *Polydora hoplura* used in the Cluster Analysis.

	Individual number of morphotype 3																		
Characters scored	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
3	1	1	1	1	1	0	0	1	0	1	0	0	0	1	0	0	0	0	1
4	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
6	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
9	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0
10	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1
11	1	1	1	0	0	1	1	1	1	0	1	1	1	1	1	1	0	1	1
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	0	0	1	1	0	1	0	1	1	0	0	1	1	1	1	1	1	1	1
14	?	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
15	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
16	1	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	0	1
17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	1	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1	0
19	1	1	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1
20	0	0	1	1	1	1	1	0	1	0	1	0	1	0	0	1	1	1	1
21	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
22	1	1	1	1	1	1	1	0	1	1	1	0	1	1	0	1	1	1	0

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Table 4 Scored Characters of morphotype 4 of *Polydora hoplura* used in the Cluster Analysis.

	Individual number of morphotype 4				
	1	2	3	4	5
1	1	1	0	1	0
2	1	1	0	1	0
3	1	1	0	1	1
4	0	1	1	0	0
5	0	0	0	0	0
6	1	0	0	0	1
7	1	1	1	1	1
8	1	1	0	1	1
9	1	1	1	0	0
10	1	1	1	1	1
11	1	1	1	1	1
12	0	1	0	0	0
13	1	0	1	1	1
14	1	1	1	0	0
15	1	0	1	1	1
16	1	1	1	1	0
17	0	0	0	0	0
18	0	0	0	1	0
19	0	0	0	0	1
20	0	0	0	1	0
21	0	1	0	1	0
22	0	0	0	0	0

Characters scored