

**An environmental parasitological
approach to marine metal pollution: The
endemic South African intertidal klipfish,
Clinus superciliosus, as model organism**

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Summary

With multiple species, particularly small bodied organisms such as parasites, still undescribed from the marine realm, new studies investigating novel taxa are highly encouraged. Linking this marine biota, such as cryptofauna, to ecosystem functions is of even greater importance. Past parasite studies, specifically in South Africa, have focussed on taxonomic descriptions. Even though their vital contribution to ecosystems have been established in other regions, this information is lacking for local species. In addition, local parasite taxonomists are scarce in South Africa. In contrast to the interactive role of parasites in ecosystems along the South African coast, contamination by metallic elements has received some attention over the last century. The next step to an integrated approach to ecosystem health would be to combine the knowledge of parasites and contamination by using environmental parasitology.

The primary aim of the present study was thus to ascertain the role of parasites as effect indicators in the marine environment. This was accomplished by conducting host-specific parasite sampling to obtain parasite species infecting *Clinus superciliosus*, as well as calculating the effect of elemental concentrations and other factors on the parasite community composition. Due to extensive background information available on multiple aspects of their biology, reproduction and diet, the endemic intertidal klipfish *Clinus superciliosus* (Clinidae), is an appropriate model organism for ecosystem health studies.

Based on previous literature, and the results obtained from the present study, it is evident that *Clinus superciliosus* is host to a conceivable 43 species of parasites. Two of the previously reported taxa were not collected during these surveys, while one of these species, *Elthusa xena*, has only been reported from outside the current sampling area. Taxa from all of the parasitic groups (Myxozoa, Digenea, Monogenea, Cestoda, Acanthocephala, Annelida, Nematoda, Copepoda, Isopoda) have now been reported to inflect *C. superciliosus*. The majority of the taxa collected during the present study are new host records, some even novel species.

In addition to the taxa found during the present study, various suggestions of possible intermediate and definitive hosts have been considered, from which future opportunities to complete life cycle studies could follow. *Octopus vulgaris* are considered as a possible host to *Proctoeces* (Trematoda: Fellodistomidae), and possibly *Acanthobothrium* (Cestoda: Onchobothriidae) species. It is recommended that the molecular data from larval species are linked to GenBank, where adults from the definitive hosts can be connected to intermediate or paratenic host species to compare their life cycles.

Prevalence (P %), mean intensity (MI) and mean abundance (MA) per host were calculated, as well as various indices on infracommunity and component community level, and compared between the various localities. Other factors affecting parasite community composition, such as host and parasite distribution patterns and species occurrences, were investigated. Condition factor (CF), hepatosomatic index (HSI), fish size (measured in grams), parasite life cycle, as well

as water temperature, host sex, and habitat type were considered. Higher water temperatures at the east coast localities, known to increase transmission of parasites, were associated with generally high abundance and diversity. Habitat availability refers to a habitat for intermediate hosts within the intertidal rocky shore, but also to the habitat within the various hosts for parasite species to thrive. This can in turn be linked to host condition, where a lower condition factor can be indicative of a weakened immune system making it easier for certain parasites to infect. These habitat conditions can also induce easy access for parasites, due to the altered hydro-morphology at the Langebaan marina.

Metallic element concentrations (As, Cd, Cu, Fe, Mn, Pb, Sb, Sn, Zn) detected in the selected host species, *Clinus superciliosus*, were regarded as naturally high, due to upwelling, geogenic weathering and larger scale oceanographic processes. From these results sources such as antifouling paints in harbours and marinas, the nearby naval base, and transport by season water currents within the False Bay area, were identified as hotspots. When compared to other monitoring species, the concentrations from the present study were lower, and were attributed to different exposure routes. The short- and long-term exposure of these organisms were also measured by considering the different tissues (muscle and liver) analysed. Evidently Cu and Sn were shown to be a short- to long-term hotspot at Simons Town marina and Cape Town harbour, which may also be attributed to the antifouling paints used within the harbour, marina and the naval base. During the present study, it was apparent that very little ecotoxicological studies are conducted on intertidal fish species along the South Africa coast, and combined with similar studies, these results can contribute to national marine pollution monitoring programmes.

Parasites are herein regarded as effect indicators of natural conditions and anthropogenic pressures. Several parasitic groups are considered as useful monitoring tools in ecosystem health studies. In particular, it was noted that acanthocephalans and cestodes associated with high concentrations of elements at the two east coast localities. This is in accordance with previous studies. Host condition at the various localities reflected these high concentrations. However, the concentrations were not considered as detrimental to these species. The lower condition factor at Tsitsikamma and Chintsa is rather attributed to the highly kinetic nature of these habitats compared to the anthropogenically altered habitat on the west coast. In addition to host condition, it was also evident that the altered habitat conditions caused changes to the ecosystem structure with reduced habitat availability for intermediate host species. The conclusion from these results was that high metallic element concentrations are not always a result from “pollution”, but in some cases due to naturally occurring high background concentrations to which taxa can adapt over time. The term “pollution” should rather be disentangled during future studies to determine the sources of, for example eutrophication, habitat degradation and overexploitation at anthropogenically impacted localities, compared to Marine Protected Areas and areas of

conservation where these activities are banned, causing species population decline and loss of ecosystem function.

Key words:

Environmental Parasitology, elemental concentrations, accumulation, South Africa, marine fish parasites

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CHAPTER 1

General Introduction



1.1 Background

The biodiversity rich southern African coast has over 270 families of fishes, with approximately 2,200 different fish species that include a large number of endemic fishes (13%) (van der Elst 2012). One of these families, with 42 species, is Clinidae (Perciformes), mostly endemic to the region from Namibia to Mozambique, resulting in this family being the largest group of endemic marine fishes in southern African waters (Holleman et al. 2012). Within this family, one of the most common and widely distributed species is *Clinus superciliosus* (Linnaeus, 1758) (Clinidae), the model organism utilised in the present study. *Clinus superciliosus* is part of a species complex that constitutes six species, specifically around the Southern Cape coast. These species are morphologically distinguishable on the basis of a combination of their meristic characteristics, form of cirrus, position of crest, number and pattern of taste buds, diet, and colouration. Furthermore, genetic analyses using S7, NADH2 and rhodopsin (von der Heyden et al. 2008, 2011), confirmed the existence of six separate species in what was called the *C. superciliosus* complex (Holleman et al. 2012). The present study focused on specimens that conformed in both morphology and genetic characteristics to *C. superciliosus* and excluded specimens of the other species in the *C. superciliosus* complex.

This species has been a topic of study in the past with regard to its reproductive biology, population genetics structure, diet, diversity and biology (Bennett et al. 1983; Prochazka and Griffiths 1992; Prochazka 1994; Fishelson et al. 2007; Gon et al. 2007; Moser 2007; von der Heyden et al. 2008; Fishelson and Gon 2009; Holleman et al. 2012; Vermaak et al. 2021). *Clinus superciliosus* is distributed from Rocky Point, Namibia, to the Kei River mouth on the east coast of South Africa (Smit et al. 2003; von der Heyden et al. 2011), occurring in the demersal zone from rocky shores to subtidal sandy areas up to 12 m (IUCN 2020). This species is viviparous and exhibits super-embryonation, where multiple stages of young develop simultaneously within the ovaries (Prochazka 1994; von der Heyden et al. 2008). This mechanism is an apparent adaptive response to overcome the small number of offspring produced, due to the high energy cost of reproduction in intertidal areas (Prochazka 1994). This also means that reproduction is not limited to specific seasons (Prochazka 1994). According to the abovementioned studies, as well as personal observations during field collection, their diet consists of various invertebrates, including amphipods, isopods, polychaetes, echinoderms, and molluscs, as well as small fish (Wilson 2009). These invertebrate species are also intermediate hosts to various parasite species that can potentially lead to the infection of *C. superciliosus*. Together with other clinids, *C. superciliosus* forms a major part of intertidal ecosystems, where it contributes to at least 40% of the biomass of intertidal rock pool fishes (Bennett 1984; Branch and Griffiths 1988).

As a significant part of the rocky shore ecosystem, *C. superciliosus* is potentially an important host to various parasite species. Considering its habitat and feeding habits, the body of each host organism offers various habitats for a magnitude of parasites to flourish (Roberts and Janovy, 2009; Vermaak 2020). However, the full extent of its involvement in parasite distribution and their life cycles, have to date not been investigated. Only a limited number of parasitological surveys have been conducted on this host species (see below), with the latest reports published from a project that ran in parallel to the present study (Vermaak et al. 2021). To date the trend in marine ichthyoparasitology, particularly in South Africa, was to focus on a specific parasite taxon and not on the total parasite community of a specific fish host (see for example publications on cestodes (van der Spuy et al. 2022); monogeneans (Acosta et al. 2021); nematodes (Svitin et al. 2019); acanthocephalans (Lisitsyna et al. 2019a); and gnathiids (Hadfield et al. 2019). These surveys were often sporadic collections scattered along the coast and in different seasons, focusing on parasite taxonomy and not ecology. Therefore, these records did not provide information on parasite community structures of a specific host fish and could not be related to the ecological role of the parasites in the system. The ubiquitous presence of parasites can assist in understanding the structure and pressures of host species and food webs. Knowledge of parasite communities of native fish is of considerable interest, since it can further be related to fish health and give a better understanding of ecosystem function and disturbance (Sures et al. 1999; Artim et al. 2020). It has been reported that habitat change can result in altered helminth and other parasite community richness and structure, and it is thus important to also investigate whole parasite communities over a period of time (Kennedy 1993; Sures et al. 1999). Therefore, parasites can be used as indicators of ecosystem health (Sures et al. 1999; Artim et al. 2020). The present study therefore aims to provide a holistic view of the biodiversity, community structures, ecology, and role as potential indicators of environmental health, specifically related to metal pollution, of the metazoan parasites of *Clinus superciliosus*. Following this brief introduction, this chapter will further provide background information on the techniques used to identify the various parasitic groups, a brief overview of the different potential parasitic groups one would expect to find on *C. superciliosus*, and the role of environmental parasitology in the present study. This chapter will be concluded with the hypotheses that were tested in this study and the aims set for the testing of the hypotheses.

1.2 Identification techniques

Identification techniques of various fish parasite species have progressed tremendously over the past 20 years and currently the norm is to use both morphological and molecular analyses to characterise a species. Taxonomic identification has been used as the main criterion to name, identify, and classify parasitic groups in the past (Crites 1962). The process involved literature studies and comparisons to previously known material, whereafter the specimens were grouped accordingly. Unknown specimens were described and named in a suitable publication (Crites

1962), where one of the described specimens was designated as the holotype specimen for future reference to avoid confusion (Crites 1962). While studying the physical features of an organism is inexpensive, and can sometimes be done in the field, advanced tools such as scanning electron microscopy and differential interference contrast light microscopy aid in morphological identification of species in the laboratory (McManus and Bowles 1996). Although the technique of morphological identification has its merits, the use of multiple analyses can aid in possible confusion based on interpretation and the variation in biological structures within a rapidly growing number of species (McManus and Bowles 1996).

Molecular analysis of a parasitic species has the advantage that a direct comparison between morphologically similar species can be done, without the influence of environmental or ontogenetic variation; obtained sequences are always available for future comparison; and that the level of divergence can easily be quantified (McManus and Bowles 1996). This application of characterisation and phylogenetic relations progressed scientific understanding of taxonomy, systematics, and diversity of species (Schwelm et al. 2021a). This is the most direct, but not the most useful, tool for the phylogeny of species since elucidating species will depend on the quality and quantity of your genetic database. This process entails the amplification of standardised regions of the genome, through specific primers and temperature cycling using polymerase chain reaction (PCR), followed by DNA sequencing (Renoux et al. 2017). Nonetheless, this technique emerged as a powerful and rapid tool with significant contribution in taxonomic and biodiversity research (Renoux et al. 2017).

Over the last few years, it has become more important to use these techniques in combination, in order to accomplish better species descriptions, differentiate inter- and infraspecies variations, phylogenetic relationships, and reduce the number of misidentifications. With the recent advances in technology, it has become standard practise to apply this integrative approach for redescriptions and new descriptions in order to reduce confusion and doubt at a later stage. The accurate identification of parasitic species can also aid in effective treatment and control of zoonotic pathogens (McManus and Bowles 1996). Knowledge on the identity of parasite species, as well as understanding the variations that occur within the group of species, is crucial for any proper species descriptions (McManus and Bowles 1996).

1.3 Metazoan parasite phyla

1.3.1 Myxozoa (Cnidaria)

From the discovery of these parasites in 1838, it took more than 140 years to finally identify and classify these multicellular species as metazoan Cnidaria (Klimpel et al. 2019). They are predominantly found parasitising teleost fish, with rare reports of amphibian, reptile, and cartilaginous fish hosts. The current stance of marine myxozoans off the coast of Africa includes 52 species from Senegal, Tunisia, and southern Africa (Reed et al. 2007). However, limited information is available on the distribution and diversity of marine species along the African

coastline (Reed et al. 2007). Myxozoans are found infecting the bile, gall bladder, eyes, muscle tissue, gills, and gill arches of their hosts, sometimes in host cysts (Fantham 1919, 1930; Gilchrist 1924; Ali 2000; Reed et al. 2007). Severe distortion of the gill lamellae caused by myxozoans have been reported (Reed et al. 2007), however, little to no physical evidence was visible during the present study when considering fish health or behaviour. In addition, an economically important species (*Kudoa thyrsites* (Gilchrist, 1923)), infecting the soft tissue of their host has been reported on commercial fishes such as snoek, hake, and pilchards in South Africa, causing post mortem tissue degradation rendering fish unmarketable (Whipps and Kent 2006). Since its original description, this species has been reported from a wide range of host species, with a cosmopolitan distribution (Henning et al. 2013; Smit and Hadfield 2015). Multiple species have been genetically identified to infect *C. superciliosus* (Table 1.1). However, the concurrent infections have sometimes proven complicated to link the molecular results to morphological characterisation (Bartošová-Sojková et al. 2018).

1.3.2 Platyhelminthes

1.3.2.1 *Trematoda*

This class represents a major component of cosmopolitan parasitic flatworms, capable of infecting all organs of vertebrate hosts, especially marine fishes. Parasites of the subclass Digenea are mainly characterised by their dorsoventrally flattened body with bilateral symmetry, oral sucker around the mouth, and ventral sucker located more distally (Roberts and Janovy 2009). A total of 87 species representing 63 genera of marine Trematoda have been described or reported from 46 South African marine fish families (Vermaak, 2020). This constitutes only 24 of a possible 46 known families of marine trematodes, including members of the Opecoelidae and Hemiuridae as the most abundant species in South Africa (Bray et al. 2016; Vermaak, 2020). Prior to the present study, and that of Vermaak et al. (2021), the majority of parasite species records were reported infecting the family Sparidae (n = 12), while there were only two species recorded parasitising *C. superciliosus* (Table 1.1).

1.3.2.2 *Monogenea*

Monogeneans are ectoparasitic platyhelminths feeding on the external layers of the skin and gills of marine and freshwater fishes (Colorni and Diamant, 2005). Only 34 valid species from a possible 470 global species, have been described from African fishes, with only two species from the marine environment (Christison et al. 2021). Two groups (Monopisthocotylea and Polyopisthocotylea) of monogeneans are morphologically divided based on the form of their opisthaptor (attachment organ) at the posterior end of the parasite (Klimpel et al. 2019). These parasites are known to only infect a single host species, genus or family, based on their specific attachment organs, with the exception of Capsalidae Baird, 1853 (see Colorni and Diamant 2005; Klimpel et al. 2019). The Monopisthocotylea group are found on the gills, skin and fins and feed

on the epidermis, while the Polyopisthocotylea parasites are only found infecting the gills and exclusively feed on host blood (Klimpel et al. 2019). The only information available on monogeneans from *C. superciliosus* is that of an unnamed *Gyrodactylus* sp. reported from Table Bay, South Africa by Christison et al. (2007) in a conference abstract.

1.3.2.3 Cestoda

The endoparasitic tapeworms, cestodes, infect many ray-finned and bony fish, with various elasmobranchs as definitive hosts (Klimpel et al. 2019). Diphylobothriidea, Bothriocephalidea, Trypanorhyncha and Tetraphyllidea are among the most dominant orders described from the marine environment (Klimpel et al. 2019). Research on their diversity, host association and distribution, specifically in South Africa, is scanty. A total of 297 species, as well as some unidentified cestodes, have been recorded globally (Alves et al. 2017). Based on a study on the annotated checklist of cestodes from South America, poor taxonomic resolution, unreliable host identification, and the absence of voucher specimens to verify parasite identification are some of the issues that hinder the advancement of adequate research on cestode diversity (Alves et al. 2017). The same would be true for South Africa as only a few species have been reported infecting teleost as intermediate hosts and elasmobranchs as definitive hosts. The monograph by Dr Linton reporting nine different species of cestodes from South Africa was only the start of Cestoda descriptions for this region (Linton 1924). Economically important teleost fish such as hake, snoek, and kingklip are known to harbour various larval stages (Smit and Hadfield 2015). The life cycle stages of cestodes, and for that matter other parasite species, still remain understudied due to a lack of data and experts working on parasitic cestodes. This can be owing to the fact that larval stages cannot be identified reliably to species level and can thus not be linked to definitive hosts and species (Palm and Caira 2007). To the authors knowledge, there are no previous records of any cestodes infecting *C. superciliosus*.

1.3.3 Acanthocephala

Acanthocephalans are heteroxenous parasites with zooplankton (isopods, copepods, amphipods and ostracods) as first intermediate hosts, whereafter the acanthor (first larvae) hatches inside a suitable invertebrate host (Smales 2015). In addition, fish can also serve as an intermediate host for the cystacanth (second larvae), while definitive hosts are typically predatory fish and piscivorous birds (Klimpel et al. 2019). Based on a checklist compiled in 2018 and a 2019 publication by Lisitsyna et al. (2019), there are only 24 species of acanthocephalan, consisting of 15 genera from 11 families, reported from South Africa (Halajian et al. 2018; Lisitsyna et al. 2019). Eleven of the possible 24 species are reported from marine hosts, consisting of only 4 families. Compared to records from other countries, such as 58 species from Australia (Smales and Weaver 2015), these numbers can be interpreted as a low biodiversity in acanthocephalan species. However, it is most probably due to a lack in parasitological surveys. These checklists

demonstrated the need for more extensive parasitological surveys. Additionally, no records of acanthocephalans infecting *C. superciliosus* are available to date.

1.3.4 Annelida

Piscicolidae leeches are known as fish leeches, specifically teleost and elasmobranch fish, with diverse life strategies (Utevsky et al. 2019; Bureson 2020). This family consists of more than 60 genera and 120 species in all oceans and freshwaters except terrestrial Antarctica, but are most dominant in marine habitats (Bureson 2020). According to the summary of species by Smit and Hadfield (2015), only four species of leeches (*Janusion stellata* (Moore, 1958), *Austrobdella oosthuizeni* Utevsky, 2004, *Lizabdella africana* Utevsky, 2007, and *Pterobdella arugamensis* (de Silva, 1963) are known infecting marine fishes in South Africa. The majority of leech species are carnivorous, with a few haemophagic, feeding on its host's blood until swollen and detaching to digest its meal (Kua et al. 2009). In the marine environment, this parasite behaviour can result in haemorrhagic sites, frayed fins, and loss of appetite by the host (Kua et al. 2009). Secondary infection at the attachment site can also occur, along with the transmission of blood parasites into the host's bloodstream (Smit et al. 2000, 2004; Davies et al. 2004; Kua et al. 2009). The classic taxonomy of this family includes diverse characters of the coelomic system and genital anatomy (Selensky 1915; Utevsky et al. 2019). Interestingly, leeches are known as the most extreme annelids, as they can survive under a number of extreme conditions such as temperature, moisture and salinity, to name a few (Phillips et al. 2020). A single record of leech from *C. superciliosus* exists (see Hayes et al. 2014) (Table 1.1).

1.3.5 Nematoda

Nematodes infect numerous different fish species (Yanong 2003). In small numbers they may be harmless, but high infection intensities have been known to cause serious illness and even death (as in the case of *Anisakis*) (Yanong 2003). Adult nematodes usually occur in the digestive tract of their fish host, but are also found in the body cavity, swim bladder, or muscle tissue (Yanong 2003). Research on South African marine nematodes are limited, with experts on this group based outside South Africa (Smit and Hadfield 2015). Camallanid nematodes have been described from five species of South African marine fishes: the dusky sweeper, *Pempheris adusta* Bleeker, 1877; the stocky hawkfish, *Cirrhitus pinnulatus* (Förster, 1801); the banded grunter, *Pomadasys furcatus* (Bloch et Schneider, 1801); the thornfish, *Terapon jarbua* (Forsskal, 1775); and the largespotted pompano, *Trachinotus botla* (Shaw, 1803) (Svitin et al. 2019). A study by Mattiucci et al. (2009) described new species from whales off the coast of New Zealand and South Africa by using morphological and molecular techniques, linking the potential intermediate host as a cephalopod (Smit and Hadfield 2015). This study emphasises the potential of using these techniques to link larval nematodes collected from teleost fish to their definitive host (Mattiucci et al. 2009; Smit and Hadfield 2015). As with the majority of the other parasitic taxa, no nematodes have been reported infecting *C. superciliosus*.

1.3.6 Arthropoda

1.3.6.1 *Hexanauplia*

Siphonostomatoida

Copepoda is the largest group of crustaceans and consists of more than 11,500 species, most of which are parasitic (Klimpel et al. 2019). This subclass includes three infraclasses (Khodami et al. 2019; Al-Quraishy et al. 2021), with the order Siphonostomatoida Burmeister, 1835 within the infraclass Podoplea Giesbrecht, 1882. Siphonostomatoida contains around 75% of the parasitic fish copepods within 39 families, mostly marine species (Williams and Bunkley-Williams 2019; Al-Quraishy et al. 2021). These parasitic species can be very damaging to commercial aquaculture species (Lafferty et al. 2015; Williams and Bunkley-Williams 2019), negatively affecting this economically important sector. The life cycle of parasitic copepods, with few exceptions, involves a single host with the first copepodid as the infective larvae (Williams and Bunkley-Williams 2019). In South Africa, only a few studies are available on ectoparasitic copepods, with only 136 of a possible 1,388 species known, also reflected by the limited experts on copepod taxonomy from this region (Dippenaar 2016b). This has an impact on its taxonomy and can reflect inadequate information of its morphology, anatomy, and molecular data (Kabata 1979; Ohtsuka et al. 2020). To date two species of *Caligus* have been reported to infect *C. superciliosus*, however, the confirmation of *Caligus labracis* Scott, 1920 has been in question owing to a lack of evidence. Thus, the present study only acknowledges the infection of *C. mortis* Kensley, 1970 as a Siphonostomatoida parasite of *C. superciliosus*. No other copepod has been reported from this host.

1.3.6.2 *Malacostraca*

Isopoda

Of the 95 families of Isopoda, only eight are known to be parasitic (Rohde 2005). Epicaridians, gnathiids and cymothoids are the three main groups, while the latter two have previously been reported to infect *C. superciliosus*. According to the Isopod checklist by Kensley (2001), only 32 parasitic isopod species were previously reported from African waters, while a total of 86 were known from the Western Indian Ocean. Based on a more recent review of Cymothoidae, at least 28 species have been reported from South African waters (Hadfield 2012). Cymothoids are found in the buccal cavity, gills, palate and on the external surface of their hosts. While cymothoids are permanent parasites of fish, gnathiids only feed temporarily and are also known as the ticks of the sea. One species of Cymothoidae, *Elthusa xena* van der Wal, Smit & Hadfield, 2019, has been reported from Alexander Bay on the west coast of South Africa, close to Namibia (Table 1). Gnathiid parasites are temporary ectoparasites of fish and elasmobranchs, with multiple juvenile stages followed by a free-living adult stage (Smit and Davies 2004; Smit and Hadfield 2015). The juvenile stages of Gnathiidae have been reported to cause damage to the gill spectrum of elasmobranchs, with possible negative impacts in cases of high infection intensities (Hayes et al.

2007; Smit and Hadfield 2015), whilst cymothoids on the other hand, are known to feed on the blood of their hosts (haematophagous) (Colorni et al. 1997; Hadfield 2012). Taxonomically, classification of gnathiid parasites is challenging as the adult males need to be morphological characterised (Hadfield et al. 2019). Hadfield et al. (2008, 2009) and Smit and Hadfield (2015) confirmed that there are at least seven species of Gnathiidae known from South Africa of which one species, *Gnathia africana* Barnard, 1914, is a confirmed parasite of *C. superciliosus* (Table 1)

1.4 Parasites of *Clinus superciliosus*

Table 1. 1: Known protozoan and metazoan parasite diversity of Clinus superciliosus from South Africa and Namibia.

Parasite species	Locality	Reference
Euglenozoa		
<i>Trypanosoma nudigobii</i> Fantham, 1919	-	Hayes et al. 2014
Myxozoa		
<i>Haemogregarina bigemina</i> (Laveran & Mesnil, 1901)	-	Smit and Davis 1999
Ciliophora		
<i>Trichodina clini</i> Fantham, 1930	-	Fantham 1930
Cnidaria		
<i>Ceratomyxa dehoopi</i> Reed, Basson, Van As & Dyková, 2007	De Hoop; Jeffreys Bay	Reed et al. 2007
<i>Ceratomyxa obovalis</i> (Fantham, 1930)	-	Reed et al. 2007
<i>Sphaeromyxa clini</i> Bartošová-Sojková, Kodádková, Pecková, Kuchta & Reed, 2015	Cape Columbine; Jacobs Bay	Bartošová-Sojková et al. 2015
<i>Henneguya clini</i> Reed, Basson, Van As & Dyková, 2007	De Hoop; Jeffreys Bay	Reed et al. 2007
Platyhelminthes		
<i>Coitocaecum capense</i> Bray, 1987	Oudekraal; Blue Hole	Bray 1987a
<i>Helicometra fasciata</i> (Rudolphi, 1819) Odhner, 1902	Blue Hole	Bray 1987a
<i>Cardiocephaloides</i> sp.	Tsitsikamma; Jeffreys Bay	Vermaak et al. 2021
<i>Cardiocephaloides physalis</i> (Lutz, 1926)	Hermanus	Vermaak et al. 2021
Annelida		
<i>Zeylanicobdella arugamensis</i> de Silva, 1963	Tsitsikamma	Hayes et al. 2006
Arthropoda		
<i>Gnathia africana</i> Barnard, 1914	De Hoop; Jeffreys Bay	Barnard 1914; Smit et al. 1999
<i>Caligus mortis</i> Kensley, 1970	Möwe point (Namibia); Saldanha Bay; De Hoop; Jeffreys Bay	Dojiri, 1989; Grobler et al. 2002
<i>Elthusa xena</i> van der Wal, Smit et Hadfield, 2019	Alexander Bay	van der Wal et al. 2019

1.5 Elemental pollution in marine ecosystems

Even though metals occur naturally in the environment, they can have major consequences in aquatic ecosystems when concentrations are increased due to anthropogenic activities (Tchounwou et al. 2012). Essential elements such as copper (Cu) and zinc (Zn) are required for biochemical and physiological processes but can be detrimental at high concentrations (Rainbow et al. 2015), while lead (Pb) and mercury (Hg) are considered toxic with no metabolic function (Rainbow and Wang 2005). Environmental contaminants of concern are metals and metalloids, here on referred to as elements, due to their ability to accumulate and even bioconcentrate in aquatic ecosystems, as well as their potential toxicity to specifically aquatic organisms (Censi et al. 2006; Zhou et al. 2008; Amoozadeh et al. 2014). Reportedly, these elements, along with

organochlorine pesticides, can pose a great threat to intertidal rocky shore organisms, however, data on the effect of elements on free-living and parasite species in the marine environment is still scarce (Sures et al. 2005; Nachev and Sures 2016). The monitoring of elemental contaminants in marine ecosystems is a major priority since ecosystems are of great ecological importance and serve as a major food source for human consumption (Nachev and Sures 2016). The biological availability of these toxic substances can be determined by analysing concentrations in species considered as bioindicators. Ideal bioindicators are species that: 1) are restricted to small areas with a known range; 2) are sufficiently abundant in the sampling area; 3) have no seasonality; 4) are easy to sample; 5) are large enough to use for chemical analyses; 6) have a predisposition for consistent uptake; 7) have high accumulation capacity; 8) have a predisposition to retain accumulated concentrations; and 9) are long lived (Barbaro et al. 1978; Beeby 2001). Some species already identified as ideal bioindicators are planktonic organisms, molluscs, annelids, crustaceans, and mammals (Reish and Gerlinger 1997; Boening 1999; van der Oost et al. 2003; Rainbow and Wang 2005; Vidal-Martínez et al. 2006; Klerks et al. 2007; Zhou et al. 2008; Degger et al. 2011a; Cáceres-Saez et al. 2013; Amoozadeh et al. 2014; Wepener and Degger 2020). Past studies have also reported on the detoxification potential of species, by means of sequestration, shedding, and metabolising compounds prior to becoming toxic to these organisms (Rainbow et al. 2015). It is important that not only bioindicator free-living taxa are considered during ecosystem studies, but the entire food web, including the parasites within.

During the last few decades, the focus of environmental monitoring has shifted to also include the parasites infecting these bioindicator species (Sures et al. 2005; Vidal-Martínez et al. 2010; Nachev and Sures 2016). This shift was brought about by the various impacts that parasites have on free-living species. In some cases, parasites have been reported to even reduce pollutant concentrations in their hosts' tissue, making free-living species unreliable indicators (Filipović Marijić et al. 2014; Nachev and Sures 2016).

1.6 Environmental parasitology

One of the recent fast-growing developments in fish parasite research is known as environmental parasitology. This has developed as a discipline where parasites are considered as bioindicators. The impact of environmental pressures is related to parasite occurrences and diversity, their sensitivity or tolerance to pollution events, anthropogenic impacts, and exposure events (Sures et al. 2017a; Biswal and Chatterjee 2020). Considering the ecological impacts, exposure to pollutants often lead to decreasing parasite diversity (MacKenzie et al. 1995; Marcogliese et al. 2005). Parasites are thus considered: i) accumulation indicators, ii) effect indicators, as well as iii) factors affecting host health (Sures et al. 2017a). Biologically available metals and metalloids can be accumulated by endoparasites, such as acanthocephalans and cestodes, since these elements can pass through the parasite's tegument and other membranes, supporting the

proposal of parasites as valuable bioindicator species. This process can have multiple consequences for the host. Various parasite-host interactions affect the homeostasis of the host, leading to antagonistic, synergistic, or additive effects, for example parasites can influence the metabolism and behaviour of their hosts (Sures et al. 2017a). However, these effects are dependent on the different species. Direct or indirect effects can often be seen as a result of intermediate host mortalities or on free-living stages of heteroxenous parasites (Sures et al. 2017a; Biswal and Chatterjee 2020). As a result, some infected hosts can often suffer deaths at certain concentrations, compared to their uninfected counterparts. These parasite communities can in return report crucial information regarding the ecosystem health and can in some cases even be beneficial to their hosts (Sures et al. 2017a). Some studies on the simultaneous exposure of hosts to parasites and pollutants have reported a consistent reduction in mortality in hosts species of *Artemia parthenogenetica* Bowen & Sterling, 1978 infected with *Flamingolepis liguloides* (Gervais, 1847) and *Confluaria podicipina* (Szymanski, 1905) (cestodes) exposed to various arsenic concentrations (Sánchez et al. 2016). Little advancement in research considering the infectivity of free-living stages of parasites have been conducted (Pietroock and Marcogliese 2003). Although this field of research has already started to develop in 1999 (Sures et al. 1999), little to no studies have applied this method in the South African marine context.

1.7 Problem statement, hypotheses, and aims

On the west coast, the cold Benguela current transports nutrient rich water northwards, while the warm Agulhas current transports water from the subtropics downwards along the east coast. This results in high biodiversity and productivity, with various phenomena, such as upwelling, contributing to species richness and biomass (Lutjeharms et al. 2000; Griffiths et al. 2010; Wepener and Degger 2019). According to Hamer (2010), only 12,098 invertebrate species (including parasites) are known from South Africa, while at least 80,000 more species are still to be discovered (Hamer 2013; Halajian et al. 2018). Fishing has been reported as a driver of parasite abundance decline, caused by a reduction in habitat availability and resources for parasites to thrive (Wood et al. 2013). As parasites are drivers of ecosystem function, these anthropogenic activities cause cryptic effects on the marine community composition and ecosystem function (Wood et al. 2013), as well as the overall biodiversity of marine species. Various checklists have been compiled in the past on different groups of parasites that demonstrate the need for more extensive parasitological studies, not only specific to South Africa. The present study approached this suggestion from a different angle, by investigating the parasite diversity of a single host species (host-specific parasite sampling) instead of focusing on a specific group of parasites (parasite-specific sampling).

This study aimed to improve our understanding of the role that parasites play within ecosystems, as well as to examine the effects of anthropogenic pressures (specifically metals and metalloids) and other factors (size, sex, water temperature, life cycle, habitat type and

seasonality, and degree of anthropogenic pressure) on these parasite communities. This was achieved by determining what drives the distribution and abundance among ichthyoparasites in *Clinus superciliosus* within its South African range. The focus of the thesis was to elucidate the number of unknown species in one host fish and connect the infection to different environmental conditions.

1.7.1 Hypotheses

The hypotheses tested during the present study:

1. Host-specific parasite sampling will yield more parasite species than parasite-specific sampling from previous studies.
2. Parasite community composition will be mostly influenced by environmental factors, such as water temperature and host habitat availability.
3. The proposed bioindicator, *Clinus superciliosus*, will reflect metal bioaccumulation based on their proximity to the surrounding land-use activities.
4. The metazoan parasite community composition will respond to element bioaccumulation in the fish host, thereby reflecting by the degree of external (environmental) element exposure.

1.7.2 Aims

To test each of the above hypotheses, the main aims of the present study were to:

- Determine the metazoan parasite taxa community of *Clinus superciliosus* by providing a comprehensive list of metazoan parasite taxa associated with selected localities along its South African distribution, providing short morphological and, where possible, phylogenetic analysis for identification to the lowest taxonomic level possible.
- Provide a full description of one newly discovered species using an integrated approach to taxonomy, through a combination of morphological and molecular techniques.
- Determine the parasite species richness and diversity at selected localities with varying environmental conditions.
- Determine which factors influence parasite community composition within this host by comparing the metazoan parasite community composition at various localities within the distribution range of *C. superciliosus*.
- Determine the bioaccumulation of selected metals and metalloids by *C. superciliosus* along the South African west and south-east coasts.
- Determine whether the elemental exposure in fish cause a change in the parasite community composition at the selected localities.

1.8 Chapter layout

The present study is divided into eight chapters:

Chapter 1 - General introduction: This chapter consists of a brief background on *Clinus superciliosus*, marine parasitology, metal pollution in the marine environment, as well as the concept of environmental parasitology. This information relates to the rationale of the present study, as well as provides the hypotheses and aims.

Chapter 2 - General methodology: Considering that not all of the localities were included in each chapter, this section gives an overview of all the localities, as well as the general methods used for collecting and investigating the fish host for various parasite species.

Chapter 3 - Parasite community of Clinus superciliosus: An overview of all the metazoan parasite taxa identified during the present study, including short morphological descriptions and phylogenetic analysis, where possible, of the various taxa was given for known and novel species.

Chapter 4 - Full morphological and molecular description of one newly discovered parasite species: As an example of how effective an integrative approach to taxonomy can aid in species descriptions, a full description of a novel species of *Alella* (Lernaeopodidae: Copepoda) was provided using differential interference contrast, scanning electron microscopy and phylogenetic characterisation with the illustration and description.

(Submitted as Erasmus, A., Hadfield, K.A., Smit, N.J. 2021. Morphological and molecular characterisation of *Alella* sp. A (Copepoda: Siphonostomatoida: Lernaeopodidae) parasitising the southern African endemic intertidal klipfish, *Clinus superciliosus*. *Systematic Parasitology*).

Chapter 5 - Factors influencing parasite community composition: An assessment of the factors influencing parasite community composition at various localities along the South African distribution was presented. Factors such as size, sex, water temperature, life cycle, habitat type and seasonality were considered.

(Accepted as Erasmus, A., Hadfield, K.A., Wepener, V., Sures, B., Smit, N.J. 2021. Metazoan parasite diversity of the endemic South African intertidal klipfish, *Clinus superciliosus*: factors influencing parasite community structure. *Parasitology International*).

Chapter 6 - Metal accumulation in Clinus superciliosus: The results from this chapter reflect the elemental concentrations from various localities, comparing the west and southeast coast concentrations in the host species, *Clinus superciliosus*. This serves as a baseline for intertidal species from these localities, as this species is a resident intertidal endemic fish.

(Published as Erasmus, A., Smit, N.J., Zimmermann, S., Nachev, M., Sures, B., Wepener, V. 2021. Metal and metalloid concentrations in the southern African endemic inter- and infratidal super klipfish, *Clinus superciliosus*, from the west and south coasts of South Africa. *Marine Pollution Bulletin*, 172:112852).

Chapter 7 - Parasites as effect indicators of pollution: An assessment on the effects of pollution on parasite community composition, combining the results from the preceding two

chapters, and disentangling the anthropogenic pressures vs natural conditions found in ecosystems was given. (Submitted in Marine Pollution Bulletin)

Chapter 8 - General conclusion and recommendations: This chapter consists of a general conclusion drawn from all of the chapters in order to verify whether *Clinus superciliosus* would be a suitable model organism for an environmental parasitological approach to marine metal pollution.

1.9 Project outputs

Research presented at national and international conferences:

Greyling* A. Wepener V, Sures B, Smit NJ, (2018) *Clinus superciliosus* as model organism for an Environmental Parasitological approach to the exposome of marine fish species. *1st UESM Prestige PhD Conference 2018*. 30 August 2018, Potchefstroom, South Africa (POSTER).

Greyling* A. Hadfield KA, Wepener V, Sures B, Smit NJ, (2018) Conservation of parasite populations through Marine Protected Areas: A South African perspective. *2nd UESM Prestige PhD Conference 2019*. 28–29 August 2019, Potchefstroom, South Africa (3 MIN SPEED TALK).

Erasmus A. Hadfield KA, Wepener V, Sures B, Smit NJ, (2021) Parasite diversity of an endemic intertidal fish, *Clinus superciliosus*: factors influencing parasite community structure. *British Society Parasitology Conference 2021*. 21–25 June 2020, Virtual Meeting, attended in Potchefstroom, South Africa (Poster).

Erasmus A.^a Vermaak A,^a Hadfield KA, Smit NJ, (2021) Gill maggots: New host and locality records for marine lernaeopodids (Lernaeopodidae) from South Africa, including the first molecular characterisation of species from this region. *49th Parasitological Society of Southern African Conference*, 14–15 September 2021, Virtual Meeting, attended in Potchefstroom, South Africa (Oral Presentation).

Erasmus A. Smit NJ, Zimmermann S, Nachev M, Sures B, Wepener V, (2021) Metal and metalloid concentrations in the southern African endemic inter- and infratidal super klipfish, *Clinus superciliosus*, from the west and south coasts of South Africa. *Society of Environmental Toxicology and Chemistry (SETAC) Africa 10th Biennial Conference*. 20–22 September 2021, Virtual Meeting, attended in Potchefstroom, South Africa (Oral Presentation).

Papers covering the work from this project have been published in peer-reviewed journals:

Erasmus A. Smit NJ, Zimmermann S, Nachev M, Sures B, Wepener V, (2021) Metal and metalloid concentrations in the southern African endemic inter- and infratidal super klipfish, *Clinus superciliosus*, from the west and south coasts of South Africa. *Marine Pollution Bulletin*, 172: 112852. [Chapter 4]

* Erasmus (néé Greyling); ^a Both authors contributed equally to this work

CHAPTER 2

General Methodology



2.1 Sampling localities

This chapter provides a general overview, selection, characterisation and photographs of the various sampling localities, as well as in which chapters they were included, for ease of referencing. In addition, general field sampling and laboratory materials and methods are also presented. Specific details concerning each study are described in their respective chapters.

In order to determine host-specific functional diversity and environmental analyses, a total of seven localities were identified along the South African distribution range of *Clinus superciliosus* (Linnaeus 1758) (Clinidae), based on their proximity to anthropogenic pressures and or conservation areas (from west to east: Langebaan marina, Cape Town harbour, Simons Town marina, Hermanus Old harbour, Tsitsikamma, Jeffery's Bay and Chintsa) (Figure 2.1; Tables 2.1–2.7).

The westernmost sampling point, Langebaan marina (2019; n = 19) (33° 02' 44.5" S; 18° 02' 19.1" E), is situated between Saldanha Bay harbour and the West Coast National Park Marine Protected Area (MPA), within a marina. Cape Town harbour (2019; n = 16) (33° 54' 29.1" S; 18° 25' 05.8" E) is a busy port located next to the city of Cape Town at the Two Oceans Aquarium. Simons Town marina (2018; n = 12) (34° 11' 30.5" S; 18° 26' 00.3" E), is a yacht club marina with a Blue Flag status, within the greater False Bay area. The old commercial harbour in Hermanus (2018; n = 24) (34° 25' 15.9" S; 19° 14' 37.6" E) has 12 established abalone farms and was known for small scale fishing activities in the past and declared a National Monument in 1970. The aforementioned localities were considered as anthropogenically altered sampling points, while the localities below were considered as natural. The Tsitsikamma (2019 and 2020; n = 10 respectively) sampling locality (34° 01' 15.2" S; 23° 52' 43.2" E) within the Storms River Mouth Camp of the Garden Route National Park is considered pristine, conserving 11% of intertidal and subtidal inshore habitats of South Africa. A very popular tourist destination and Chokka fishing industry hotspot, Jeffreys Bay (2018; n = 14) (34° 1' 35.0004" S; 24° 55' 50.9982" E), is located just south of Gqeberha (previously Port Elizabeth). The easternmost sampling point, Chintsa (2019 and 2020; n = 10 respectively) (32° 50' 11.5" S; 28° 07' 01.2" E), is another popular tourist spot involving controlled recreational fishing located next to the Amathole MPA between the Gonubie and Kei marine areas. Geographic representations of the selected localities in each Chapter were generated using ArcGIS 10.6.1 software.

Prior to sampling, the necessary ethical clearance (North-West University (NWU) AnimCare – NWU-00440-16-A5, NWU-0051-19-A5;), as well as permits (Department of Agriculture, Forestry and Fisheries (DAFF) – RES2018/35, RES2019/103, RES2020/29); South African National Parks (SANParks) MALH-K/2016-005a, SMIT-NJ/2020-004), were obtained.

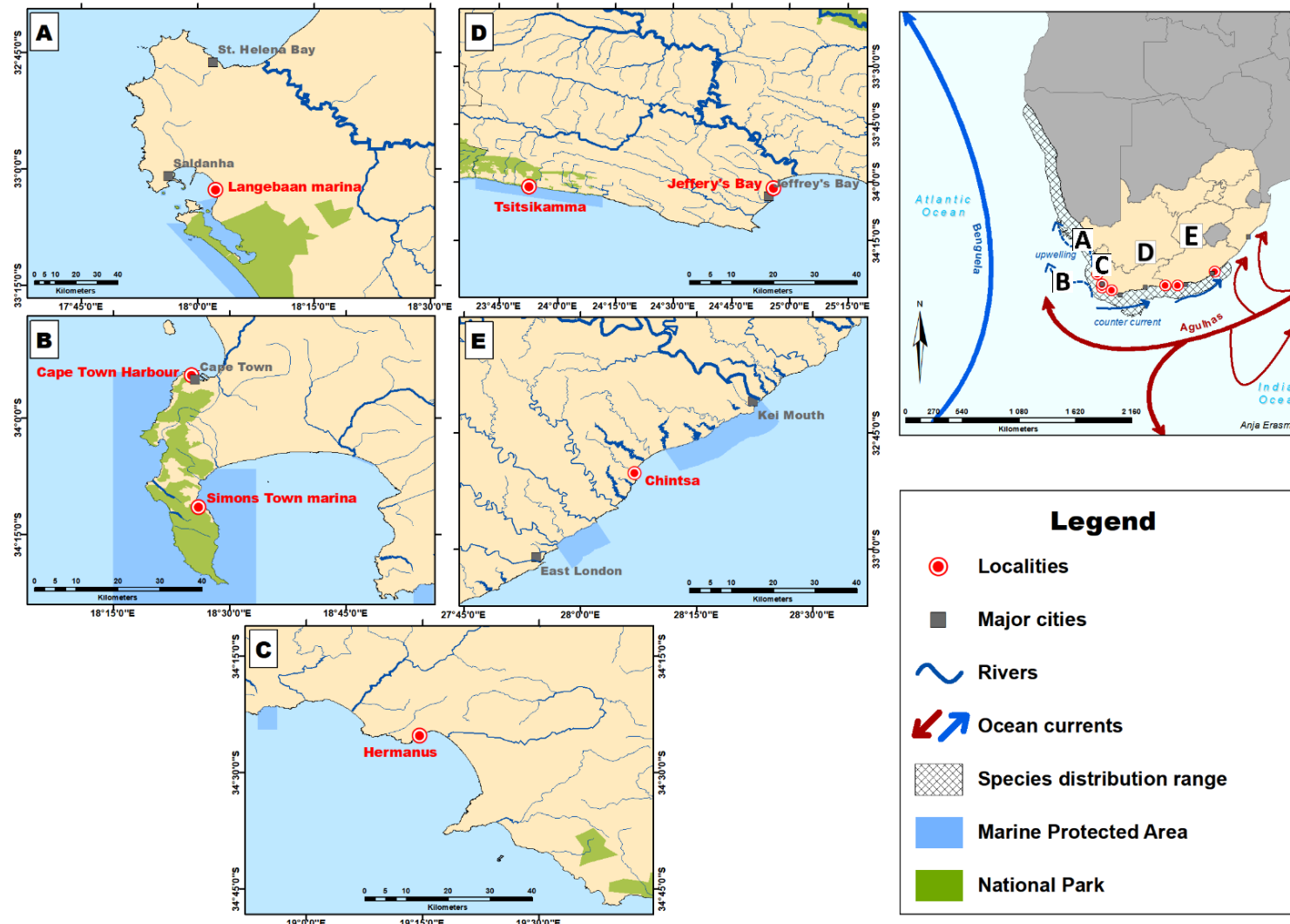


Figure 2. 1: General map of sampling localities surveyed throughout the project. A) Langebaan marina on the west coast; B) Cape Town harbour, and Simons Town marina in False Bay; C) Old harbour Hermanus as the southernmost sampling point; D) Tsitsikamma and Jeffery's Bay on the south coast; and E) Chintsa as the easternmost point.

Table 2. 1: Characteristics of Langebaan marina (LM19) as seen in map, listed from west to south along the South African coast, with the abbreviated locality name given in brackets.

Langebaan marina (LM19)	
	
Coordinates	S 33°02'44.5"; E 18°02'19.1"
Survey period	August 2019
Biogeographic region	Cold temperate
Habitat type	Infratidal
Condition of locality	Anthropogenically altered
Water temperature (average for survey period)	15.9 °C
Salinity	36 ppt
Number of fish sampled	n = 19
Chapters included	Chapters 3; 4; 5; 6; and 7

Table 2. 2: Characteristics of Cape Town harbour (CT19) as seen in map, listed from west to south along the South African coast, with the abbreviated locality name given in brackets.

Cape Town harbour (CT19)	
	
Coordinates	S 33°54'29.1"; E 18°25'05.8"
Survey period	September 2019
Biogeographic region	Cold temperate
Habitat type	Infratidal
Condition of locality	Anthropogenically altered
Water temperature (average for survey period)	16 °C
Salinity	35.8 ppt
Number of fish sampled	n = 16
Chapters included	Chapters 3; 4; 5; 6; and 7

Table 2.3: Characteristics of Simons Town marina (ST18) as seen in map, listed from west to south along the South African coast, with the abbreviated locality name given in brackets.

Simons Town marina (ST18)	
	
Coordinates	S 34°11'30.5"; E 18°26'00.3"
Survey period	April 2018
Biogeographic region	Cold temperate
Habitat type	Infratidal
Condition of locality	Anthropogenically altered
Water temperature (average for survey period)	16.6 °C
Salinity	35 ppt
Number of fish sampled	n = 12
Chapters included	Chapters 4; and 6

Table 2.4: Characteristics of Old harbour Hermanus (Herm19) as seen in map, listed from west to south along the South African coast, with the abbreviated locality name given in brackets.

Old harbour Hermanus (Herm19)	
	
Coordinates	S 34°25'15.9"; E 19°14'37.6"
Survey period	October 2019
Biogeographic region	Warm temperate
Habitat type	Infratidal
Condition of locality	Anthropogenically altered
Water temperature (average for survey period)	16.7 °C
Salinity	35.4 ppt
Number of fish sampled	n = 24
Chapters included	Chapters 3; 4; and 6

Table 2.5: Characteristics of Tsitsikamma (Tsi19; Tsi20) as seen in map, listed from west to south along the South African coast, with the abbreviated locality name given in brackets.

Tsitsikamma (Tsi19; Tsi20)	
	
Coordinates	S 34°01'15.2"; E 23°52'43.2"
Survey period	March 2019; March 2020
Biogeographic region	Warm temperate
Habitat type	Intertidal
Condition of locality	Natural
Water temperature (average for survey period)	19.9 °C
Salinity	35 ppt
Number of fish sampled	n = 10; respectively from each survey
Chapters included	Chapters 3; 4; 5; 6; and 7

Table 2.6: Characteristics of Jeffery's Bay (Jeff19) as seen in map, listed from west to south along the South African coast, with the abbreviated locality name given in brackets.

Jeffery's Bay (Jeff19)	
	
Coordinates	S 34° 1' 35.0004"; E 24° 55' 50.9982"
Survey period	March 2018
Biogeographic region	Warm temperate
Habitat type	Intertidal
Condition of locality	Natural
Water temperature (average for survey period)	19.9 °C
Salinity	35 ppt
Number of fish sampled	n = 10
Chapters included	Chapter 3

Table 2.7: Characteristics of Chintsa (Chin19; Chin20) as seen in map, listed from west to south along the South African coast, with the abbreviated locality name given in brackets.

Chintsa (Chin19; Chin20)	
	
Coordinates	S 32°50'11.5"; E 28°07'01.2"
Survey period	March 2019; October 2020
Biogeographic region	Warm temperate
Habitat type	Intertidal
Condition of locality	Natural
Water temperature (average for survey period)	22.6 °C
Salinity	36.4 ppt
Number of fish sampled	n = 10; respectively from each survey
Chapters included	Chapters 3; 4; 5; 6; and 7

2.2 Field methods

During each survey a minimum of 10 fish were collected using small fyke net traps (20 mm mesh size), with frozen squid and sardines as bait, due to their non-invasive nature in reducing harm to the fish. Fish were sampled at random (with no size or sex preference), removed from the traps, placed in individual aerated bags with water from the collection site, and kept alive for transportation to the field laboratory (Figure 2.2). At the field laboratory, they were individually placed into clear aerated containers (with lids) along with the contents of the bag to allow for external parasites, such as gnathiid isopod juveniles, to complete feeding (~3 hours) and detach naturally (Welicky et al. 2017). Gnathiid isopods were also collected directly from the seawater after the detachment period by pouring the container water through a sieve (100 µm) and collecting the gnathiids with a broad mouth pipette (Grutter et al. 2020). The fish were then subjected to an external parasitological examination using adapted methods (from Scholz et al. 2018 and Klimpel et al. 2019), starting by first inspecting the skin and fins for ectoparasites, followed by collection of blood and fin clippings for molecular identification. Each fish was measured (total and standard length), weighed, photographed and subsequently humanely killed by means of percussive stunning and severing the spinal cord behind the head (AnimCare Ethics approved SOP NWU-00267-17-A5). Eyes, gills, and opercula were screened for parasites before dissection, whereafter muscle tissue and internal organs (body cavity, mesentery, intestine, stomach, gallbladder, liver, and reproductive organs) were placed in the appropriate solutions (either fresh seawater from the site of collection, for the gills, or saline water as indicated in Klimpel et al. 2019) and fully examined for endoparasites under a stereomicroscope in the field. Where possible, photomacrographs of live parasites were also captured using a Zeiss Stemi 305 compact stereo microscope with an AxioCam camera and ZEN lite imaging software (Zeiss, Oberkochen, Germany) (see Chapter 3). Observed parasites were collected, enumerated, fixed, and preserved according to standard methods for each parasite group, following adapted guidelines (from Scholz et al. (2018) and Klimpel et al. (2019)).

Methods described by Klimpel et al. (2019) were adapted for external and internal parasitological examination. Externally, eyes, gills and operculum were screened prior to dissection, whereafter internal organs (body cavity, mesentery, intestine, stomach, gallbladder, liver and reproductive organs) and muscle tissue were placed in the appropriate solutions and examined for endoparasites under a Zeiss Stemi 305 compact stereo microscope with an AxioCam camera (Zeiss, Oberkochen, Germany). Photographs and video clippings of parasites were taken using Zen lite imaging Software (Zeiss, Oberkochen, Germany). Any parasites present were thereafter removed from the organs using fine needles, glass pipettes and forceps, cleaned and fixed in appropriate solutions (Klimpel et al. 2019). Fixed specimens were placed in respective, labelled tubes and preserved accordingly. All of the field collection data was written on a standardised field sheet for each fish and subsequently entered into an electronic database

held at the North-West University, Water Research Group (NWU-WRG). All collected material was stored in freezers at the NWU-WRG until further analyses and identification.

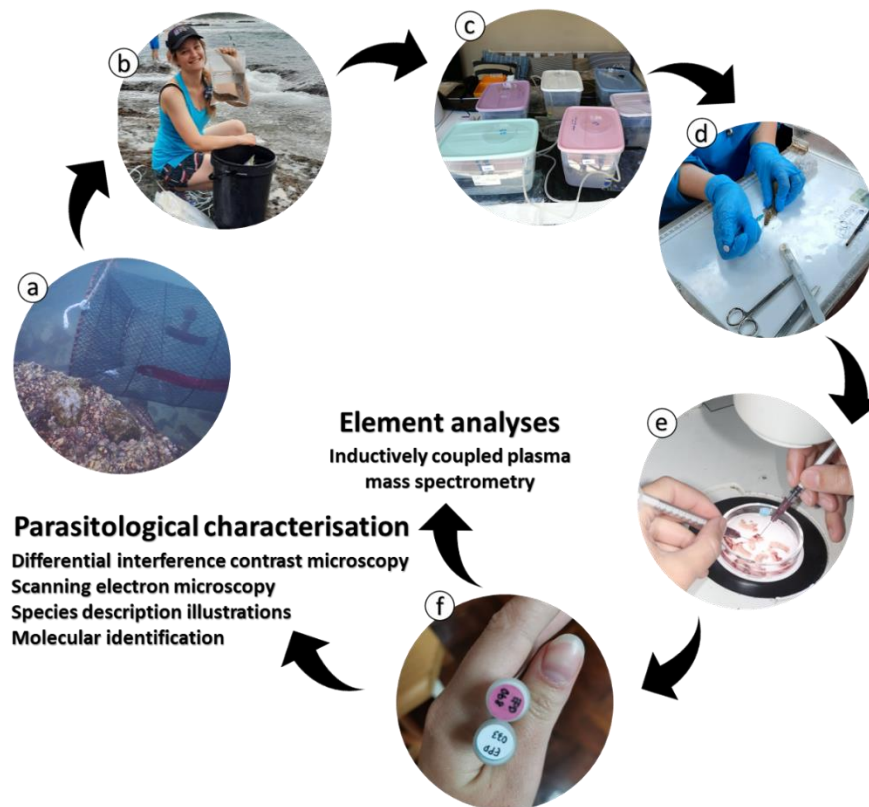


Figure 2. 2: Workflow of sampling procedure: a) collect Clinus superciliosus using baited traps; b) bag each fish individually for collection of ectoparasites; c) place each fish in aerated container to allow for ectoparasites (gnathiids, caligids, leeches) to detach naturally; d) full external inspection for ectoparasites, extract blood, note morphometrics of fish host, and start with necropsy; e) investigate each organ for parasites and remove with the aid of a field microscope; and f) sort the fixed and preserved morphotypes in separate tubes for identification and further analyses in the laboratory.

2.3 Laboratory methods

Preserved parasite specimens were grouped according to morphological differences, and prepared for further morphological and molecular analyses. If possible, the principle of ten specimens per morphotype was followed for species identification: one holotype, three paratypes, two specimens for scanning electron microscopy (SEM), two specimens for molecular analyses, and two for further staining and dissection. Type and voucher specimens will be deposited in museum collections following submissions of species description for publication. Taxa names followed the World Register of Marine Species (WoRMS Editorial board, 2020), where all of the parasites were identified up to the lowest taxonomic level possible.

DNA isolation was done by extracting an excised piece or the whole specimen, depending on their size, using various extraction kits for each species (see details in Chapter 3 and Chapter 4). The respective primers and PCR conditions for the molecular extractions are presented below (Table 2.8, Figure 2.3), while further details regarding the molecular and morphological characterisation of each parasite group are discussed in Chapter 3.

Table 2. 8: Selected genes and primers used for parasite specific molecular extractions.

Primer name	Sequence	Reference
MYXOZOA		
18S		
SPH (F)	5'-GTT ACC ATT GTA GCG CGCGT-3'	Eszterbauer and Székely 2004
SPH (R)	5'-ACT CGT TGG TAA GGTAGT GGC T-3'	Eszterbauer and Székely 2004
Myxsp (F)	5'-TTC TGC CCT ATC AAC TWG TTG-3'	Fiala 2006
Myxsp (R)	5'-GGT TTC NCD GRG GGM CCA AC-3'	Fiala 2006
Kud4R (R)	5'- GTG CTT TAT TCA AGG CAC-3'	Whipps et al. 2003
Myx4R (R)	5'-CTG ACA GAT CAC TCC ACG AAC-3'	Hallett and Diamant 2001
CESTODA		
18S		
Worm A (F)	5'–GCGAATGGCTCATTAATCAG–3'	Littlewood and Olson 2001
Worm B (R)	5'–CTTGTTACGACTTTTACTTCC-3'	
28S		
LSU5 (F)	5'-TAGGTCGACCCGCTGAAYTTAAGCA-3'	Scholz et al. 2013
1500R (R)	5'-GCTATCCTGAGGGAACTTCG-3'	
HIRUDINEA		
COI		
LCO4190 (F)	5'-GGTCAACAAATCATAAAGATATTGG-3'	Ravi and Yahaya 2017
HCO2198 (R)	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	
COPEPODA		
COI		
LCO4190 (F)	5'-GGTCAACAAATCATAAAGATATTGG-3'	Folmer et al. 1994; Hayes et al. 2021
HCO2198 (R)	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	
18S		
18S-E (F)	5'-CCGAATTCGTCGACAACCTGGTTGATCCTGCCAGT-3'	Neethling and Avenant-Oldewage 2020
NEM18SR (R)	5'-GGGCGGTATCTGATCGCC-3'	
28S		
28SF (F)	5'-ACAACTGTGATGCCCTTAG-3'	Song et al. 2008
28SR (R)	5'-TGGTCCGTGTTTCAAGACG-3'	
(F) – forward primer (R) - reverse primer		

(F) – forward primer
(R) - reverse primer

The parasite samples for the present study were freshly collected and preserved for the various taxonomic characterisation methods, applied in combination where possible, to aid in elucidating the role of parasites in the ecosystem. Detailed methods applicable the various analysis of each chapter will be discussed subsequently.

2.3.1 PCR thermocycle protocols

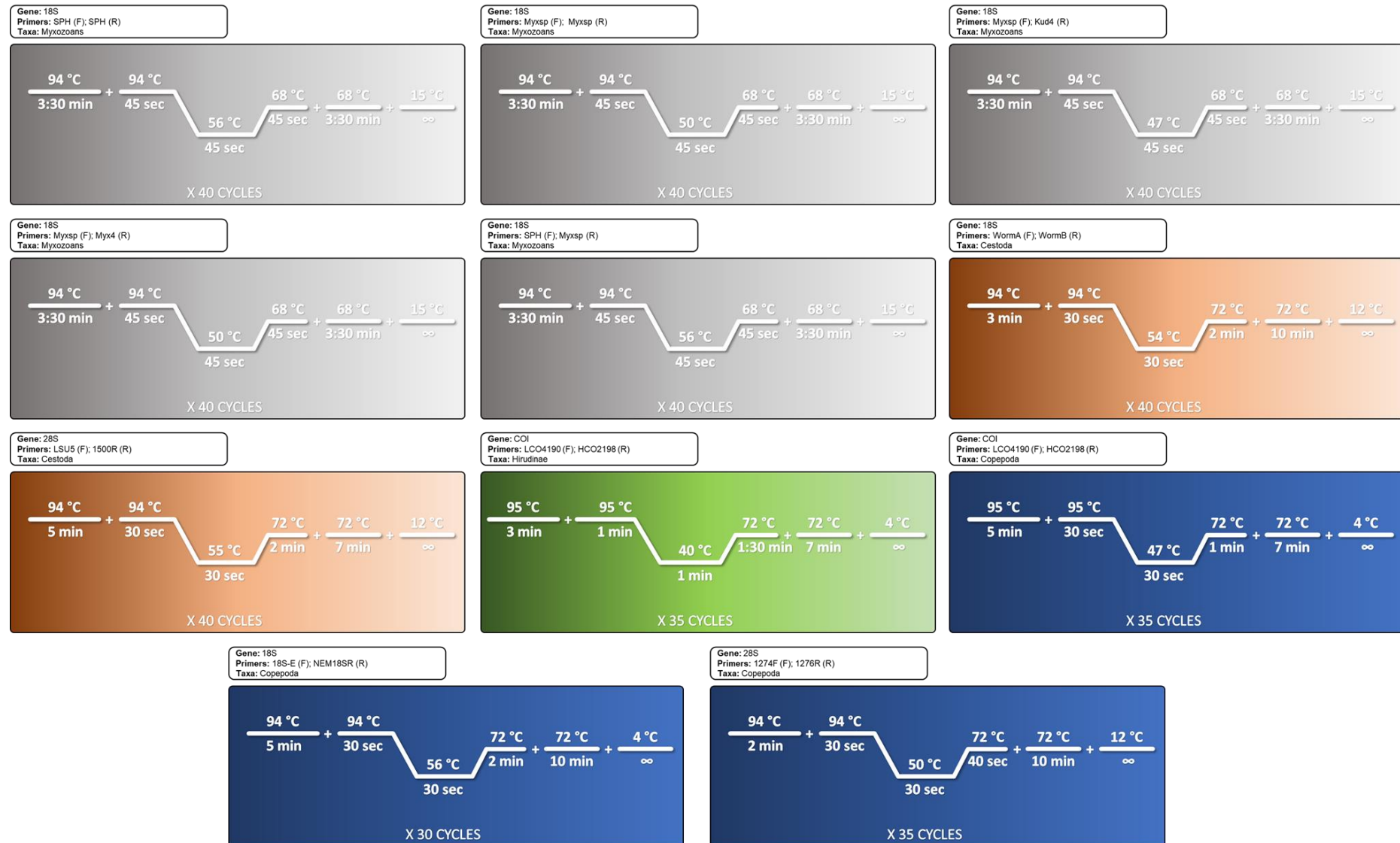
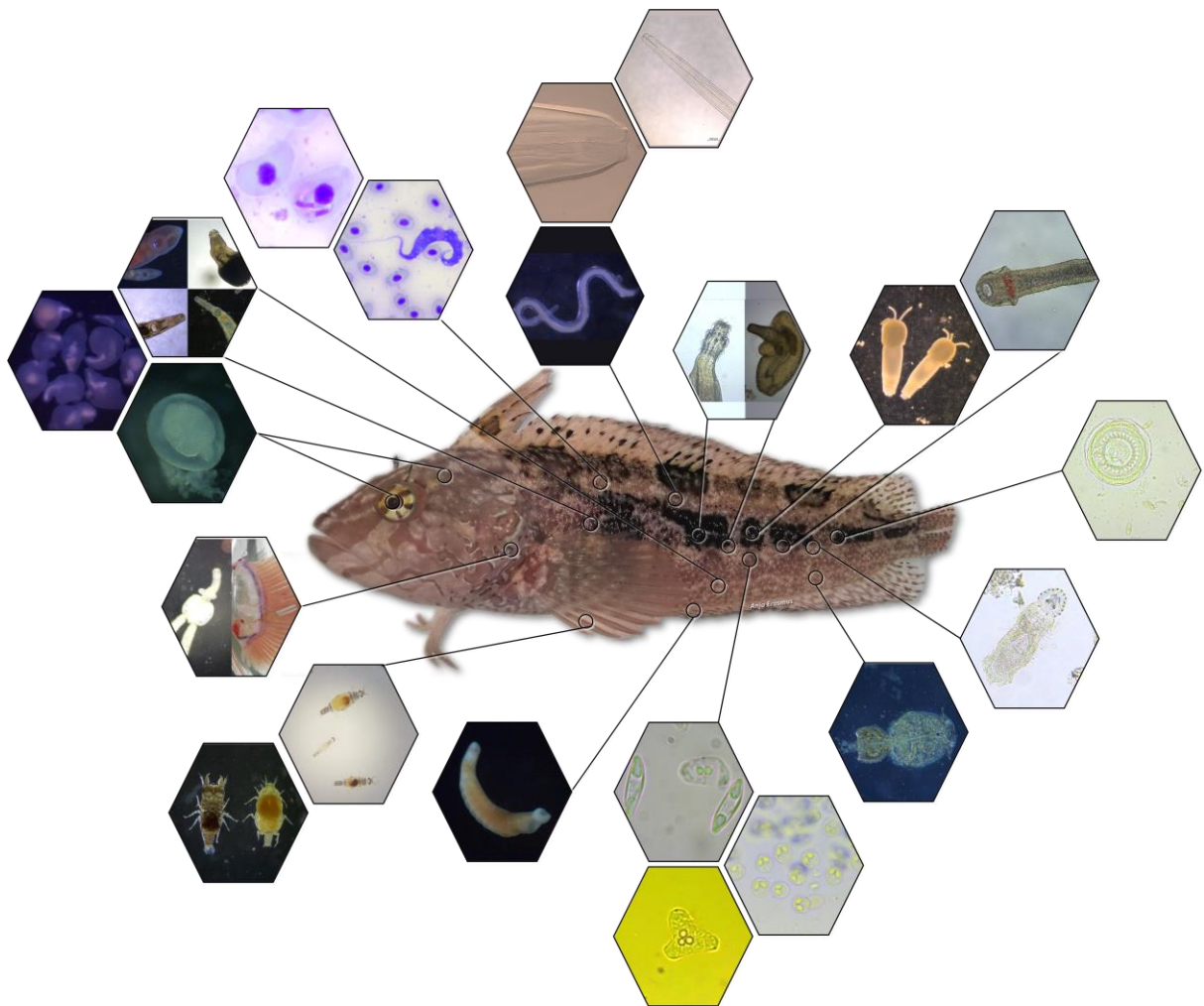


Figure 2. 3: PCR thermocycle protocols of the various genetic markers used to amplify the extracted DNA from the various taxa (indicated in different colours).

CHAPTER 3

Parasite biodiversity of *Clinus superciliosus*



3.1 Introduction

Marine biodiversity research in South Africa follows the trend with vertebrates as the most speciose to macro algae as least speciose taxa with regards to documented reports and overall richness (Nunkoo 2019). From a total of 12,915 species, only 2.7% account for parasites (acanthocephalan, cestode, trematode, hirudinean, monogenean, myxozoan) (Griffiths et al. 2010). The lack of parasite species reported from South Africa has been attributed to the paucity of local experts (Smit and Hadfield 2015; Nunkoo 2019). The majority of parasite studies in South Africa have been taxonomic descriptions, and only since the 1980s has more complex research involving parasite-host interactions, distributions, and aspects of ecology been considered. In one of the most recent developments in South African marine ichthyology research, parasites are being considered as bioindicators of pollution, where the sensitivity or tolerance to pollution events and exposures are assessed. To aid in this need for more complex research, one must first consider the taxa involved.

A common intertidal klipfish, *Clinus superciliosus* (Linnaeus 1758) (Clinidae), serves as a great model organism for the present study, due to its feeding habits, the fact that they are resident fish and are found along a wide range of the South African coast. These factors will aid in reflecting the parasite community at each specific habitat. To date, the parasite fauna of *C. superciliosus* has not been fully investigated. A total of 12 confirmed metazoan species have been reported from this host in the past, as well as three protozoan species (see Chapter 1 Table 1.1). During the present study, further investigation on the complete parasite community of *C. superciliosus* was conducted by incorporating various localities along the South African coasts and by using an integrative taxonomic approach that combines various morphological and molecular techniques, where possible. We hypothesise that host-specific parasite sampling will yield more parasite species than parasite-specific sampling from previous studies. Thus, this section of the present study aimed to characterise parasites infecting the common intertidal klipfish, *C. superciliosus*, using morphological and molecular methods. Due to the sheer diversity and number of parasite species collected and the lack of both morphological and molecular data on similar species from this region for comparison, it was not possible to identify all of them to species level. However, future outputs from this thesis will include full descriptions of species identified herein as possibly new to science, as well as new species and host records. Since this fish host is endemic to southern Africa, it can be assumed that many of the parasite species collected during the present study are also endemic and therefore may be new to science. Thus, to test the above stated hypothesis, the aim of this chapter was to determine the metazoan parasite taxa community of *C. superciliosus* by providing a comprehensive list of metazoan parasite taxa associated with selected localities along its South African distribution. This will be achieved by providing short morphological and, where possible, phylogenetic analysis for identification to the lowest taxonomic level possible. These novel data will contribute to the marine parasite diversity of South

Africa, elucidate the role of *C. superciliosus* in the transmission of parasite species and establish their host-range and distribution of newly discovered species.

3.2 Materials and methods

3.2.1 Study area

The present study forms part of a larger project investigating the marine hidden diversity, particularly parasites, along the South African coast, inside and outside Marine Protected Areas. *Clinus superciliosus* ($n = 121$) was collected within its distribution range at various localities from the west to the south coast of South Africa (Figure 3.1). These sampling localities are all unique in their own way owing to the species that occur here because of the highly dynamic nature of the coast (Goble et al. 2014; Wepener and Degger 2019). Details regarding these sampling localities are fully described in Chapter 2.

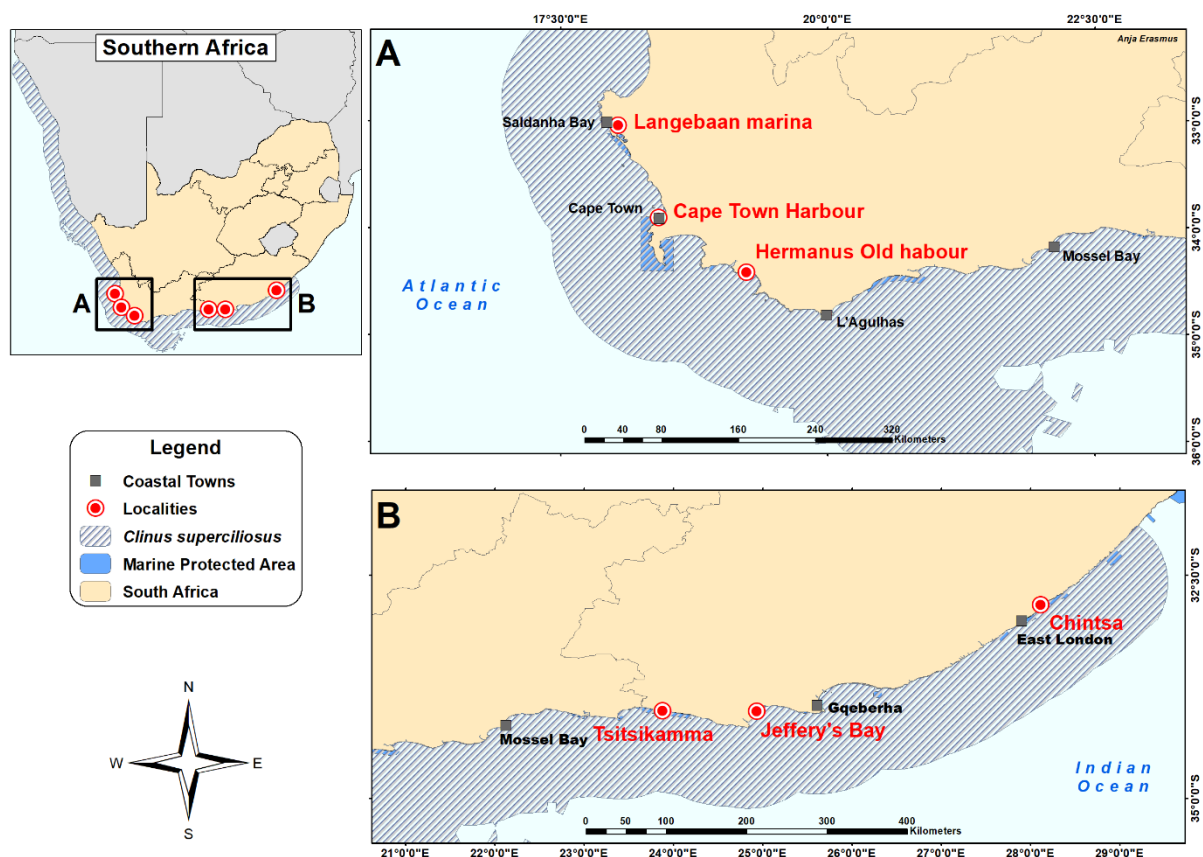


Figure 3. 1: Study map of sampling localities where *Clinus superciliosus* was collected for parasitological surveys. Species distribution data was obtained from Holleman et al. (2014); GBIF.org (2020).

3.2.2 Field collection

Fish ($n = 121$) and associated parasites were collected using the sampling procedure described in Chapter 2, with the necessary permits and ethical approval.

3.2.3 Laboratory process

3.2.3.1 *Morphological analysis*

Further investigation of representative samples of each taxon was done using either the Zeiss Stemi 305 compact stereo microscope with an AxioCam camera (Zeiss, Oberkochen, Germany), the Nikon Eclipse Ni differential interference contrast (DIC) with phase-contrast light microscopy (Nikon Instruments, Tokyo, Japan) or Phenom Pro Desktop scanning electron microscopy (SEM) (ThermoScientific, Waltham, Massachusetts, USA) to differentiate between key morphological characteristics. The z-dimensional stacking (z-stack) function was used to examine the different structures by using DIC micrographs of temporary mounts. In addition, some specimens were prepared for SEM, cleaned and dehydrated using a graded ethanol series and placed in hexamethyldisilazane (HMDS) for ± 2 h, or until dry. Specimens were then mounted onto an aluminium stub using glue or carbon tape and sputter-coated with gold/palladium (SPI-MODULE™ Sputter Coater; Structure Probe, Inc.), whereafter photomicrographs were taken of the characteristic structures to aid in morphological identification. Morphometric measurements were obtained where possible using ImageJ software v1.53j (Schindelin et al. 2012) (available at <https://imagej.net/software/fiji/>). Ratios and measurements were based on the maximum values of each measured structure, while all proportional values were rounded to two decimal places. Metric data were indicated as a range followed by the mean. Digital species description illustrations were made from z-stacked micrographs, Adobe Illustrator CC v25.4.1, Adobe Photoshop CC v25.0 and a WACOM Intuos Pro v6.3.44-1 (Saitama, Japan) drawing tablet with a grip pen. Species descriptions were prepared in DELTA (DEscriptive Language for TAXonomy) (available at <https://www.delta-intkey.com/>) based on a comparative character set.

Myxozoan samples were screened directly in the field using a 100X oil immersion objective on a Zeiss Axioskop photomicroscope with AxioCam camera (Zeiss, Oberkochen, Germany) and preserved in 70–90% ethanol for subsequent examination in the laboratory. Whereafter a Olympus microscope with Nomarski interference contrast was used to photograph morphologically different specimens. DNA was extracted from a random selection of samples within the different sites of infection. A description of standard morphological characters (presented in the section below) was based on fixed specimens and images. The digenean parasites were processed as part of the MSc project of Vermaak (2020). For identification to the lowest possible level, selected digenean morphotypes were stained with Mayer's haematoxylin and mounted with Dammar gum as permanent slides (Vermaak 2020). Monogeneans were mounted in the field using glycerine ammonium picrate (GAP) (Malmberg 1970). Acanthocephalans with everted proboscis were fixed in 80% ethanol, and viewed as temporary mounts in lactophenol (200 mL lactic acid, 200 g/L phenol, 400 mL glycerol and 200 mL deionized water) (Amin and Christison 2005). Nematodes and everted tentacles of trypanorhynchid cestodes were fixed in the field using 80% ethanol and examined as semi-permanent mounts in lactophenol and glycerol, respectively, to determine if there are any species level differences

(Moravec et al. 2006; Amin 2013; Moravec et al. 2016; Caira and Jensen 2017). Other species of cestode specimens were put through a hydration series of graded ethanol and stained using Delafield's haematoxylin, thereafter they were dehydrated in a series of graded ethanol, cleared in clove oil and mounted permanently in Canada balsam for identification (Oosthuizen et al. 2020; van der Spuy et al. 2020). Annelida were fixed in the field and placed in a lactophenol solution prior to examination in the laboratory (Ravi and Yahaya 2017) whereafter characterisation of the species was done following 25 characters defined by Williams and Bureson (2006). Copepods were cleared and stained in lactic acid with lignin pink and mounted as a temporary preparation in lactophenol on a cavity slide with a cover slip for observation using a DIC microscope (Kabata 1979; Boxshall and Halsey 2004; Hayes et al. 2021). As mentioned previously, gnathiid isopod juveniles were kept alive, for up to 28 days post feeding, in the NWU marine facility following the protocol by Davies and Smit (2001) and Grutter et al. (2020) until the final moult, in order to ensure positive identification of the adult male (Smit et al. 2002; Hadfield et al. 2008).

3.2.3.2 Molecular analysis

Where possible, considering the number of individuals present per morphotype, specific gene DNA were extracted from the entire small specimen or from excised parts of larger specimens, by making use of the various methods described in detail below. Comparable sequences for the majority of parasite groups were limited, thus hindering definitive and accurate conclusions for species identification. For details regarding the primers and PCR conditions see Chapter 2.

Extraction and sequence generation

Small subunit (SSU) rDNA sequences of the various genera were extracted using OneTaq Hot Start Quick Load Master Mix (NEB), 0.5 μ M of each primer, 1 μ l DNA in a total reaction volume of 20 μ l. Amplification was done in a PeqStar 2x Cyclor (VWR) (for Myxozoa). Utilising a KAPA Express Extraction Kit (Kapa Biosystems, Cape Town, South Africa), as well as a PCRBiosystems Rapid DNA Extraction Kit (PCRBiosystems available from Analytical Solutions, Randburg, South Africa), the various DNA were extracted and amplified. The PCRBio kit was modified to 10 μ l of lysis buffer and 5 μ l of protease containing buffer added to the sample. Further, the reaction was diluted with 450 μ l molecular grade water instead of the 900 μ l as per the manufacturer's recommendation. The relevant information regarding the primers used for the various species are listed in Chapter 2: Table 2.2 and any specifics regarding the parasite groups will be discussed in detail in each of the following sections. Amplified PCR products were visualised using agarose gel electrophoresis, as EZ-Vision Bluelight DNA dye (VWR AMRESCO Life Science available from Inqaba Biotechnical Industries Pty. Ltd.) added to 1% agarose gel, and when successful, sent for cleaning, purification and sequencing to a commercial sequencing company, Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa). The detailed polymerase chain reaction (PCR) thermocycle protocols for each gene and primer set are presented in Chapter 2 Figure 2.3.

Forward and reverse sequences were assembled and edited with Geneious v11.1.4 bioinformatics software (Biomatters, Auckland, New Zealand).

Phylogenetic analyses

All of the novel sequences were verified using BLAST (NCBI, <https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to validate the taxa identity, and a basic local alignment was performed with other sequences in GenBank. As a general rule, newly generated sequences were aligned with sequences from the same genus of the respective species, when available, otherwise with species within the same family, based on the amplified gene. In addition, an outgroup was selected based on literature relevant to the genus or family. In Geneious, MUSCLE or ClustalW was used to align and assemble the selected sequences, and the best nucleotide substitution model (specifically Akaike information criterion, AIC) was determined statistically using jModelTest 2.1.4 software (Guindon and Gascuel 2003; Darriba et al. 2012). Appropriate statistical methods will be discussed in detail per parasite group below.

In order to determine the phylogenetic position of the various species, Bayesian Inference (BI) phylogenetic trees were constructed using MrBayes parameters in CIPRES Science Gateway v3.3 (available at <https://www.phylo.org/>), and maximum likelihood (ML) trees were constructed using PhyML v3.0 (available at <http://www.atgc-montpellier.fr/phyml/>) (Guindon et al. 2010; Miller et al. 2010). Nodal support for ML analyses were estimated at 100 bootstrap repetitions. Phylogenetic trees for BI and ML were edited and visualised in FigTree v1.4.3 software (Rambaut 2012). Pairwise genetic distance matrices (p-distances) were calculated in MEGA v11 (Tamura et al. 2021).

3.2.4 Data analyses

For the purpose of this chapter, number of infected fish ($n = 93$), overall prevalence ($P \%$), mean abundance (MA) and mean intensity (MI) were calculated per species across all of the localities (Bush et al. 1997; Poulin 2015):

$$\begin{aligned} \text{Prevalence } (P \%) &= \frac{\text{no. of infected fish}}{\text{total no. of fish examined}} \times 100 \\ \text{Mean abundance } (MA) &= \frac{\text{no. of parasites}}{\text{no. of fish examined}} \\ \text{Mean intensity } (MI) &= \frac{\text{no. of collected parasites}}{\text{total no. of infected fish}} \end{aligned}$$

3.3 Results

From the species known to infect *C. superciliosus*, only nine metazoan parasites were collected during the present study, as well as some of the protozoan parasites. A possible 43 parasite taxa were collected and identified, with the majority as new host records and some are even new to science. One of these novel species has been described in Chapter 4, using an integrated approach to taxonomy.

3.3.1 Discovery, diversity, distribution and site of infection

The most species rich phylum reported in the present study is Platyhelminthes, with the class Trematoda including more than 14 different species (Table 3.1). A relatively high diversity of Acanthocephala and Nematoda was also reported, since no species have been described as parasitising clinid fish prior to this study.

Table 3. 1: Sampled diversity including quantitative descriptors of parasite populations of Clinus superciliosus. SOI – Site of infection; (in order of appearance: LM19 – Langebaan marina 2019; CT19 – Cape Town harbour 2019; Her18 – Hermanus 2018; Tsi19 – Tsitsikamma 2019; Tsi20 – Tsitsikamma 2020; Chin19 – Chintsa 2019; Chin20 – Chintsa 2020)

Parasite taxa	SOI	Life cycle stage	Locality	No. of infected fish (n=93)	Prevalence (P%)	Abundance (n)	Mean Abundance (MA)	Mean Intensity (MI)
Cnidaria								
<i>Myxidium</i> sp.	Gallbladder	H	LM19; Chin19	2	2.2	-	-	-
<i>Henneguya</i> sp.	Eye	H	LM19; Tsi19; Chin19	10	10.8	-	-	-
<i>Myxobolus</i> sp.	Eye (cyst)	H	LM19; CT19; Tsi19; Chin19	13	14.0	-	-	-
<i>Ceratomyxa</i> spp.	Gallbladder	H	LM19; CT19; Tsi19; Chin19; Tsi20; Chin20	62	66.7	-	-	-
<i>Sphaeromyxa clini</i>	Gallbladder	H	LM19; CT19; Chin19	24	25.8	-	-	-
<i>Kudoa</i> sp.	Urinary bladder wall	H	LM19; CT19	2	2.2	-	-	-
Platyhelminthes								
<i>Coitocaecum capense</i>	Intestine	HA	LM19; Her18; Tsi19; Tsi20	19	20.4	82	0.20	4.32
<i>Coitocaecum</i> sp. 1	Intestine	HA	Her18	1	1.1	1	0.01	1.00
<i>Coitocaecum</i> sp. 2	Intestine	HA	Chin19	1	1.1	1	0.01	1.00
<i>Coitocaecum</i> sp. 3	Intestine	HA	LM19; Her18; Tsi19; Tsi20; Chin19; Chin20	5	5.4	34	0.05	6.80
<i>Helicometra</i> sp. 1	Intestine	HA	Her18	33	35.5	175	0.35	5.30
<i>Helicometra</i> sp. 2	Intestine	HA	LM19	1	1.1	1	0.01	1.00
<i>Proctoeces</i> sp.	Intestine	HA	Tsi19; Chin19	5	5.4	10	0.05	2.00
Zoogonidae gen. sp.	Intestine	HA	Tsi20; Chin19; Chin20	6	6.5	9	0.06	1.50
<i>Cardiocephaloides</i> sp.	Eyes/ Brain	HL*	LM19; Her18; Tsi19; Tsi20; Chin19; Chin20	21	22.6	170	0.23	8.10
<i>Cardiocephaloides physalis</i>	Eyes	HL*	Her18	1	1.1	3	0.01	3.00
Hemiuridae gen. sp. 1	Gonads/ Ovaries/ Intestine	HA	Tsi19; Tsi20; Chin19; Chin20	22	23.7	61	0.24	2.77
Hemiuridae gen. sp. 2	Intestine	HA	LM19	1	1.1	4	0.01	4.00
<i>Stephanostomum</i> sp.	Body cavity/ Intestine	HL*	Chin19; Chin20	4	4.3	5	0.04	1.25

Table 3. 1 (continued)

Parasite taxa	SOI	Life cycle stage	Locality	No. of infected fish (n=93)	Prevalence (P%)	Abundance (n)	Mean Abundance (MA)	Mean Intensity (MI)
<i>Dollfusotrema</i> sp.	Eyes/ Brain/ Body cavity/ Liver/ Muscle tissue/ Skin/ Gills/ Heart/ Gallbladder/ Gonads/ Intestine	HL*	LM19; Chin20	21	22.6	4645	0.23	221.19
<i>Gyrodactylus</i> sp.	Mucus	M	LM19; CT19	18	19.4	79	0.19	4.39
<i>Nybelinia</i> sp.	Intestine/ Mesentery/ Body cavity	HL	Tsi19; Chin19; Chin20	12	12.9	87	0.13	7.25
<i>Acanthobothrium</i> sp.	Intestine	HL	Chin19; Chin20	18	19.4	265	0.19	14.72
Acanthocephala								
Polymorphidae sp.	Mesentery/ Intestine/ Liver/ Body cavity	HL	LM19; Tsi19; Tsi20; Chin19; Chin20	28	30.1	102	0.30	3.64
Arhythmacanthidae gen. sp. 1	Intestine	HA	LM19; Chin20	2	2.2	2	0.02	1.00
Arhythmacanthidae gen. sp. 2	Intestine	HA	Her18	5	5.4	8	0.05	1.60
Rhadinorhynchidae sp.	Intestine	HA	Chin19; Chin20	1	1.1	1	0.01	1.00
Annelida								
<i>Pterobdella arugamensis</i>	Fins/ Skin	M	Tsi19; Tsi20; Chin19; Chin20	8	8.6	8	0.09	1.00
Piscicolidae gen. sp.	Fins/ Skin	M	LM19	2	2.2	3	0.02	1.50
Nematoda								
<i>Anisakis</i> Type II	Intestine/ Gallbladder	HL	LM19	5	5.4	30	0.05	6.00
<i>Cucullanus</i> sp.	Intestine	HA	LM19	7	7.5	23	0.08	3.29
Rhabdochonidae sp.	Intestine	HA	LM19	1	1.1	2	0.01	2.00
Ascarididae sp.	Intestine	HA & HL	LM19	2	2.2	3	0.02	1.50
Camallanidae sp.	Intestine	HL	Chin19	1	1.1	1	0.01	1.00
Arthropoda								
<i>Caligus mortis</i>	Skin	M	LM19	3	3.2	5	0.03	1.67
<i>Caligus</i> sp.	Skin	M	Chin19; Chin20	4	4.3	6	0.04	1.50
<i>Allea</i> sp. A	Gills	M	LM19	8	8.6	23	0.09	2.88
<i>Gnathia africana</i>	Fins/ Skin	M	LM19; Tsi19; Tsi20	29	31.2	104	0.31	3.59
<i>Gnathia pilosus</i>	Fins/ Skin	M	Chin19; Chin20	5	5.4	10	0.05	2.00

* metacercariae; M – monoxenous; H – heteroxenous; HL – heteroxenous larval; HA – heteroxenous adult

3.3.2 Descriptions of parasite biodiversity

3.3.2.1 Characterisation of Myxozoa (Cnidaria)

Phylum:	Cnidaria Hatschek, 1888
Class:	Myxozoa Grassé, 1970
Order:	Bivalvulida Schulman, 1959
Family:	Myxidiidae Lom & Noble, 1984
Genus:	<i>Myxidium</i> Buetschli, 1882
Species:	<i>Myxidium</i> sp.

Material examined

Molecular extraction was complicated by concurrent infections, however, the gallbladders of two fish (one from Langebaan marina, and one from Chintsa) were found infected with a species of *Myxidium* (2.2% prevalence).

Morphological characteristics

The spores ($n = 2$) (Figure 3.2) were spindle-shaped with rounded ends, measuring 20.4–21.2 (20.8) μm in length, 17.0–18.7 (17.8) μm in width. The two polar capsules were approximately round and equal in size, measuring 7.9–10.5 (9.2) μm long and 7.8–9.4 (8.6) μm wide. The polar filament coils were not visible during examination. Sporoplasm filling the cavity opposite polar capsules.



Figure 3. 2: Photomicrograph of fixed *Myxidium* sp. taken with an Olympus microscope with Nomarski interference contrast. Scale bar: 10 μm ; Photo credit: Dr Daniel Grabner.

Phylogenetic characterisation

The resulting alignment was 1293 bp in length, with a great divergence (92.6–59.7%) within genus (Table 3.2). This led to the assumption that no species similar to the present study have been described and sequenced to date.

Table 3. 2: Evolutionary distances between species of *Myxidium* isolated from the 18S SSU gene region, including phylogenetic analysis, expressed as percent similarity (below diagonal) and *p*-distances (above diagonal). Newly generated sequence is indicated in the shaded row.

	<i>Myxidium</i> sequences	1	2	3	4	5	6	7	8	9	10	11
1	EPD077_ <i>Myxidium</i> sp. (gallbladder)		0.064	0.064	0.065	0.077	0.070	0.106	0.229	0.230	0.255	0.234
2	JN033230_ <i>Myxidium laticurvum</i>	92.6		0.049	0.044	0.055	0.044	0.082	0.192	0.182	0.212	0.215
3	DQ377711_ <i>Myxidium gadi</i>	92.5	94.1		0.005	0.027	0.038	0.075	0.194	0.190	0.219	0.219
4	DQ377707_ <i>Myxidium gadi</i>	92.4	94.6	99.5		0.032	0.041	0.080	0.187	0.183	0.215	0.218
5	DQ377702_ <i>Myxidium bergense</i>	91.1	93.3	97.1	96.7		0.051	0.079	0.200	0.192	0.221	0.213
6	GQ890672_ <i>Myxidium finnmarkicum</i>	90.3	93.0	94.2	93.8	92.7		0.069	0.204	0.194	0.230	0.233
7	DQ377708_ <i>Myxidium incurvatum</i>	88.1	90.4	91.8	91.3	91.3	90.5		0.210	0.196	0.219	0.213
8	X76639_ <i>Myxidium lieberkuehni</i>	63.2	69.8	69.9	70.2	69.3	70.3	68.2		0.209	0.243	0.254
9	DQ377697_ <i>Myxidium coryphaenoideum</i>	64.1	71.6	71.3	71.5	71.0	72.0	70.3	76.5		0.179	0.272
10	AJ582061_ <i>Myxidium truttae</i>	59.2	66.6	66.2	66.0	65.9	65.9	66.0	71.9	77.4		0.220
11	AJ582213_ <i>Myxidium giardi</i>	59.7	62.2	61.9	60.8	62.2	62.8	61.9	71.7	67.9	73.5	

Remarks

Myxidium Butschli 1882 is one of the most speciose genera of Myxosporidia consisting of both marine and freshwater species. The genus reportedly has 205 species described from fish, amphibians, and reptiles (Lom and Dykova 2006; Abdel-Baki 2009). Of the 205 species, only 12 have been described from Africa. It is expected that, with more sampling, a large number of *Myxidium* species will still be described from this region.

Genetic divergence of the specimens found in the present study with the most closely related sequence on GenBank was 92.2% (55 nt) similar. These results indicate that this is the first molecular characterisation of this species. Based on the morphological and molecular analysis, the specimens found in the gallbladders of the two fish belong to the genus *Myxidium*, however, more sampling is needed to confirm species level identification.

Family: Myxobolidae Thélohan, 1892
Genus: *Henneguya* Thélohan, 1892
Species: *Henneguya clini* Reed, Basson, Van As & Dyková 2007

Material examined

The eyes of ten *Clinus superciliosus* (from Langebaan marine, Tsitsikamma and Chintsa) were infected with specimens of *Henneguya* (10.8% prevalence).

Morphological characteristics

The spores ($n = 5$) (Figure 3.3) had a total length of 25.2–30.6 (28.2) μm , with ovoid to spherical body shape measuring 7.2–8.5 (7.8) μm in length and 4.2–6.4 (5.7) μm in width. The two polar capsules are located anteriorly, equal in size and approximately round to pyriform, measuring 3.2–4 (3.7) μm long and 2.3–2.8 (2.6) μm wide. Three to four coils in the polar filaments were noted in the majority of the spores. Sporoplasm was situated behind the two polar capsules, filling the remaining cavity.

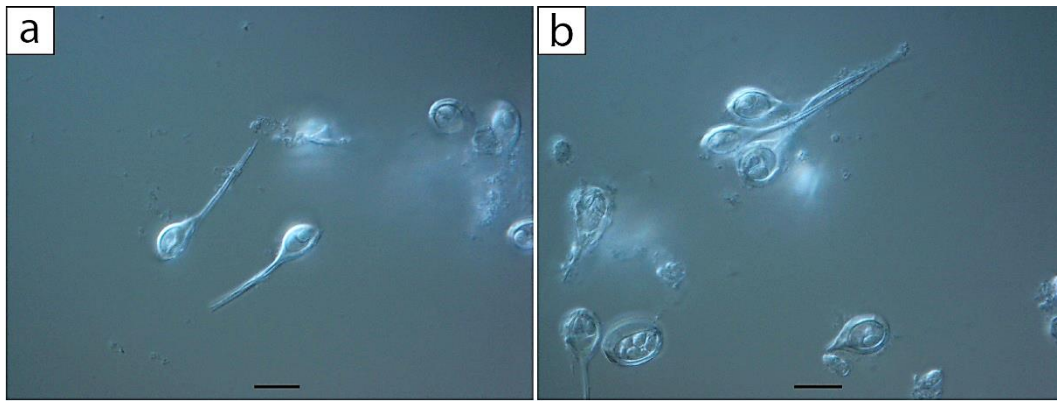


Figure 3. 3: Photomicrographs of fixed *Henneguya clini* taken with an Olympus microscope with Nomarski interference contrast. Scale bar: 10 μ m; Photo credit: Dr Daniel Grabner.

Phylogenetic characterisation

The resulting alignment was 1322 bp in length, indicating a great divergence within this genus, and the newly generated sequence the most closely related to *H. sinova* (98.7–59.7% similar) infecting a species of Blenniidae, which is a family of fishes closely related to Clinidae (Table 3.3).

Table 3. 3: Evolutionary distances between species of *Henneguya* isolated from the 18S SSU gene region, including phylogenetic analysis, expressed as percent similarity (below diagonal) and *p*-distances (above diagonal). Newly generated sequence is indicated in the shaded row.

	<i>Henneguya</i> sequences	1	2	3	4	5	6	7	8	9	10	11	12
1	EPD063_ <i>Henneguya clini</i> (eye)		0.005	0.063	0.139	0.195	0.205	0.176	0.174	0.187	0.183	0.221	0.270
2	KU726089_ <i>Henneguya sinova</i>	98.7		0.079	0.118	0.183	0.188	0.154	0.149	0.162	0.154	0.192	0.239
3	KP099967_ <i>Henneguya bicaudi</i>	92.0	90.3		0.070	0.170	0.167	0.097	0.067	0.087	0.069	0.065	0.211
4	MK007473_ <i>Henneguya aegae</i>	81.3	84.9	92.2		0.207	0.227	0.178	0.156	0.169	0.181	0.211	0.253
5	MH744155_ <i>Henneguya peruviansis</i>	76.6	78.1	79.3	74.8		0.238	0.039	0.170	0.177	0.168	0.195	0.260
6	AF031411_ <i>Henneguya salminicola</i>	74.5	77.2	80.3	75.1	71.5		0.209	0.223	0.222	0.215	0.266	0.259
7	MT415832_ <i>Henneguya multiradiatus</i>	79.2	81.6	87.9	78.1	94.5	74.7		0.143	0.149	0.137	0.168	0.238
8	AF021881_ <i>Henneguya exilis</i>	78.9	82.3	93.1	82.9	78.8	75.0	82.0		0.069	0.099	0.178	0.222
9	DQ673465_ <i>Henneguya gurlei</i>	77.1	80.7	90.9	81.8	77.8	75.2	81.0	92.7		0.100	0.183	0.237
10	MH230064_ <i>Henneguya quelen</i>	77.9	81.3	91.2	80.3	78.6	75.8	82.3	88.9	89.0		0.169	0.222
11	AF306794_ <i>Henneguya lesteri</i>	72.8	76.6	92.3	76.9	75.2	71.3	78.3	79.0	78.6	80.0		0.225
12	AF001579_ <i>Ceratomyxa shasta</i>	59.7	64.8	76.1	64.7	62.2	63.9	64.7	66.3	65.2	66.4	67.3	

Remarks

The identification of previously described species of *Henneguya* was made based on overlaps in dimensions and host family, as well as host tissue affinity (Wagner 2016). The specimens collected during the present study were morphologically similar to *Henneguya clini* Reed, Basson, Van As & Dykova, 2007, collected from the same host from the Cape south coast, however, they were found infecting different tissues. Size differences were attributed to measurements taken prior to fixation compared to fixed specimens from the present study. The newly generated sequence does not conform to any reference sequence on GenBank, indicating that this is the first sequence for the species morphologically identified as *Henneguya clini*.

Genus: *Myxobolus* Bütschli, 1882
Species: *Myxobolus* sp.

Material examined

Various forms of cysts were squashed on microscope slides and placed under a coverslip, followed by examination using a light microscope. Cysts were found behind the eyes and in the gills, as well as in muscle tissue of 13 of the fish (14% prevalence) from all of the localities investigated during the present study.

Morphological characteristics

The spores (n = 10) (Figure 3.4) had a total length of 10.3–13.7 (11.5) μm and width of 9.7–12.3 (10.7) μm at the widest region. The two polar capsules are located anteriorly, equally pyriform in shape being 4.5–5.8 (5) μm long and 2.9–3.5 (3.4) μm wide. No suture ridges were visible during the examination; however, four polar filament coils were observed in the majority of the specimens.

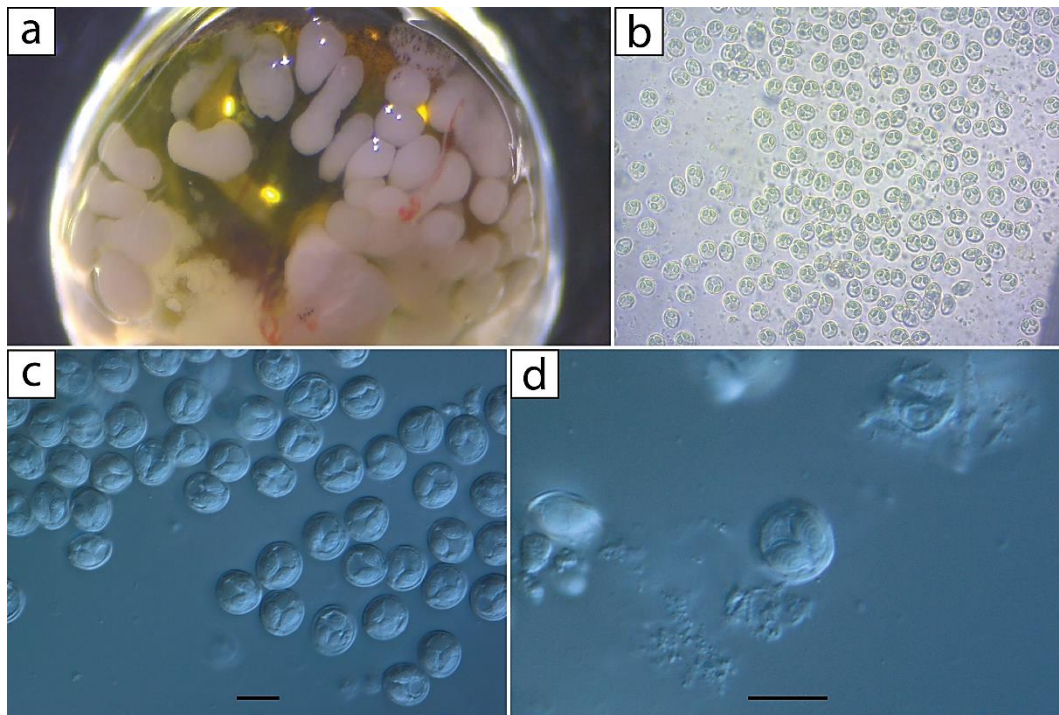


Figure 3. 4: a) Cysts formed behind *Clinus superciliosus* eyes, containing *Myxobolus* sp. b) Cyst squash of live *Myxobolus* sp. from behind the eye of *Clinus superciliosus*, c-d) Photomicrographs of fixed *Myxobolus* sp. taken with an Olympus microscope with Nomarski interference contrast. Scale bar (c, d): 10 μm ; Photo credit (c, d): Dr Daniel Grabner.

Phylogenetic characterisation

The resulting alignment was 1313 bp in length, indicating a great divergence (96.9–68.1%) within the genus (Table 3.4). This led to the assumption that no species similar to the present study have been described and sequenced to date.

Table 3. 4: Evolutionary distances between species of *Myxobolus* isolated from the 18S SSU gene region, including phylogenetic analysis, expressed as percent similarity (below diagonal) and *p*-distances (above diagonal). Newly generated sequence is indicated in the shaded row.

	Myxobolus sequences	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	EPD031_Myxobolus sp. (gallbladder)		0.008	0.098	0.102	0.107	0.127	0.124	0.124	0.124	0.131	0.128	0.182	0.186	0.169	0.191	0.143	0.252	0.329	0.323	0.291
2	AF085182_Myxobolus portucalensis	96.9		0.185	0.184	0.202	0.223	0.197	0.200	0.193	0.217	0.200	0.212	0.209	0.210	0.216	0.222	0.309	0.329	0.323	0.322
3	AB121146_Myxobolus cultus	87.8	80.2		0.002	0.179	0.204	0.176	0.176	0.169	0.196	0.180	0.196	0.198	0.194	0.196	0.203	0.294	0.312	0.308	0.306
4	HQ613409_Myxobolus cultus	87.4	80.3	99.8		0.180	0.207	0.176	0.176	0.168	0.198	0.179	0.196	0.198	0.193	0.196	0.204	0.295	0.312	0.309	0.306
5	U96492_Myxobolus cerebrealis	87.0	77.0	80.1	80.0		0.193	0.175	0.174	0.170	0.196	0.179	0.186	0.189	0.191	0.169	0.194	0.291	0.305	0.305	0.300
6	KP751908_Myxobolus mucosus	84.7	73.9	76.3	76.1	77.6		0.156	0.156	0.157	0.113	0.158	0.217	0.222	0.210	0.211	0.122	0.307	0.327	0.329	0.321
7	HQ613412_Myxobolus wulii	84.3	75.6	78.3	78.4	79.0	81.7		0.042	0.032	0.147	0.005	0.203	0.200	0.201	0.193	0.146	0.292	0.307	0.311	0.301
8	FJ710800_Myxobolus koi	84.3	75.3	78.4	78.3	79.1	81.7	95.6		0.044	0.150	0.045	0.207	0.202	0.205	0.199	0.148	0.293	0.312	0.317	0.306
9	HQ613411_Myxobolus pyramidis	84.3	75.9	78.9	79.0	79.4	81.5	96.7	95.5		0.147	0.034	0.205	0.201	0.199	0.183	0.151	0.294	0.309	0.315	0.303
10	GU574808_Myxobolus hearti	84.3	75.0	77.6	77.5	77.9	88.1	83.2	82.9	83.1		0.148	0.208	0.219	0.211	0.201	0.050	0.303	0.323	0.324	0.317
11	KY784580_Myxobolus wulii	83.9	75.4	78.0	78.1	78.6	81.4	99.5	95.4	96.5	83.1		0.207	0.204	0.205	0.197	0.147	0.291	0.305	0.309	0.299
12	AF378337_Myxobolus ichkeulensis	79.1	75.7	77.7	77.8	77.8	73.5	76.0	75.5	75.7	75.0	75.6		0.045	0.100	0.222	0.210	0.310	0.320	0.329	0.313
13	AF378341_Myxobolus spinacurvatura	78.7	76.5	78.0	78.1	77.9	73.5	76.6	76.3	76.5	74.5	76.2	94.7		0.096	0.220	0.221	0.311	0.319	0.327	0.313
14	AY129318_Myxobolus bizerti	80.3	76.5	78.5	78.6	77.9	74.9	76.7	76.3	76.9	75.4	76.3	89.0	90.0		0.205	0.204	0.306	0.323	0.327	0.316
15	U96495_Myxobolus squamalis	75.6	75.7	78.5	78.5	80.3	74.7	76.5	76.0	77.4	76.3	76.1	74.2	74.7	76.2		0.204	0.313	0.330	0.332	0.326
16	KY203795_Myxobolus sp. LP-2017	83.1	74.5	77.0	76.8	78.0	87.2	83.3	83.1	82.8	95.0	83.2	74.8	74.2	76.1	76.1		0.303	0.329	0.325	0.323
17	JF694785_Myxobolus groenlandicus	72.0	63.9	65.9	65.8	66.4	65.7	67.9	67.7	67.7	66.1	68.1	64.6	64.6	65.1	63.6	66.0		0.033	0.032	0.033
18	KX886731_Myxobolus aeglefini	64.3	61.6	63.7	63.7	64.5	63.3	66.2	65.6	65.9	63.7	66.4	63.2	63.6	63.2	61.6	63.2	96.4		0.021	0.000
19	KX886734_Myxobolus platessae	64.9	62.2	64.2	64.1	64.6	63.3	65.9	65.2	65.4	63.7	66.1	62.5	62.9	62.9	61.4	63.6	96.4	97.8		0.021
20	EU420055_Myxobolus albi	68.1	62.4	64.5	64.5	65.1	64.0	66.9	66.2	66.6	64.4	67.0	63.9	64.3	63.9	62.0	63.8	96.4	100.0	97.8	

Remarks

The morphological characters noted in the present study, as well as molecular results, correspond to the genus *Myxobolus*. There are currently no described species of *Myxobolus* infecting marine fish from South Africa. The most closely related species sequence available on GenBank is that of *Myxobolus portucalensis* Saraiva & Molnár, 1990, however it is only 96.9% similar to the newly generated sequence. Further morphological and molecular analyses are needed to confirm lower-level identification.

Family: Ceratomyxidae Doflein, 1899
Genus: *Ceratomyxa* Thélohan, 1892
Species: *Ceratomyxa* spp.

Material examined

During the present study, 62 fish (66.7% prevalence) sampled were found to be infected with *Ceratomyxa* spp. Morphologically distinct species were observed during examination, however, based on the molecular results, a simultaneous infection of multiple species of *Ceratomyxa* occurred that could not be linked to morphological differences.

Morphological characteristics

At least three morphologically different species were observed during the present study. Morphotype I (n = 1) was transversely elongate with a narrow crescent-shaped spore measuring 23.9 µm long and 9.4 µm wide. Two equally sized ovoid polar capsules were observed in the centre of the spore, along the sutural line, measuring 3.6 µm in length and 2.6 µm in width. Morphotype II (n = 1) was elongated with a narrow crescent-shaped spore slightly convex anteriorly, measuring 25.7 µm in length and 9.6 µm in width. Two equally sized polar capsules were observed in the mid-section of the spore, equally sized, approximately round and measuring 2.9 µm in length and 2.6 µm in width. The sporoplasm was noted to not fill the entire cavity of the spore. The suture line was noted across the midline of the spore. Morphotype III (n = 1) measured 24.3 µm long and 10.7 µm wide, transversely elongate, broader crescent-shaped and slightly convex anteriorly. The two polar capsules were observed in the mid-section of the spore, as equally sized, approximately round in shape measuring 2.8 µm in length and 2.3 µm in width. The sporoplasm was noted to fill the entire cavity, with no suture line visible.

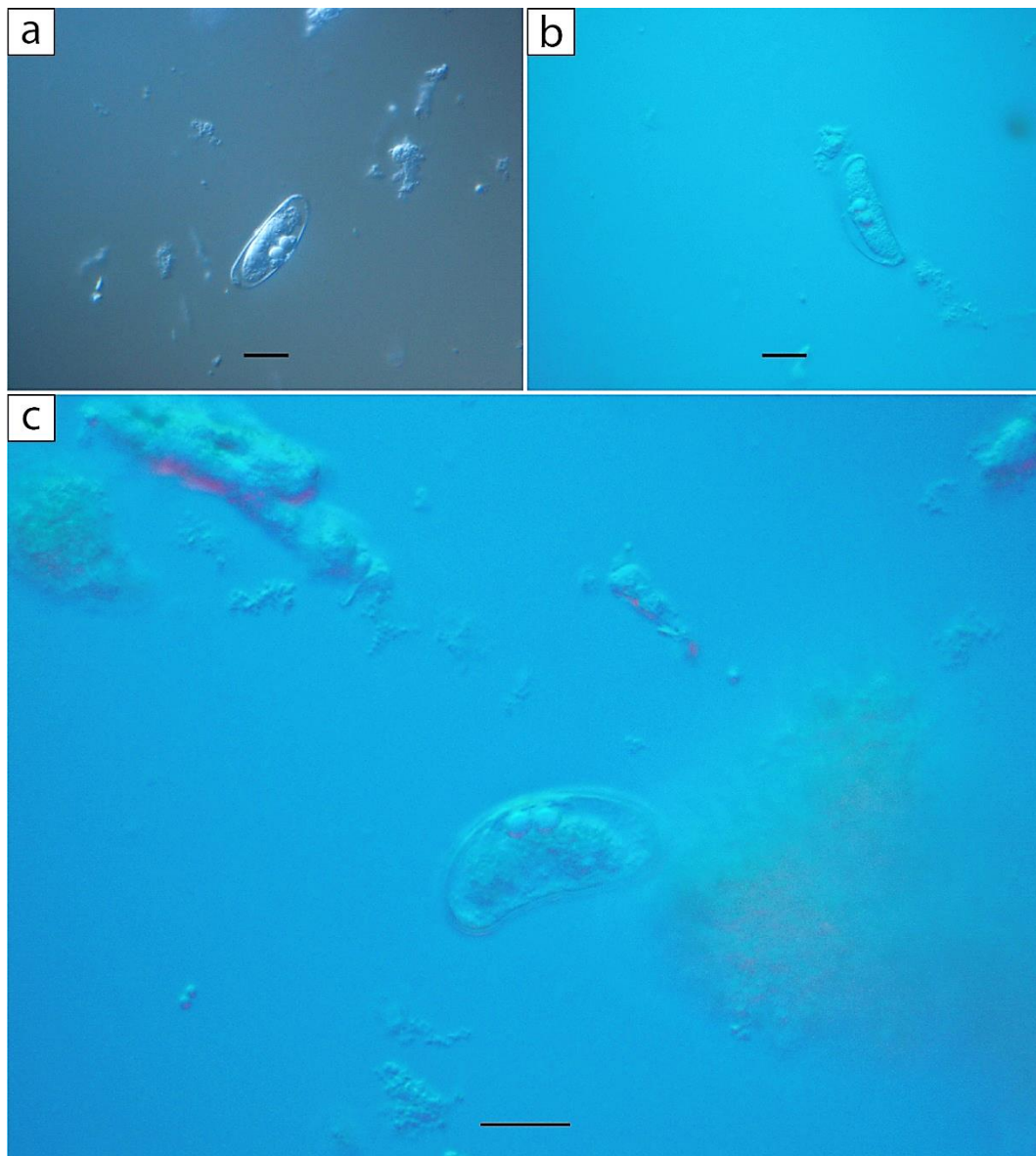


Figure 3. 5: Photomicrographs of *Ceratomyxa* sp. taken with an Olympus microscope with Nomarski interference contrast. Scale bar: 10 μ m; Photo credit: Dr Daniel Grabner.

Phylogenetic characterisation

Alignment obtained were 601 bp in length, indicating some divergence within the genus, owing to concurrent infections. Some of the sequences conformed to reference sequences available on GenBank (Table 3.3; see Appendix Table A. 4 for representing sequence identification; and Table A. 5 for GenBank reference sequences).

Table 3. 5: Evolutionary distances between species of *Ceratomyxa* isolated from the 18S SSU gene region, including phylogenetic analysis, expressed as percent similarity (below diagonal) and *p*-distances (above diagonal). See Table A. 4 for representing sequence identification. Newly generated sequences are indicated in the shaded rows.

	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	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57
1		0.009	0.009	0.000	0.009	0.009	0.000	0.000	0.009	0.009	0.009	0.000	0.000	0.009	0.009	0.000	0.009	0.009	0.000	0.009	0.000	0.000	0.009	0.009	0.009	0.009	0.009	0.000	0.000	0.024	0.009	0.010	0.000	0.623	0.625	0.623	0.631	0.000	0.000	0.000	0.019	0.009	0.066	0.038	0.085	0.009	0.009	0.057	0.019	0.028	0.075	0.644	0.613	0.632	0.596	0.009	
2	95		0.000	0.003	0.000	0.000	0.000	0.000	0.007	0.007	0.007	0.004	0.009	0.000	0.000	0.000	0.200	0.012	0.000	0.004	0.203	0.200	0.200	0.200	0.200	0.200	0.200	0.206	0.212	0.099	0.017	0.007	0.204	0.642	0.628	0.636	0.647	0.256	0.245	0.258	0.344	0.318	0.320	0.285	0.332	0.286	0.274	0.239	0.238	0.330	0.292	0.646	0.660	0.661	0.634	0.276	
3	95	100		0.003	0.000	0.000	0.000	0.000	0.007	0.000	0.007	0.007	0.004	0.009	0.000	0.000	0.000	0.200	0.012	0.000	0.004	0.204	0.200	0.200	0.200	0.200	0.200	0.207	0.212	0.099	0.017	0.007	0.205	0.640	0.628	0.636	0.645	0.251	0.243	0.257	0.343	0.317	0.317	0.284	0.331	0.285	0.270	0.235	0.234	0.327	0.288	0.646	0.660	0.661	0.634	0.271	
4	96	100	100		0.003	0.003	0.000	0.000	0.005	0.003	0.005	0.005	0.000	0.000	0.004	0.000	0.003	0.178	0.003	0.004	0.004	0.178	0.178	0.178	0.178	0.178	0.178	0.177	0.187	0.099	0.023	0.011	0.176	0.629	0.630	0.635	0.635	0.229	0.231	0.238	0.325	0.304	0.301	0.264	0.323	0.273	0.274	0.239	0.237	0.272	0.291	0.649	0.660	0.661	0.634	0.245	
5	95	100	100	100		0.000	0.000	0.000	0.008	0.000	0.008	0.008	0.004	0.009	0.004	0.000	0.000	0.182	0.013	0.000	0.004	0.185	0.182	0.182	0.182	0.182	0.182	0.188	0.201	0.102	0.017	0.007	0.187	0.633	0.634	0.634	0.638	0.244	0.239	0.249	0.333	0.313	0.310	0.274	0.328	0.274	0.272	0.237	0.236	0.279	0.290	0.653	0.665	0.661	0.635	0.258	
6	95	100	100	100	100		0.000	0.000	0.007	0.000	0.007	0.007	0.004	0.009	0.000	0.000	0.000	0.200	0.012	0.000	0.004	0.203	0.200	0.200	0.199	0.199	0.199	0.199	0.205	0.212	0.102	0.017	0.007	0.204	0.643	0.628	0.634	0.648	0.256	0.248	0.258	0.346	0.320	0.319	0.282	0.333	0.289	0.278	0.244	0.242	0.334	0.295	0.645	0.657	0.661	0.635	0.276
7	94	99	99	99	99	99		0.000	0.007	0.002	0.007	0.007	0.000	0.000	0.000	0.000	0.204	0.007	0.000	0.004	0.204	0.205	0.205	0.204	0.204	0.204	0.204	0.202	0.212	0.100	0.018	0.008	0.201	0.639	0.621	0.629	0.642	0.275	0.265	0.274	0.365	0.337	0.336	0.295	0.350	0.307	0.295	0.261	0.257	0.347	0.310	0.643	0.659	0.657	0.631	0.297	
8	96	100	100	100	100	100	99		0.010	0.002	0.010	0.009	0.000	0.000	0.000	0.000	0.206	0.010	0.000	0.004	0.206	0.206	0.207	0.205	0.205	0.205	0.205	0.204	0.214	0.102	0.018	0.008	0.202	0.638	0.620	0.628	0.641	0.274	0.262	0.272	0.365	0.338	0.334	0.297	0.348	0.308	0.296	0.263	0.261	0.347	0.309	0.646	0.658	0.661	0.635	0.296	
9	95	99	99	99	99	99	98	98		0.007	0.000	0.000	0.004	0.009	0.000	0.005	0.006	0.195	0.005	0.000	0.004	0.199	0.196	0.196	0.195	0.195	0.195	0.195	0.201	0.208	0.099	0.017	0.007	0.022	0.646	0.632	0.638	0.651	0.252	0.252	0.255	0.344	0.312	0.325	0.282	0.331	0.285	0.269	0.239	0.243	0.331	0.288	0.634	0.663	0.661	0.636	0.270
10	95	99	99	99	100	100	99	98	98		0.007	0.007	0.004	0.009	0.000	0.000	0.000	0.196	0.012	0.000	0.004	0.200	0.197	0.197	0.196	0.196	0.196	0.196	0.201	0.209	0.102	0.017	0.007	0.199	0.656	0.628	0.635	0.652	0.298	0.296	0.299	0.379	0.354	0.351	0.320	0.356	0.328	0.308	0.286	0.279	0.360	0.336	0.645	0.657	0.661	0.635	0.316
11	95	99	99	99	99	99	98	99	100	98		0.000	0.004	0.009	0.000	0.005	0.006	0.197	0.005	0.000	0.004	0.200	0.198	0.198	0.197	0.197	0.197	0.197	0.203	0.210	0.102	0.017	0.007	0.202	0.646	0.633	0.639	0.651	0.250	0.249	0.252	0.344	0.310	0.323	0.283	0.329	0.283	0.269	0.239	0.243	0.329	0.288	0.645	0.662	0.661	0.635	0.267
12	95	99	99	99	99	99	98	99	100	98	100		0.004	0.009	0.000	0.005	0.006	0.197	0.005	0.000	0.004	0.200	0.198	0.198	0.197	0.197	0.197	0.197	0.203	0.210	0.102	0.017	0.007	0.201	0.646	0.633	0.639	0.652	0.252	0.252	0.255	0.346	0.312	0.325	0.282	0.331	0.285	0.271	0.241	0.245	0.330	0.290	0.645	0.662	0.661	0.635	0.270
13	96	100	100	100	100	100	99	100	100	100	100	100		0.000	0.004	0.004	0.025	0.000	0.004	0.004	0.144	0.141	0.141	0.141	0.141	0.141	0.141	0.148	0.162	0.103	0.017	0.007	0.147	0.633	0.632	0.637	0.644	0.252	0.252	0.255	0.344	0.312	0.325	0.282	0.331	0.285	0.269	0.239	0.243	0.331	0.288	0.634	0.663	0.661	0.636	0.158	
14	95	95	95	95	95	95	94	95	95	95	95	95	95		0.009	0.000	0.009	0.009	0.000	0.009	0.000	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.000	0.000	0.022	0.009	0.009	0.000	0.622	0.624	0.622	0.636	0.037	0.039	0.030	0.045	0.036	0.101	0.073	0.144	0.046	0.018	0.063	0.027	0.036	0.072	0.625	0.622	0.624	0.594	0.036
15	95	99	99	99	99	99	98	99	99	99	99	99	99	95		0.000	0.000	0.004	0.007	0.000	0.000	0.004	0.004	0.004	0.004	0.004	0.004	0.011	0.007	0.012	0.006	0.000	0.011	0.630	0.631	0.630	0.635	0.146	0.154	0.150	0.148	0.152	0.157	0.204	0.177	0.105	0.085	0.107	0.081	0.096	0.140	0.642	0.638	0.650	0.618	0.151	
16	95	100	100	100	100	100	100	100	99	100	99	99	99	94	99		0.000	0.178	0.006	0.000	0.004	0.178	0.178	0.178	0.178	0.178	0.178	0.178	0.179	0.189	0.103	0.018	0.008	0.178	0.622	0.623	0.627	0.633	0.229	0.227	0.234	0.329	0.310	0.304	0.263	0.329	0.280	0.280	0.244	0.240	0.277	0.292	0.650	0.664	0.661	0.635	0.247
17	95	100	100	100	100	100	100	99	100	99	100	99	99	95	100	0.141	0.012	0.000	0.004	0.004	0.144	0.141	0.141	0.141	0.141	0.141	0.141	0.148	0.162	0.103	0.017	0.007	0.147	0.633	0.632	0.637	0.644	0.252	0.252	0.255	0.344	0.312	0.325	0.282	0.331	0.285	0.269	0.239	0.243	0.331	0.288	0.634	0.663	0.661	0.636	0.171	
18	95	79	79	81	81	79	78	79	79	79	79	79	97	95	99	81	85		0.183	0.004	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.083	0.007	0.000	0.004	0.005	0.648	0.639	0.645	0.648	0.235	0.243	0.247	0.333	0.300	0.330	0.284	0.349	0.279	0.259	0.242	0.252	0.329	0.300	0.686	0.671	0.661	0.655	0.269	
19	96	97	97	98	97	97	96	97	98	96	98	98	100	95	99	97	97	80		0.007	0.000	0.181	0.183	0.184	0.183	0.183	0.183	0.183	0.179	0.185	0.083	0.017	0.007	0.178	0.644	0.632	0.639	0.647	0.238	0.240	0.243	0.340	0.307	0.324	0.280	0.320	0.277	0.261	0.232	0.239	0.325	0.285	0.643	0.661	0.661	0.631	0.269
20	95	100	100	99	100	100	99	99	100	100	100	100	100	99	99	99	100	99	99		0.000	0.004	0.004	0.004	0.004	0.004	0.004	0.011	0.007	0.012	0.006	0.000	0.011	0.630	0.631	0.630	0.635	0.146	0.154	0.150	0.148	0.152	0.157	0.204	0.177	0.105	0.085	0.107	0.081	0.096	0.140	0.642	0.638	0.650	0.618	0.151	
21	96	99	99	99	99	99	98	99	99	99	99	99	99	95	99	99	99	99	99	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.004	0.000	0.004	0.005	0.649	0.639	0.645	0.649	0.237	0.246	0.249	0.335	0.302	0.332	0.283	0.350	0.281	0.261	0.244	0.254	0.330	0.302	0.686	0.671	0.661	0.655	0.271
22	96	79	79	81	81	79	78	79	79	79	79	98	95	99	81	85	100	80	99	99		0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.007	0.000	0.004	0.000	0.642	0.633	0.638	0.650	0.235	0.243	0.247	0.339	0																

Table 3. 5: (continued)

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	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57					
43	95	57.7	57.7	64.5	63.8	57.6	55.7	56.1	58.4	54.8	58.5	58.4	81.8	91.3	83.5	63.8	70.2	59.5	58.1	83.6	83.1	58.8	59.4	59.5	59.4	59.4	59.4	54.2	60	73	77.6	83.4	49.4	25.7	28.6	29.7	25	82.9	81.3	82.6	71.5		0.352	0.232	0.400	0.379	0.246	0.246	0.248	0.273	0.278	0.708	0.681	0.655	0.645	0.189					
44	89.3	59.2	59.5	65.7	64.9	59.3	57.5	58	58.9	56.4	59	58.9	80.6	84	81.4	65.2	69.9	58.3	58.2	81.5	80.9	57.8	58.4	58.5	58.1	58.1	58.1	53.7	58.2	73.3	72.7	80.7	49.1	26.5	31.2	31.7	25.8	52.2	51.7	52.7	55	57.2		0.371	0.331	0.366	0.360	0.351	0.343	0.347	0.384	0.730	0.716	0.681	0.654	0.343					
45	92.3	50.5	50.5	55.8	55.3	50.6	49.4	49.5	50.7	48.5	50.6	50.7	73.6	87.9	75.5	55.4	60.9	50.6	50.3	75.5	75.1	50	50.5	50.6	50.7	50.7	50.7	47.2	50.7	64.3	68.8	75.6	43.2	22.1	22.7	23.9	23.3	72.8	74.1	71.5	59.2	65.3	48.4		0.411	0.372	0.267	0.270	0.261	0.285	0.292	0.689	0.667	0.677	0.641	0.256					
46	87.9	59.9	60	65.4	64.9	59.7	58	58.5	60	57.4	60.2	60	79	82.1	80.5	64.6	69.1	58.3	60.1	80.4	80.2	57.6	58.2	58.3	58.1	58.1	58.1	58.1	53.5	58.5	70.5	71.6	80	51.1	27.9	31.6	31.9	27.8	51.2	52.1	51.9	54.5	56.4	61.9	48.3		0.357	0.402	0.381	0.377	0.390	0.401	0.685	0.670	0.653	0.639	0.384				
47	94.5	62	62.1	68.6	67.9	61.8	59.9	60.1	62.2	58	62.3	62.2	85.1	88.1	86.2	67.9	72.9	62.4	62.1	86.2	85.8	61.8	62.6	62.5	62.3	62.3	62.3	56.3	62.9	76.2	79.8	85.9	51.3	27.4	31	32.3	27.3	52.9	52.3	52.7	52.8	55.8	58.4	50.1	61.1		0.364	0.359	0.368	0.374	0.383	0.688	0.656	0.632	0.631	0.358					
48	95	62.2	62.5	70.1	68.9	61.9	60	60.3	62.6	59.5	62.7	62.5	89.9	93.8	90.6	69.7	76.3	63.3	62.4	90.7	90.3	62.7	63.4	63.6	63.1	63.1	63.1	57.7	62.6	81.3	85.4	90.5	52.5	27.4	30.6	32.1	26.6	64.7	63.7	65.1	66	68.3	54.9	58.7	54.6	56.1		0.109	0.176	0.203	0.233	0.650	0.642	0.664	0.642	0.248					
49	90.6	61.5	61.8	69.3	68.1	61.2	59.3	59.6	61.8	58.2	61.9	61.8	87.7	89.6	88.4	68.9	74.6	61.7	61.5	88.5	88	61.1	61.9	62	61.6	61.6	61.6	61.6	56	61.3	79.6	82.1	88.2	50.7	26.1	27.9	30	25.2	64.7	64.4	65.2	63.8	66	53.9	58.4	54.5	54.4	84.7		0.170	0.167	0.216	0.655	0.652	0.668	0.642	0.234				
50	94.1	63.4	63.7	71.5	70.3	63.2	61.4	61.6	63.3	60.4	63.4	63.3	90.3	92.9	91	71.5	78	62.8	62.8	91.1	90.7	62.2	62.9	63.1	62.6	62.6	62.6	62.6	56.6	62.4	80.9	86	90.8	51.6	26.1	28.5	30.1	25.4	63.8	64.1	65.1	64	66.8	55.5	59.6	56.1	54.9	79.7	80.9		0.170	0.211	0.665	0.666	0.656	0.632	0.227				
51	93.2	66.3	66.6	71.9	71.2	65.9	64.4	64.7	65.9	62.7	66.2	66.1	89.2	92.1	89.5	71.3	77.7	65.7	65.6	89.6	89.2	65.2	65.9	66.1	65.6	65.6	65.6	65.6	60.2	65.8	79.9	83.8	89.4	54.4	28.6	31.8	32.6	27.4	60.6	59.3	60.2	61.7	64.2	55.8	55.1	55.7	54.2	71	71.9	72.9		0.219	0.685	0.680	0.647	0.626	0.252				
52	88.8	59.9	60.2	67.5	66.3	59.6	57.9	58.4	60.4	56.3	60.5	60.4	84.1	88.8	85.3	67.6	72.8	59.6	59.9	85.4	85.6	59.2	59.8	59.9	59.5	59.5	59.5	55	59.7	77.7	78.2	85.6	50.2	24.4	27.2	28.9	23.7	61.3	62.3	63	63.9	65.1	52.6	56.3	54.4	54.1	73.8	75.6	77.2	69.3		0.671	0.656	0.657	0.619	0.256					
53	29.8	30.9	30.9	30.4	30.1	30.9	31.1	30.8	30.9	30.9	30.9	30.9	31.8	31.6	31.2	30.4	30.9	27.2	30.7	31.3	31.2	27.2	27.2	27.2	27.2	27.2	27.2	27.3	26.5	27.8	30.8	31.4	27.2	29.1	29	29	26.4	23	22	22.9	26.7	23.5	22.5	21.5	26.8	25.3	28.8	26.6	26.6	27.7	26.5		0.208	0.254	0.352	0.688					
54	37.2	32.7	32.7	32.7	32.2	32.9	32.7	32.8	32.2	32.9	32.4	32.4	34.5	36.4	34.4	32.1	33.1	31.4	32.2	34.3	34.4	31.4	31.4	31.4	31.4	31.4	31.4	31.4	31.8	31.2	32.9	32.9	34.4	31.6	29.5	29.3	29.4	27.6	26	24.8	25.1	31.9	28.5	26	24.2	31	31	32.4	29.7	29.2	30.9	30.7	71.1		0.230	0.405	0.669				
55	33	31.3	31.3	31.3	31.3	31.3	31.6	31.2	31	31.3	31.3	31.3	32.2	33.8	32.3	31.3	31.3	31.3	31	32.2	32.3	31.4	31.3	31.3	31.3	31.3	31.3	31.4	31.6	31.3	31.2	32.5	31.3	28	28	28	25.8	29.6	28.7	28.5	29.9	30.3	28.4	26.3	31.4	32.4	31.3	30.9	31.9	32.9	31.9	69.6	75		0.316	0.672					
56	37.3	34	34	34	34	34	34.2	34	34.1	34	34	34	35.7	37.9	35.5	34	34	32.2	34.1	35.3	35.5	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.2	32.9	32.7	32.4	35.7	32.2	29	29	29	29.7	29.6	28.5	28.2	33.3	31.5	30.9	27.4	33	32.7	33.3	33.3	34	35.1	35.8	58.1	57.9	65.8		0.649
57	95	54.8	55.1	60.9	60.1	54.7	52.9	53.2	55.3	52.1	55.4	55.3	79.4	91.3	81.2	60.2	65.7	55.1	54.7	81.3	80.6	54.3	55.2	55.3	54.9	54.9	54.9	54.9	50.1	55.3	69.7	76	81.1	44.9	23.7	25.4	25.7	23.1	76.7	76.8	76.6	65.8	69.7	53.9	69.5	53.9	54.9	61.5	62.3	63.1	60.3	60.5	23.4	26	27.5	26.9					

Remarks

This genus of myxozoans represents a diverse and rapidly evolving group of species that are closely related (Bartošová-Sojková et al. 2018). Previous studies on intertidal species report that these parasites are found with high prevalence, little host specificity, and concurrent infections (Bartošová-Sojková et al. 2018). Only nine species of *Ceratomyxa* have been described from marine fish off the African coast (Reed et al. 2007): *Ceratomyxa acanthuri* Kpatcha, Diebakate, Faye & Toguebaye, 1996; *Ce. australis* Gaevskaya & Kovaleva, 1979; *Ce. cottoidii* Reed, Basson, Van As & Dykova, 2007; *Ce. dehoopi* Reed, Basson, Van As & Dykova, 2007; *Ce. fistulariae* Kpatcha, Diebakate, Faye & Toguebaye, 1996; *Ce. honckenii* Reed, Basson, Van As & Dykova, 2007; *Ce. lagocephali* Kpatcha, Diebakate, Faye & Toguebaye, 1996; *Ce. schulmani* Dubina & Isakov, 1976; *Ce. syacii* Kpatcha, Diebakate, Faye & Toguebaye, 1996; *Ce. trachinocephali* Kpatcha, Diebakate, Faye & Toguebaye, 1996; *Ce. trichiuri* Kpatcha, Diebakate, Faye & Toguebaye, 1996; and *Ce. truncata* Thélohan, 1895.

The species collected during the present study was characterised by crescent shaped spores with two polar capsules, conforming to the genus *Ceratomyxa* (see Bartošová-Sojková et al. 2018). According to literature, these species are highly host-specific and rarely infect more than one host species (Gunter et al. 2008; Heiniger and Adlard 2013; Bartošová-Sojková et al. 2018), however, concurrent infections of several species of different morphology are a common occurrence (Bartošová-Sojková et al. 2018). This was also the case during the present study, where multiple morphologies and molecular results were noted, however, they could not be linked to each other. Newly generated sequences of the present study conform to species collected and sequences by Bartošová-Sojková et al. (2018): *Ceratomyxa dehoopi*, *Ceratomyxa* sp. 2, *Ceratomyxa* sp. 4, and *Ceratomyxa* sp. 5. Further characterisation of the taxa is needed to determine species level identification.

Family:	<i>Sphaeromyxidae</i> Lom & Noble, 1984
Genus:	<i>Sphaeromyxa</i> Thélohan, 1892
Species:	<i>Sphaeromyxa clini</i> Bartošová-Sojková, Kodádková, Pecková, Kuchta & Reed, 2015

Material examined

Of all the fish sampled, only 24 out of 93 fish were infected with *Sphaeromyxa* sp. (25.8% prevalence) from the gallbladders and eyes of fish from Langebaan marina, Cape Town harbour, Tsitsikamma and Chintsa.

Morphological characteristics

The spores were arcuate from the frontal view, tapering towards the ends and slightly sigmoid in shape. Spores from this genus (n = 6) measured 17.4–20 (18.7) µm in length and 4.9–6.1 (5.6) µm in width, with polar capsules located on either end, equal in size, and of oval shape. The polar capsules measured 5.1–6.5 (5.9) µm in length and 2.7–3.1 (2.9) µm in width. Coil filaments were not visible during examination and the suture line was indistinctly visibly.

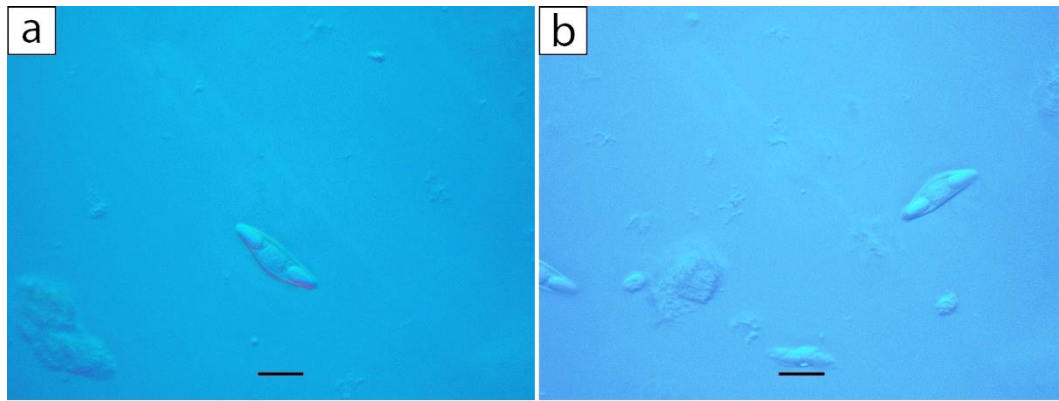


Figure 3. 6 Photomicrographs of *Sphaeromyxa clini* taken with an Olympus microscope with Nomarski interference contrast. Scale bar: 10 μ m; Photo credit: Dr Daniel Grabner.

Phylogenetic characterisation

The newly generated sequences were aligned, resulting in 890 bp in length alignment, indicating some divergence within the genus, owing to concurrent infections. Some of the sequences conformed to references sequences available on GenBank (Table 3.6).

Table 3. 6: Evolutionary distances between species of *Sphaeromyxa* isolated from the 18S SSU gene region, including phylogenetic analysis, expressed as percent similarity (below diagonal) and p-distances (above diagonal). Newly generated sequence is indicated in the shaded row

	<i>Sphaeromyxa</i> sequences	1	2	5	3	4	6	7	8	9	10	11	12	13	14	15	16
1	EPD069_ <i>Sphaeromyxa clini</i> (gallbladder)		0.000	0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.002	0.112	0.083	0.083	0.081	0.145	0.148
2	EPD087_ <i>Sphaeromyxa clini</i> (gallbladder)	100		0.000	0.000	0.000	0.000	0.000	0.002	0.000	0.002	0.112	0.083	0.083	0.081	0.145	0.148
5	EPD032_ <i>Sphaeromyxa clini</i> (gallbladder)	99.9	99.9		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.178	0.149	0.144	0.142	0.225	0.227
3	KM201337_ <i>Sphaeromyxa</i> sp. 1512	100	100	100		0.000	0.000	0.000	0.000	0.000	0.000	0.179	0.150	0.146	0.143	0.227	0.229
4	KM201339_ <i>Sphaeromyxa</i> sp. 1456	100	100	100	100		0.000	0.000	0.002	0.000	0.002	0.113	0.083	0.083	0.081	0.145	0.148
6	KM201338_ <i>Sphaeromyxa clini</i>	99.9	99.9	99.9	99.9	99.9		0.000	0.000	0.000	0.000	0.177	0.151	0.146	0.144	0.225	0.227
7	KM201340_ <i>Sphaeromyxa</i> sp. 1542	99.9	99.9	99.9	99.9	99.9	99.8		0.000	0.000	0.000	0.174	0.146	0.141	0.138	0.221	0.223
8	KM201336_ <i>Sphaeromyxa</i> sp. 813	99.8	99.8	99.7	100	100	99.9	99.9		0.000	0.002	0.112	0.083	0.083	0.081	0.145	0.150
9	KM201341_ <i>Sphaeromyxa</i> sp. 931	99.6	99.6	99.6	99.6	99.6	99.4	99.6	99.6		0.000	0.204	0.174	0.168	0.165	0.251	0.247
10	KM201342_ <i>Sphaeromyxa</i> sp. 933	99.4	99.4	99.4	99.6	99.6	99.5	99.6	99.4	99.7		0.188	0.158	0.153	0.150	0.236	0.236
11	KM201343_ <i>Sphaeromyxa limocapitis</i>	86.2	86.2	86.2	77.6	77.4	77.4	77.9	86.2	74.9	76.1		0.130	0.132	0.130	0.136	0.142
12	DQ377692_ <i>Sphaeromyxa hellandi</i>	91.3	91.3	91.2	84.3	84.2	84.0	84.6	91.3	81.4	83.1	84.6		0.009	0.007	0.138	0.143
13	DQ377701_ <i>Sphaeromyxa hellandi</i>	91.3	91.3	91.2	84.8	84.6	84.5	85.0	91.3	81.9	83.6	84.4	99.1		0.005	0.141	0.147
14	DQ377693_ <i>Sphaeromyxa hellandi</i>	91.5	91.5	91.4	85.0	84.9	84.8	85.3	91.5	82.2	83.8	84.6	99.3	99.5		0.138	0.145
15	AY538662_ <i>Sphaeromyxa zaharoni</i>	83.9	83.9	83.8	74.7	74.4	74.4	74.9	83.9	71.1	73.1	84.8	84.6	84.2	84.6		0.076
16	KM201344_ <i>Sphaeromyxa longa</i>	83.8	83.8	83.7	74.7	74.4	74.4	74.9	83.5	71.7	73.3	84.5	84.2	83.9	84.1	91.9	

Remarks

The specimens from the present study morphologically conform to *Sphaeromyxa clini*, (Bartošová-Sojková et al. 2015), and are molecularly most closely related to *Sphaeromyxa* sp. presented in Table 3.6. The species have been reported to infect multiple species of Clinidae on the Western Cape coast, and specifically *Clinus superciliosus* from Cape Columbine, South Africa. This is thus a new locality record for this species as the easternmost point of distribution for this parasite species at Chintsa, as well as Tsitsikamma.

Order: Multivalvulida Shulman, 1959
Family: Kudoidae Meglitsch, 1960
Genus: Kudoa Meglitsch, 1947
Species: *Kudoa thyrsites* (Gilchrist, 1923)

Material examined

No specimens of *Kudoa* Meglitsch, 1947 were observed during microscopical screening, however, molecular results indicated that the urinary bladder wall of one fish each from Langebaan marina and Cape Town harbour, was infected with a species of *Kudoa*.

Phylogenetic characterisation

Two sequences were generated and aligned (1102 pbp) with other species of *Kudoa*, including *Kudoa thyrsites* (Gilchrist, 1923) from various localities around the world. Genetic distances were determined based on the alignment (Table 3.7).

Remarks

This is the first record of *Kudoa thyrsites* reported to infect *Clinus superciliosus* from the coast of South Africa. This species of Myxozoa is economically important, infecting the muscle of commercial fish species and causing post mortem tissue degradation rendering them unmarketable (Whipps and Kent 2006; Jones and Long 2019). *Kudoa thyrsites* is known to infect the muscle fibres of their hosts and have not been reported from the urinary bladder prior to this study. It would be of great interest to investigate the muscle of *C. superciliosus* using histological methods to determine if the infection is confined to the urinary bladder, or widespread throughout its other organs.

The sequences obtained during the present study were 100% similar to one another, and are most closely related to *Kudoa thyrsites* found to infect *Sardinops ocellatus* from South Africa (Whipps and Kent 2006) (Appendix Table A. 7). The newly generated sequences are only 0.9% different to the same species collected from the Great Barrier Reef, Australia (Whipps et al. 2003b).

Table 3. 7: Evolutionary distances between species of *Kudoa* isolated from the 18S SSU gene region, including phylogenetic analysis, expressed as percent similarity (below diagonal) and *p*-distances (above diagonal). Newly generated sequence is indicated in the shaded row

	Kudoa sequences	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	EPD071_ <i>Kudoa thyrsites</i> (urinary bladder wall)		0.000	0.007	0.008	0.008	0.008	0.008	0.008	0.012	0.020	0.019	0.023	0.020	0.027	0.028	0.029	0.023	0.031
2	EPD092_ <i>Kudoa thyrsites</i> (urinary bladder wall)	100.0		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.005	0.009	0.009	0.005	0.005	0.005	0.000	0.005
3	AY542481_ <i>Kudoa thyrsites</i>	99.3	100.0		0.003	0.001	0.000	0.000	0.003	0.006	0.010	0.010	0.014	0.010	0.016	0.018	0.020	0.013	0.021
4	AF031412_ <i>Kudoa thyrsites</i>	99.2	100.0	99.7		0.005	0.004	0.004	0.005	0.007	0.012	0.011	0.015	0.012	0.016	0.019	0.021	0.014	0.022
5	AY078430_ <i>Kudoa thyrsites</i>	99.2	100.0	99.9	99.5		0.001	0.001	0.004	0.008	0.011	0.011	0.015	0.011	0.018	0.019	0.021	0.014	0.022
6	AY542482_ <i>Kudoa thyrsites</i>	99.2	100.0	100.0	99.6	99.9		0.000	0.003	0.007	0.010	0.010	0.014	0.010	0.017	0.018	0.020	0.013	0.021
7	MH899081_ <i>Kudoa thyrsites</i>	99.2	100.0	100.0	99.6	99.9	100.0		0.003	0.007	0.010	0.010	0.014	0.010	0.017	0.018	0.020	0.013	0.021
8	AY152747_ <i>Kudoa thyrsites</i>	99.2	100.0	99.7	99.5	99.6	99.7	99.7		0.008	0.009	0.011	0.013	0.009	0.016	0.017	0.019	0.014	0.022
9	AY382607_ <i>Kudoa thyrsites</i>	98.8	100.0	99.3	99.3	99.2	99.3	99.3	99.2		0.012	0.010	0.015	0.012	0.019	0.017	0.018	0.016	0.022
10	AB188529_ <i>Kudoa megacapsula</i>	98.0	99.1	99.0	98.8	98.9	99.0	99.0	99.1	98.8		0.013	0.013	0.000	0.018	0.015	0.017	0.015	0.020
11	LC493825_ <i>Kudoa guangdongensis</i>	98.0	99.1	98.9	98.8	98.8	98.9	98.9	98.8	98.9	98.6		0.011	0.013	0.016	0.012	0.013	0.012	0.015
12	AY152749_ <i>Kudoa thyrsites</i>	97.7	99.1	98.6	98.5	98.5	98.6	98.6	98.7	98.5	98.7	98.8		0.013	0.015	0.019	0.018	0.012	0.018
13	AB263074_ <i>Kudoa megacapsula</i>	97.9	99.1	98.9	98.8	98.9	99.0	99.0	99.0	98.8	100.0	98.6	98.7		0.018	0.015	0.017	0.015	0.020
14	LC190926_ <i>Kudoa empresnichikoe</i>	97.3	99.5	98.3	98.4	98.2	98.3	98.3	98.4	98.1	98.2	98.3	98.5	98.1		0.019	0.019	0.006	0.022
15	FJ792712_ <i>Kudoa gunterae</i>	97.2	99.5	98.1	98.1	98.1	98.2	98.2	98.3	98.3	98.5	98.7	98.1	98.4	98.1		0.007	0.018	0.015
16	JX090292_ <i>Kudoa whippsi</i>	97.1	99.5	98.0	97.9	97.9	98.0	98.0	98.1	98.2	98.3	98.6	98.2	98.2	98.1	99.3		0.018	0.015
17	LC128647_ <i>Kudoa parathyrsites</i>	97.7	100.0	98.7	98.6	98.6	98.7	98.7	98.6	98.4	98.5	98.7	98.8	98.4	99.4	98.2	98.2		0.019
18	AY382606_ <i>Kudoa lateolabracis</i>	96.9	99.5	97.9	97.8	97.8	97.9	97.9	97.8	97.8	98.0	98.5	98.2	98.0	97.8	98.5	98.5	98.1	

3.3.2.2 Characterisation of various helminths (Trematoda, Monogenea, Cestoda)

As mentioned previously the present study formed part of a larger study investigating the parasite diversity of South African nearshore marine fish parasites. As part of this larger study the Digenea of *C. superciliosus* formed the topic of the MSc dissertation by Anja Vermaak entitled: “Diversity of digenean trematodes of *Clinus superciliosus* (Linnaeus, 1758) from the coast of South Africa”. For the full descriptions of the Digenea reported below please see Vermaak (2020).

Phylum:	Platyhelminthes Minot, 1876
Class:	Trematoda Rudolphi, 1808
Order:	Plagiorchiida La Rue, 1957
Family:	Opecoelidae Ozaki, 1925
Genus:	<i>Coitocaecum</i> Nicoll, 1915
Species:	<i>Coitocaecum capense</i> Bray, 1987
Species:	<i>Coitocaecum</i> sp. 1
Species:	<i>Coitocaecum</i> sp. 2
Species:	<i>Coitocaecum</i> sp. 3
Genus:	<i>Helicometra</i> Odhner, 1902
Species:	<i>Helicometra</i> sp. 1
Species:	<i>Helicometra</i> sp. 2

Material examined

Specimens from the family Opecoelidae were collected from all of the localities, except for Cape Town harbour. Prevalence per taxa were as follows: *Coitocaecum capense* (20.4%); *Coitocaecum* sp. 1 (1.1%); *Coitocaecum* sp. 2 (1.1%); *Coitocaecum* sp. 3 (5.4%); *Helicometra* sp. 1 (35.5%); *Helicometra* sp. 2 (1.1%). All of the taxa found were reported to infect the intestine of *C. superciliosus*.

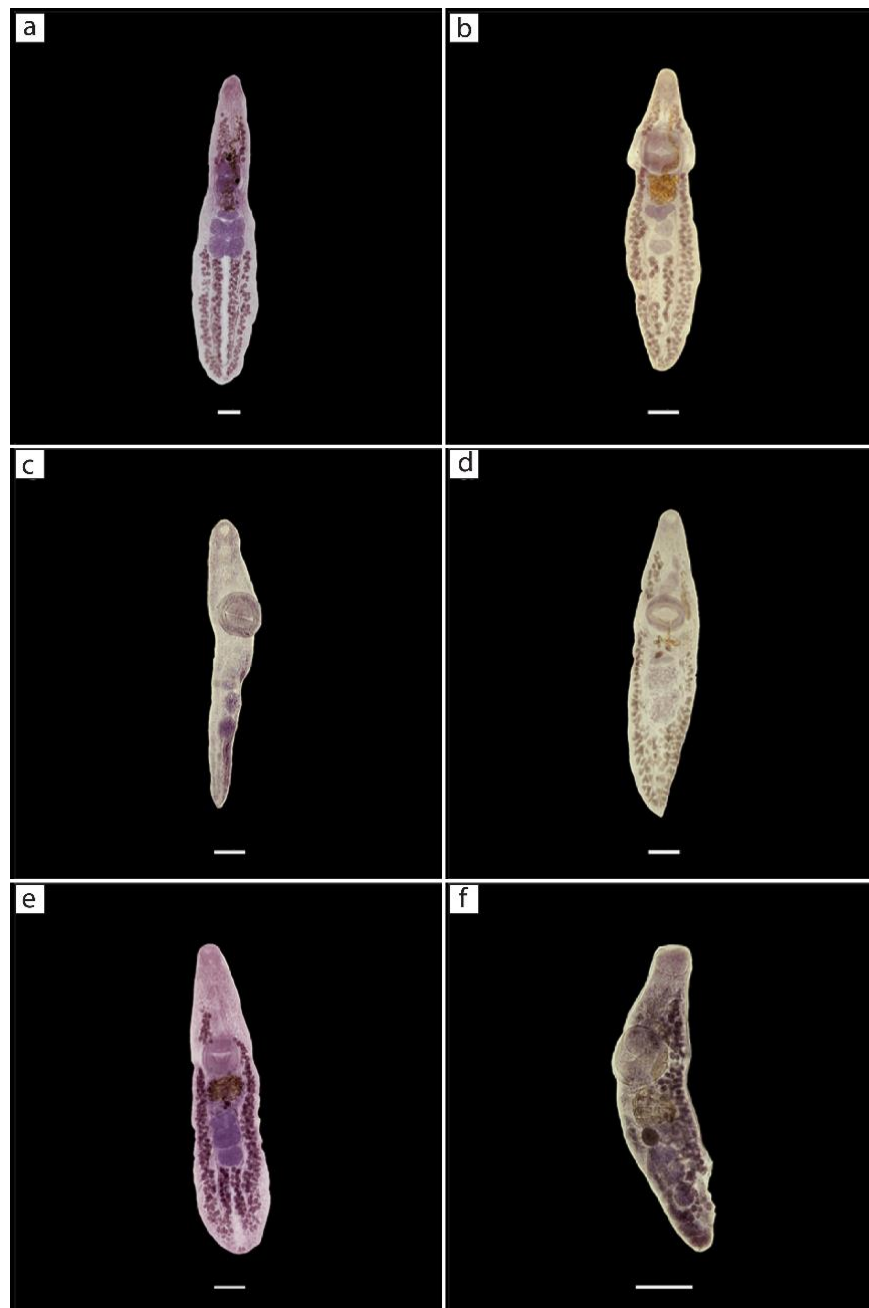


Figure 3. 7: Photomicrographs of adult Opecoelidae species ex *Clinus superciliosus* – a) *Coitocaecum capense*; b) *Coitocaecum* sp. 1; c) *Coitocaecum* sp. 2; d) *Coitocaecum* sp. 3; e) *Helicometra* sp. 1; and f) *Helicometra* sp. 2. Photo credit: Anja Vermaak.

Remarks

The family Opecoelidae is the largest family of the subclass Digenea, with more than 90 genera worldwide. It is also the family with the greatest number of species infecting *C. superciliosus*. There are currently only 15 opecoelid species described from South Africa and, considering the high endemicity of this biodiversity rich region, it is assumed that this number is an under representation of Opecoelidae species. In addition to *Coitocaecum capense* Bray, 1987, there were three unidentified species of *Coitocaecum* and two unidentified species of *Helicometra*, while *Helicometra fasciata* (Rudolphi, 1819) was not found throughout any of the surveys at Langebaan marina, Cape Town harbour, old harbour Hermanus, Tsitsikamma, Jeffreys Bay and Chintsa.

One species identified as *Coitocaecum capense* during the present study conforms to the description of Bray (1987), with differences in body size, oral sucker, pharynx, oesophagus, ovary, egg length and size of testes (Vermaak, 2020). However, there are three species that were morphologically identified as *Coitocaecum* with distinct character differences, and molecularly differentiated as separate species. To the knowledge of the researchers, there has been no other reports of *Coitocaecum* sp. 1, *Coitocaecum* sp. 2 and *Coitocaecum* sp. 3 from any other fish host along the coast of South Africa, or worldwide.

In the case of the two species of *Helicometra* collected during the present study, consistent with the genus as described by Cribb (2005), *Helicometra* sp. 1 is morphologically similar to *H. fasciata*. The differentiating structures include a smaller oral sucker, shorter pharynx, longer oesophagus, smaller ovary, and a smaller testis than what was described by Bray (1987). Additionally, *Helicometra* sp. 2 is morphologically similar to *H. scorpaenae* Prudhoe & Bray 1973. However, positive comparison with certainty is not possible since the original specimens were contracted and distorted.

Family: Fellodistomidae Nicoll, 1909
Genus: *Proctoeces* Odhner, 1911
Species: *Proctoeces* sp.

Material examined

Proctoeces sp. specimens were collected from Tsitsikamma (2019) (30% prevalence), and Chintsa 2019 (20% prevalence), from the intestine.



Figure 3. 8: Stained photomicrograph of *Proctoeces* sp. Photo credit: Anja Vermaak

Remarks

Marine fish of South Africa have been reported as hosts to five species from this family: *Fellodistomum* sp. 1; *Steringotrema pagelli* (van Beneden, 1871) Odhner, 1911; *Tergestia pauca* Teixeira de Freitas & Kohn, 1965; *Theledera pectinata* (Linton, 1905) Linton, 1910; and *Tergestia laticollis* (Rudolphi, 1819) Stossich, 1899 (see Parukhin 1976; Bray 1984; Parukhin 1989; le Roux 2013). *Proctoeces maculatus* (Looss, 1901) Odhner, 1911 was also reported from the octopus, *Octopus vulgaris* Cuvier, 1797 (Bray 1984).

The intraspecific variation for the 28S gene for *Proctoeces* is reportedly 0–0.42% (0–5 nt) (Antar and Gargouri 2015). Based on the phylogenetic results of the novel sequences and the GenBank sequences, there is an intraspecific difference between *Proctoeces* sp. from the present study and *P. maculatus* retrieved from the bream, *Sparus aurata* Linnaeus, 1758 in Tunisia (Wee et al. 2017; Vermaak 2020), while *P. maculatus* has also been reported from *O. vulgaris* in Durban, South Africa (Bray 1984). It is very likely that the same species was found during the present study since they share the same habitat and are exposed to the same cercariae (Vermaak 2020).

Family: Zoogonidae Odhner, 1902

Genus: Zoogonidae gen. sp.

Material examined

Specimens from the family Zoogonidae were collected from Chintsa (2019), Tsitsikamma (2020), and Chintsa 2019, with 20% prevalence at each locality, infecting the intestine of their hosts.



Figure 3. 9: Photomicrograph of live Zoogonidae gen. sp.

Remarks

Based on previous studies, a total of five species from the family Zoogonidae have been reported from marine fish in South Africa: *Cephaloporus bakeri* Bray, 1985; *Lecithostaphylus retroflexus* (Molin, 1859) Odhner, 1911; *Overstreetia sodwanaensis* Bray, 1985; *Steganoderma* sp. and *Steganodermatoides allocytti* (Tkachuk, 1979) Bray, 1985 (Fantham 1938; Bray 1985; Bray 1987b; Parukhin 1989). During the present study, a species of Zoogonidae was collected at Chintsa, ex *Clinus superciliosus*.

The phylogenetic analyses did not aid greatly in elucidating the species identification since only five other sequences (excluding the outgroup) were available on GenBank. The high genetic diversity in the phylogenetic tree confirms that further investigation, morphological characterisation and comparisons, will be needed before a full identification to a lower taxonomic level can be confirmed (Vermaak 2020). It is possible that this Zoogonidae gen. sp. might be from the genus *Zoogonoides*, based on its placement in the phylogenetic tree.

Family: Strigeidae Railliet, 1919
Genus: *Cardiocephaloides* Sudarikov, 1959
Species: *Cardiocephaloides physalis* (Lutz, 1927)
Species: *Cardiocephaloides* sp.

Material examined

Cardiocephaloides sp. were reported to infect fish collected from all of the localities, except for Cape Town harbour. With an overall prevalence of 22.6%, they were found infecting the eyes and brain of *Clinus superciliosus*. *Cardiocephaloides physalis* was only collected from Hermanus (1.1% prevalence).

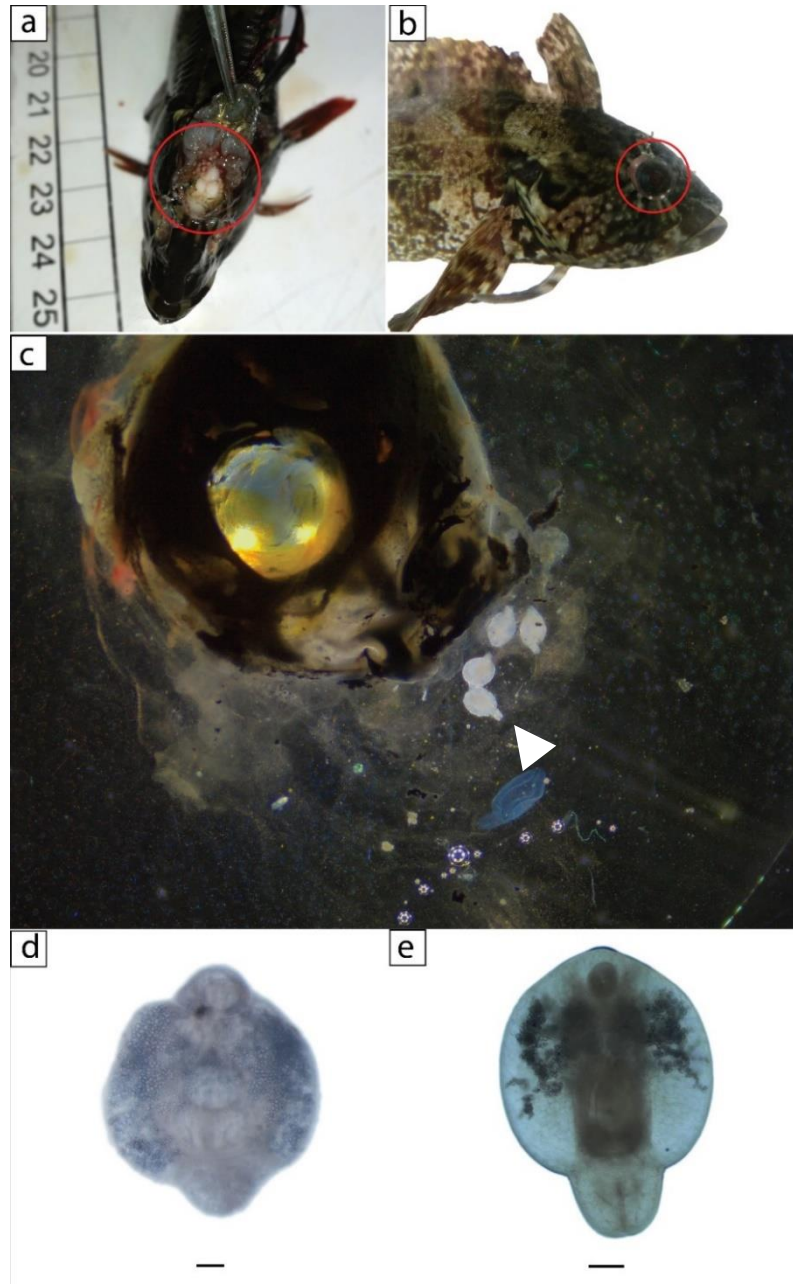


Figure 3. 10: *Cardiocephaloides* sp. infecting the brain (a) and eyes (b) of *Clinus superciliosus*; c) photomicrograph of the *Cardiocephaloides* specimens next to the eye (arrow); and ventral view of excysted, fixed specimen of d) *Cardiocephaloides physalis* ex *Clinus superciliosus* from Hermanus and e) *Cardiocephaloides* sp. ex *Clinus superciliosus* from Jeffrey's Bay, from Vermaak et al. 2021.

Remarks

With only seven known species of the genus *Cardiocephaloides* (Strigeidae Railliet, 1919) worldwide, this host, *Clinus superciliosus*, harbours two. Of these, only one is known. This three-host parasite utilises *C. superciliosus* as a second intermediate host infecting the eyes and brains of fish before infecting seabirds as its definitive host (Niewiadomska 2002; Donald and Spencer 2016; Vermaak et al. 2021). *Cardiocephaloides* is known to cause numerous mortalities in penguin chicks (Vermaak et al. 2021).

The present study demonstrated that the species diversity of *Cardiocephaloides* from South Africa is higher than expected, as well as that the intermediate hosts are not limited to sardines (Vermaak et al. 2021). The hosts reported in these different studies suggests that cercariae of this species are not host specific, nevertheless further studies are required to elucidate the life cycle and transmission of this species (Vermaak et al. 2021). Specimens of *C. physalis* are morphologically consistent with species found to infect *Sardinops sagax* (Jenyms) (Ukomadu 2017), and *Cardiocephaloides* sp. (digital vouchers) reported to infect *Engraulis anchoita* Hubbs & Marini (see Timi et al. 1999). Some differences in morphometrics were ascribed to variation in maturity of metacercarial stages reported in the various studies (Vermaak et al. 2021). Based on the COI gene analyses from the present study, the intraspecific variation between specimens from different geographic regions was low (0.4–0.9%).

A second species from the present study, *Cardiocephaloides* sp., was molecularly confirmed by phylogenetic characterisation (Vermaak et al. 2021). The ITS1 region comparison revealed this species to be conspecific to sporocysts reported from whelks (*Cominella adspersa* (Bruguière), *Cominella glandiformis* (Reeve) and *Cominella virgata* H. Adams & A. Adams) from New Zealand Donald and Spencer (2016). However, the adult molecular and morphological descriptions are needed to confirm the full identification of these trematode larvae (Vermaak et al. 2021).

During the present study, the excretory granules are suggested as a species differentiating character, where *C. physalis* had medium-sized, dense granules, distributed in two lateral fields between the posterior margin of the oral sucker and the posterior margin of the holdfast organ (as described in Vermaak et al. 2021). However, *Cardiocephaloides* sp. from the present study had small, less dense granules distributed between the posterior margin of the oral sucker and the mid-level of the holdfast organ (as described in Vermaak et al. 2021). It was not possible to compare this character with species identified by Timi et al. (1999) and Ukomadu (2017), since the authors did not report on this character in detail. Nonetheless, this character has been used previously to differentiate between metacercariae of the genus *Diplostomum* von Nordmann, 1832 (see Pérez-del-Olmo et al. 2014; Kudlai et al. 2017).

Family: Hemiuridae Looss, 1899

Genus: Hemiuridae gen. sp. 1

Genus: Hemiuridae gen. sp. 2

Material examined

Two morphotypes of Hemiuridae were collected from the intestine and reproductive organs of fish collected from Tsitsikamma (65% prevalence) and Chintsa (45% prevalence), as well as Langebaan marina (1.1% prevalence).

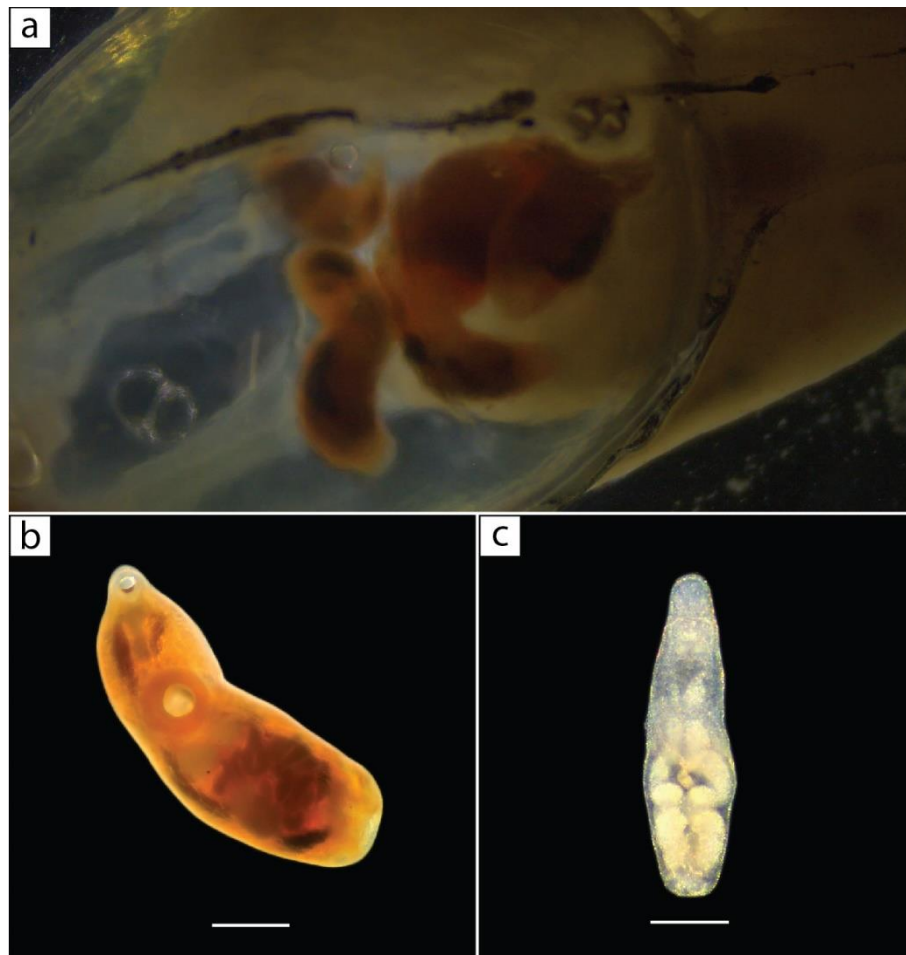


Figure 3. 11: a) Photo of live Hemiuridae gen. sp. 1 in the reproductive organs; b) Hemiuridae gen. sp. 1 and c) Hemiuridae gen. sp. 2. Photo credit (b, c): Anja Vermaak.

Remarks

The family Hemiuridae, currently the most diverse family infecting South African marine fish species, has 18 reports along the coast from Cape Town to Sodwana (Vermaak 2020). The present study reports the westernmost distribution point for this family, with one species in Langebaan marina, and the other on the south to southeast coast at Tsitsikamma and Chintsa. This was also the only species found in the reproductive organs of *Clinus superciliosus*.

Most commonly found in the stomach of marine hosts (Pankov et al. 2006), Hemiuridae from the present study was found infecting the intestine and reproductive organs of *C. superciliosus*. Based on morphological characterisation, two distinct species were sampled, one (adult) infecting the intestine and ovaries, and the other (pregnoid adult) infecting the male

gonads (Vermaak 2020). No similar DNA sequences have been characterised, leading to the conclusion that these novel sequences either represent species new to science, or species not molecularly characterised prior to the present study. Results from the 28S rRNA region indicate that these species probably belong to the genus *Pulmovermis* (genetic divergence of 26–30 nt).

Family: Acanthocolpidae Lühe, 1906
Genus: *Stephanostomum* Looss, 1899
Species: *Stephanostomum* sp.

Material examined

Stephanostomum sp. were collected from the intestine and body cavity of fish collected from Chintsa (4.3% prevalence).

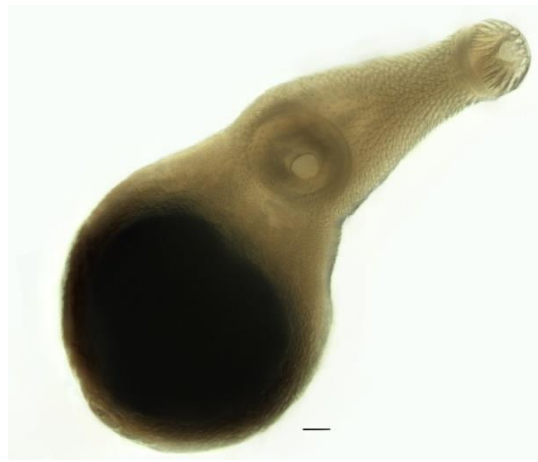


Figure 3. 12: Unstained, z-stacked photomicrograph of a fixed specimen, *Stephanostomum* sp. Photocredit: Anja Vermaak

Remarks

There are currently five known species of this family reported from marine fish in South Africa: *Pleorchis sciaenae* Yamaguti, 1938; *Stephanostomum ditrematis* (Yamaguti, 1939) Manter, 1947; *Stephanostomum imparispine* (Linton, 1905) Manter, 1940; and *Stephanostomum solontschenkae* Parukhin, 1968 (see Parukhin 1976; Bray 1985; Bray 1986). None of the abovementioned species have been reported from the host family Clinidae, which makes this the first host and distribution record.

The novel sequences generated from the present study do not match any of the representative sequences available for this genus, however, they might represent the same species as was collected from *Chaetodon marleyi* Regan or *Merluccius capensis* Castelnau, based on the relatively close proximity of the sampling localities (Vermaak 2020).

Family: Bucephalidae Poche, 1907
Genus: *Dollfustrema* Eckmann, 1934
Species: *Dollfustrema* sp.

Material examined

Dollfustrema sp. were collected from almost every organ of the host at Langebaan marina (100% prevalence), with limited records at Chintsa (10% prevalence).

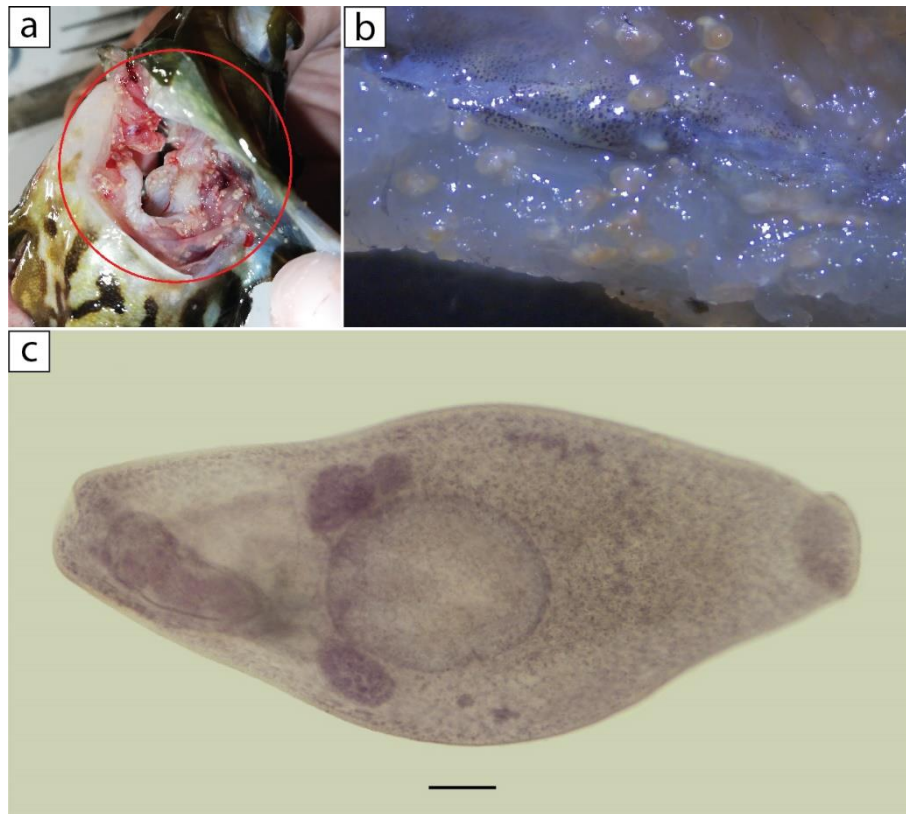


Figure 3. 13: Photograph taken in the field of the gills (a) and muscle tissue (b) of *Dollfustrema* sp. during collection of *Clinus superciliosus*; c) Fixed light photomicrograph of *Dollfustrema* sp. Photo credit (c): Anja Vermaak.

Remarks

A total of five species have been reported from Bucephalidae from marine fishes of South Africa: *Bucephalus margaritae* Ozaki & Ishibashi, 1934; *Proisorhynchoides arcuatus* (Linton, 1900) Love & Moser, 1983; *Proisorhynchus caudovatus* Manter, 1940; *Rhipidocotyle paruchini* Gavriljuk-Tkachuk, 1979; and *Rhipidocotyle* sp. (see Bloch and Schneider 1801; Parukhin 1976; Gavriljuk-Tkachuk 1979; Bray 1984; Nunkoo et al. 2017). The present study reports the first record of *Dollfustrema* sp. from *Clinus superciliosus*. This is the first host record of the genus *Dollfustrema* to infect clinid fish from South African marine waters. *Dollfustrema* infecting fish at Langebaan marina was remarkably present in all organs and tissues. Fish from Langebaan marina on the west coast, hosted the greatest number of individuals, with some infections by the family Bucephalidae reaching more than an estimated 1,000 individuals of this species.

As with all digenean metacercariae, the adult gene sequences are needed to identify the lower taxonomic level. The species was found to infect all organs and tissues of

Clinus superciliosus at the Langebaan marina locality, which was attributed to the low turbulence and current movement present within the marina (Vermaak 2020). This, in combination with the general passive behaviour of the fish, makes this host highly susceptible to parasitic infection. Further investigation can aid in understanding the life cycle and transmission of this parasite species.

Class: Monogenea Van Beneden, 1858
Order: Gyrodactylidea Bychowsky, 1937
Family: Gyrodactylidae Cobbold, 1864
Genus: *Gyrodactylus* von Nordmann, 1832
Species: *Gyrodactylus* sp.

Material examined

Monogenea samples collected (Langebaan marina (52.6% prevalence) and Cape Town harbour (50% prevalence)) were fixed on slides and preserved in 70–80% ethanol during field collection, and used for further investigation. Specimens were studied morphologically using the coverslip-flattened technique with a DIC microscope. *Gyrodactylus* sp. was found attached to the skin (mucus) of *Clinus superciliosus*.

Morphological characteristics

A morphological description of the basic characters of coverslip-flattened specimens (n = 3) (Shinn et al. 2004; Christison et al. 2021) was compiled from multiple images captured on a Nikon Eclipse Ni compound microscope (with 40x magnification lens). Only the adult structures were measured where the specimens measured 302.4–368.4 (337.8) µm long, and 95.1–135.5 (109.1) µm wide at the level of the uterus (Figure 3.14b). The haptor was approximately circular, however, due to the coverslip-flattened specimen the outline was not clearly discernible (Figure 3.14g). Detailed measurements of the haptoral central hook complex are provided in Table 3.8 below. This species contains 16 marginal hooks in the haptoral complex. The entire structure of the marginal hooklets could not be seen in the majority of the specimens on 40x magnification.

Table 3. 8: Detailed measurements (in μm) of the haptoral central hook complex of the hamuli of *Gyrodactylus* sp., according to Shinn et al. (2004). ND – not determined (due to flattened specimens).

	Characters measured	n	min	max	mean
1	Total length	3	302.4	368.4	337.8
2	Total width (at uterus)	3	95.1	135.5	109.1
3	Haptor length	ND	ND	ND	ND
4	Haptor width	ND	ND	ND	ND
5	Hamulus aperture distance	2	10.7	11.1	10.9
6	Hamulus proximal shaft width	2	5.4	7.6	6.5
7	Hamulus point length	2	17.4	18.6	18.0
8	Hamulus distal shaft width	2	4.7	4.9	4.8
9	Hamulus shaft length	2	20.4	25.2	22.8
10	Hamulus inner curve length	2	0.9	1.1	1.0
11	Hamulus aperture angle	2	30.1	32.4	31.3
12	Hamulus point curve angle	2	5.1	6.8	5.9
13	Inner hamulus aperture angle	2	36.0	40.4	38.2
14	Hamulus root length	2	8.5	9.3	8.9
15	Hamulus total length	2	31.0	31.3	31.2
16	Ventral bar total width	2	16.8	17.2	17.0
17	Ventral bar total length	ND	ND	ND	ND
18	Ventral bar process-to-mid length	2	3.1	3.3	3.2
19	Ventral bar median length	2	1.3	1.4	1.3
20	Ventral bar process length	ND	ND	ND	ND
21	Ventral bar membrane length	ND	ND	ND	ND
22	Marginal hook total length	1	23.9	23.9	23.9
23	Marginal hook shaft length	1	18.0	18.0	18.0
24	Marginal hook sickle length	1	5.9	5.9	5.9
25	Marginal hook sickle proximal width	1	3.9	3.9	3.9
26	Marginal hook toe length	ND	ND	ND	ND
27	Marginal hooks sickle distal width	1	3.8	3.8	3.8
28	Marginal hook aperture	1	4.3	4.3	4.3
29	Marginal hook instep/arch height	ND	ND	ND	ND

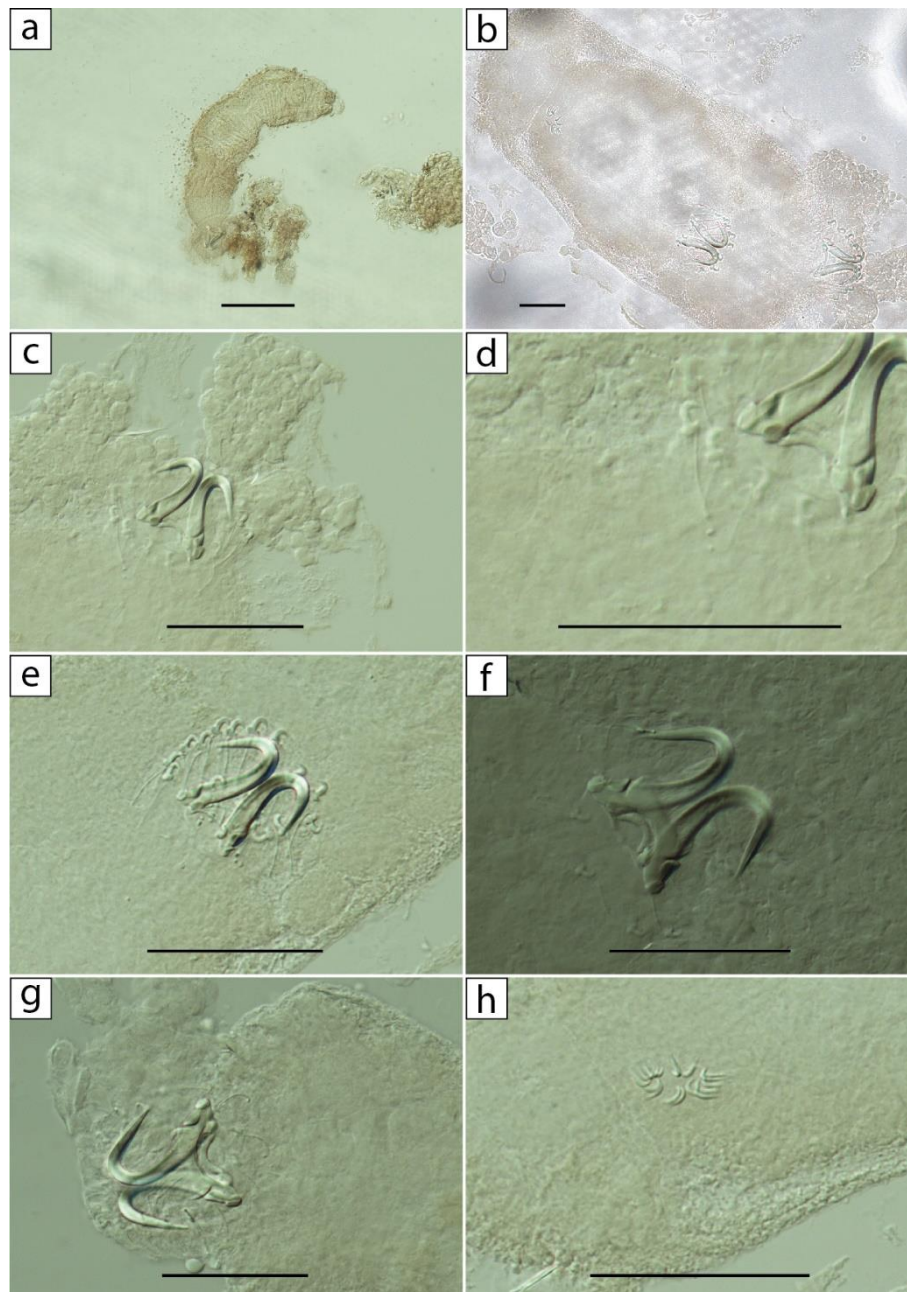


Figure 3. 14: Photomicrographs of coverslip-flattened monogeneans with the haptor central hook complex visible.

Remarks

From the subclass Monopisthocotylea, the genus *Gyrodactylus* has more than 470 species described from marine, freshwater and brackish-water hosts globally (Harris et al. 2004; Boeger et al. 2020; Christison et al. 2021; Dmitrieva et al. 2022). Of these, only 34 valid species of *Gyrodactylus* have been described from 12 African fish families (Přikrylová et al. 2012; Dos Santos et al. 2019; Christison et al. 2021), with only two species currently described from the African marine environment: *G. eyipayipi* Vaughan, Christison, Hansen & Shinn, 2010 from the greater pipefish, *Sygnathus acus* (Linnaeus), and *G. molweni* Christison, Vaughan, Shinn & Hansen, 2021 from the grey mullet, *Chelon richardsonii* (Smith) (Vaughan et al. 2010; Christison et al. 2021). Although there has been an observation of a *Gyrodactylus* species infecting

Clinus superciliosus from Table Bay, South Africa (Conference abstract: Christison et al. 2007), no description of the species has to date been published.

This specimen differs from the newly described species from South Africa, *G. molweni*, by the shape and size of the ventral bar, and the morphology of the marginal hook sickles. Dmitrieva et al. (2022) reported species of *Gyrodactylus* infecting different Bleniidae fish, a closely related fish family to Clinidae, from the Mediterranean and Black seas. Comparable morphometrics of specimens from the present study (*Gyrodactylus* sp.) and from Dmitrieva et al. (2022) (*G. sphinx* Dmitrieva & Gerashev, 2000 and *G. gerashevi* Dmitrieva, Sanna, Vodiasova, Prokhorova, Casu, Burreddu, Piras, Garippa, & Merella, 2022) can be seen in Table A. 8, where overlap is visible in the size of some structures. These specimens differ in hamulus aperture distance (10.9 vs 11.3 vs 11.0), hamulus proximal shaft width (6.5 vs 5.2 vs 5.1), hamulus point length (18 vs 15.6 vs 15.3), hamulus total length (31.2 vs 30.1 vs 29.9), ventral bar total width (17.0 vs 11.8 vs 11.5) and ventral bar median length (1.3 vs 5.4 vs 5).

Since this is not the first observation of *Gyrodactylus* sp. infecting *Clinus superciliosus*, comparison between the specimens is required to confirm the identification. Further morphological analysis of the haptor central hook complex, as well as molecular sequences of the internal transcribed spacer 2 (ITS2) are needed for a full species comparison and description.

Class: Cestoda
Order: Trypanorhyncha Diesing, 1863
Family: Tentaculairidae Poche, 1926
Genus: *Nybelinia* Poche, 1926
Species: *Nybelinia* sp.

Material examined

Trypanorhynch samples with a prevalence of 12.5% at Hermanus, 5% at Tsitsikamma, and 40% at Chintsa were collected during the present study. The morphological descriptions were based on six specimens of postlarvae Trypanorhyncha (2 specimens were used for SEM; 2 specimens were used for molecular extraction; 2 whole temporary mounts in glycerol). A total of ten *Clinus superciliosus* were infected (body cavity) with postlarvae stages of the cestode *Nybelinia* sp. on the south and southeast coasts of South Africa.

Morphological characteristics

The postlarvae has a total body length of 2311–3366 (2838) µm and width of 771–832 (801) µm at the level of the bothridia, and a body width/ length ratio of 1 : 3.5 (Figures 3.15a; 3.15b). The scolex is craspedote, length measures at 819–1015 (917) µm with bothria measuring 881–925 (903) µm (Figures 3.15 and Figure 3.16). Four slender cylindrical tentacles are present, 341–393 (367) µm long (not all tentacles were fully everted) with short, curved hooks arranged in quincunxes. Armature is homeoacanthous, homeomorphous, with uncinata metabasal hooks. The width of the tentacle at the base is 79–80 (79) µm, with a width / length ratio of 1 : 4.6 (Figure 3.16e). The bulbs are connected by the retractor muscle and are attached to the inside of

the bulb, which runs through the tentacle sheaths towards the tip of each tentacle, and measures 424–459 (442) μm in length and 119–135 (127) μm in width (Figure 3.15c). The body was terminated by a short velum 432–778 (605) μm , varying in size between specimens (Figure 3.15). The distal bothrial surface is covered with filitriches and lineate spinitriches (Figure 3.16b), while the proximal bothrial surface is covered with longer lineate spinitriches.

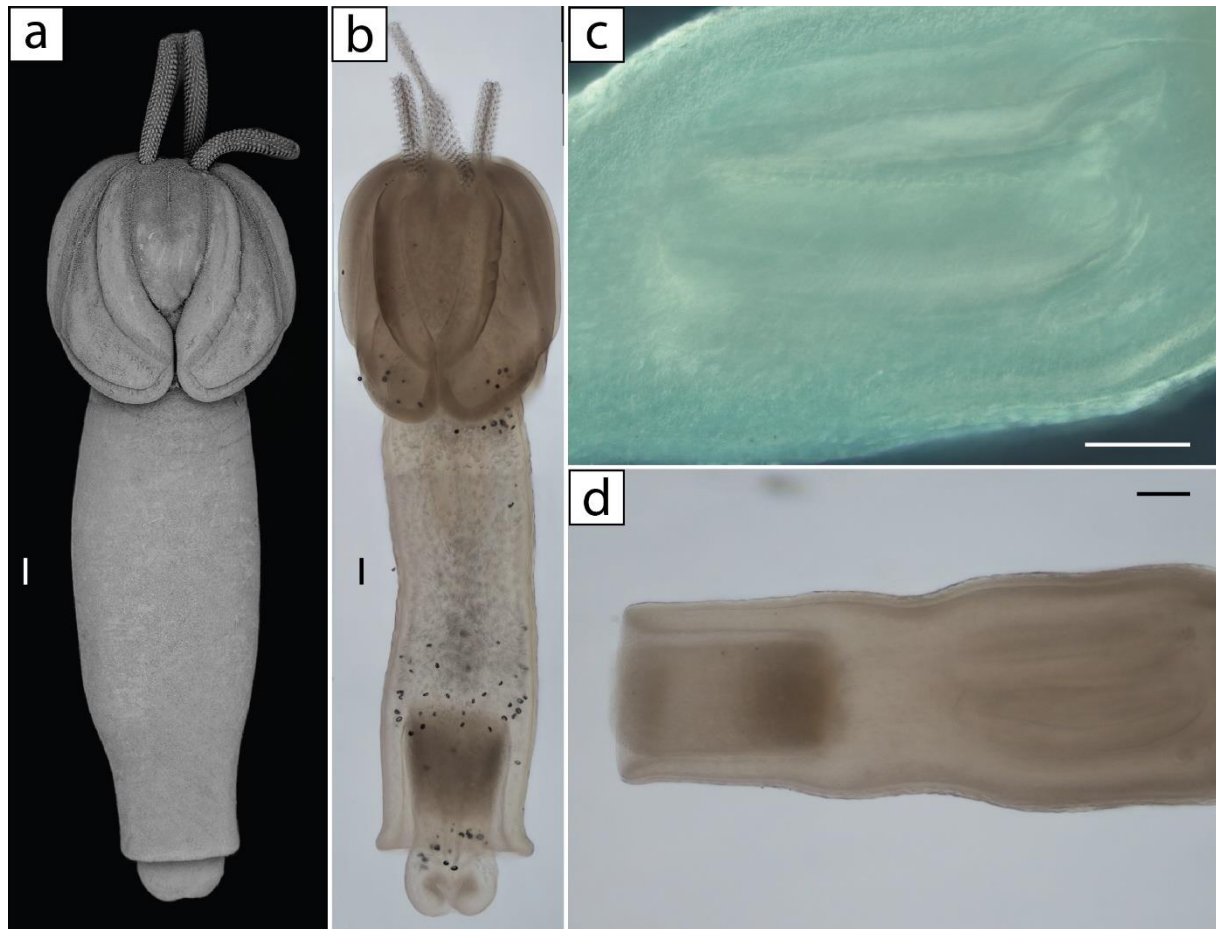


Figure 3. 15: Photomicrographs of *Nybelinia* sp. postlarvae ex *Clinus superciliosus*: a) SEM of whole specimen; b) whole mount in glycerol; c) DIC micrograph of bulbs; d) velum.

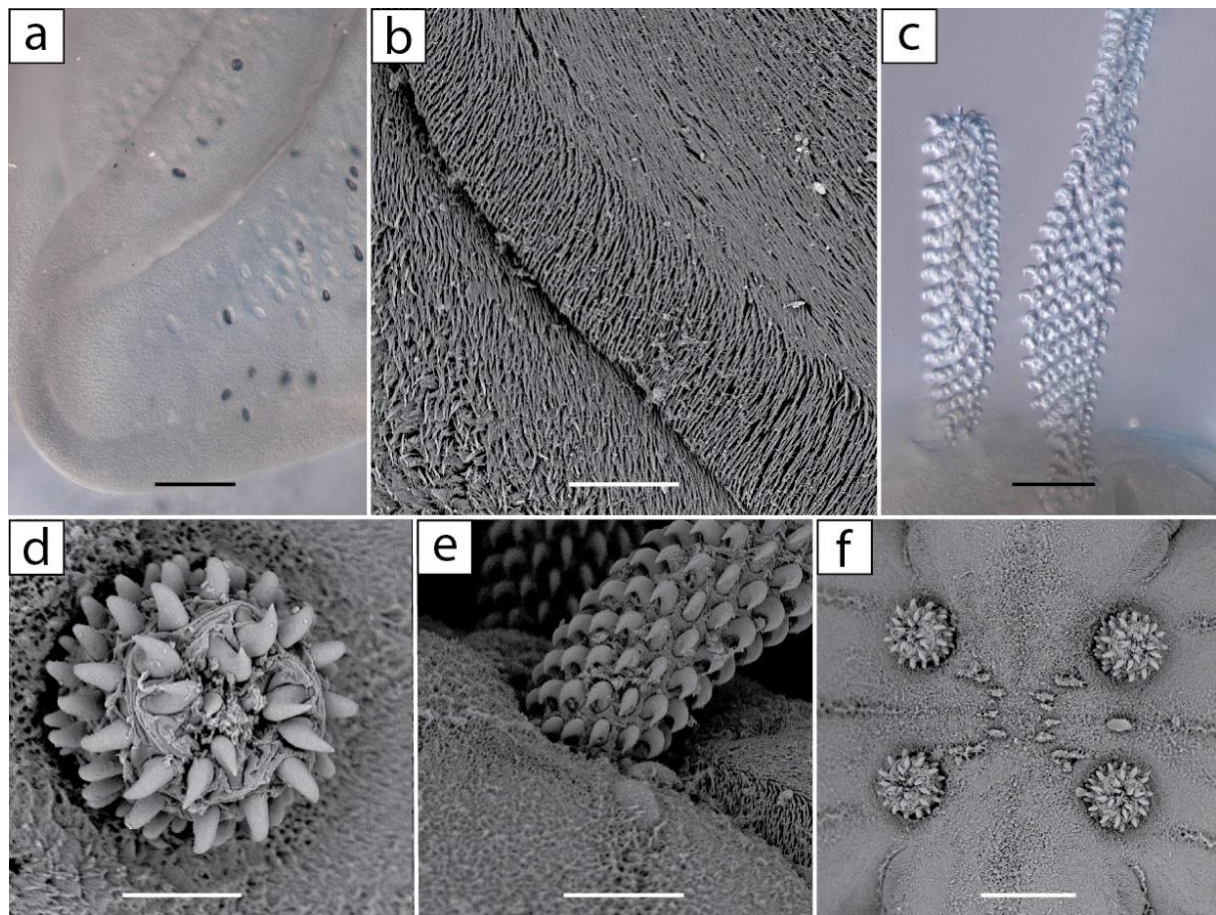


Figure 3. 16: Photomicrographs of *Nybelinia* sp. postlarvae ex *Clinus superciliosus*: a) DIC photomicrograph of the bothria; b) SEM of the bothria with filitriches visible; c) tentacles with rosethorn-shaped hooks arranged in quincunxes; d) lateral view of tentacles not fully everted; e) base of tentacle; f) lateral view of the scolex with tentacles partially everted.

Phylogenetic analysis

The 28S rDNA of two specimens was amplified by PCR using methods described by Scholz et al. 2013. Resulting sequences were 1297–1440 bp in length with no divergence between them. Novel sequences aligned with 18 cestode taxa with a total length of 1449 bp, including *Tentacularea coryphaenae* Bosc, 1802 as outgroup (Palm et al. 1997) (Table A. 9). The BLAST results indicated the highest identity similarity (99.8%) with the postlarvae of *Nybelinia* sp. 4 from Vidal-Martínez et al. (2019) (MK558805), infecting the dusky flounder from Mexico (Table 3.9). The overall genetic divergence between species of *Nybelinia* Poche, 1926 in the 28S dataset was 0–7.6% (0–119 nt). Bayesian inference (BI) and maximum likelihood (ML) criteria was used for phylogenetic analysis, applying the best substitutional model (AIC model: GTR+I+G). Computational resources CIPRES and PhyML was used for BI and ML analyses, respectively. The phylogenetic tree was constructed in FigTree v1.4.4 (Rambaut 2009) and concatenated as BI / ML (Figure 3.17).

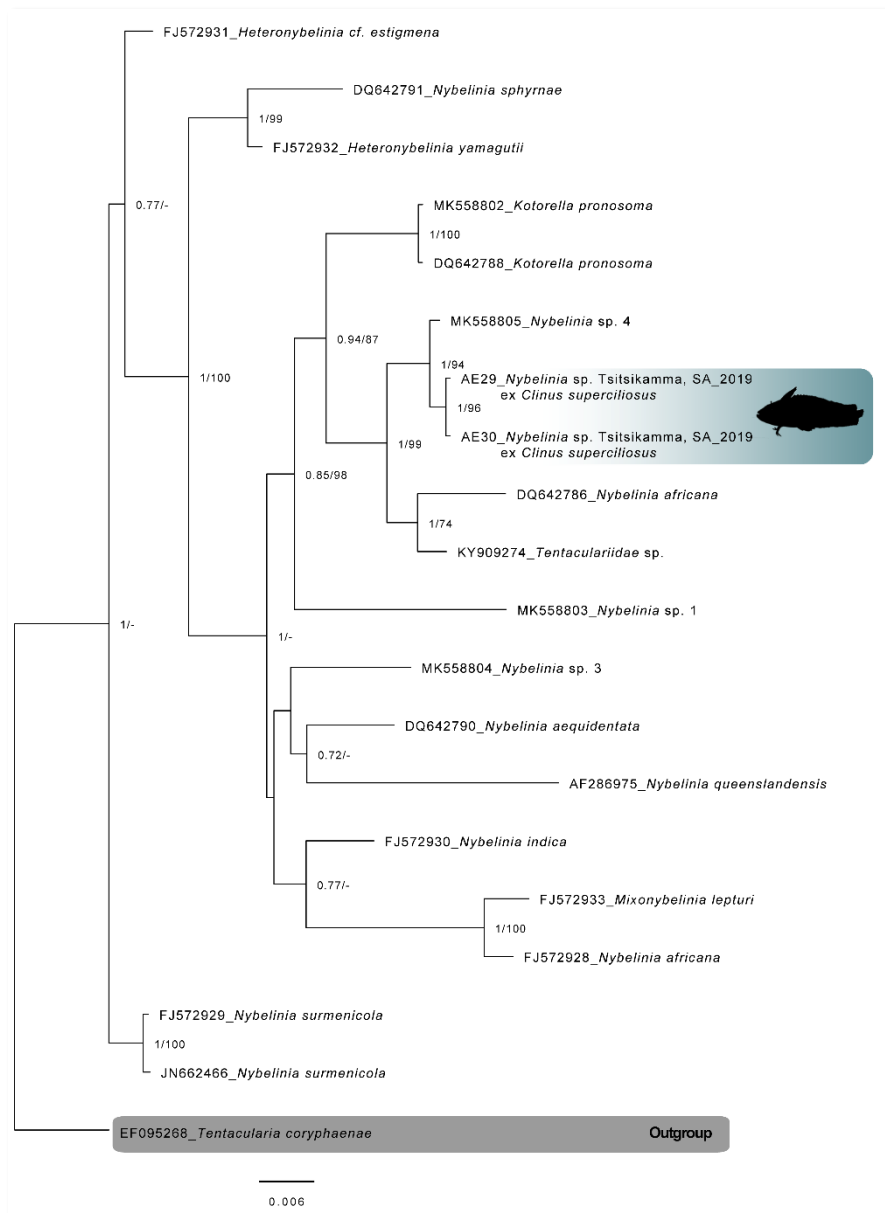


Figure 3. 17: Bayesian inference (BI) and maximum likelihood (ML) phylogenetic tree for representatives of *Tentaculariidae* spp. on 28S rDNA sequences. Novel sequences are highlighted in bold, and can be seen in the coloured box; the outgroup is displayed in the grey box. Nodal support for BI > 0.7 and ML > 70 is indicated (BI/ML); scale bar indicates the expected number of substitutions per site.

Table 3. 9: Evolutionary distances between species of *Tentaculariidae* isolated from the 28S rDNA gene region, including phylogenetic analysis, expressed as percent similarity (below diagonal) and *p*-distances (above diagonal). Newly generated sequences are indicated in the shaded row.

	Tentaculariidae species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	AE29 <i>Nybelinia</i> sp.		0.000	0.002	0.011	0.017	0.020	0.042	0.021	0.025	0.027	0.024	0.026	0.037	0.031	0.033	0.036	0.038	0.035	0.041	0.053
2	AE30 <i>Nybelinia</i> sp.	100.0		0.002	0.012	0.017	0.022	0.042	0.023	0.028	0.027	0.027	0.029	0.037	0.034	0.037	0.040	0.041	0.039	0.045	0.053
3	MK558805 <i>Nybelinia</i> sp. 4	99.8	99.8		0.009	0.018	0.021	0.040	0.020	0.026	0.026	0.024	0.027	0.038	0.030	0.032	0.036	0.037	0.034	0.041	0.053
4	KY909274 <i>Tentaculariidae</i> sp.	98.8	98.6	99.0		0.013	0.021	0.039	0.020	0.026	0.027	0.024	0.028	0.037	0.030	0.032	0.036	0.038	0.034	0.043	0.050
5	DQ642786 <i>Nybelinia africana</i>	98.0	98.0	98.0	98.5		0.023	0.037	0.023	0.029	0.031	0.029	0.031	0.037	0.034	0.035	0.041	0.044	0.042	0.047	0.052
6	MK558802 <i>Kotorella pronosoma</i>	97.8	97.5	97.7	97.9	97.5		0.039	0.000	0.024	0.028	0.024	0.027	0.037	0.029	0.030	0.033	0.034	0.034	0.042	0.051
7	JN662466 <i>Nybelinia surmenicola</i>	95.7	95.7	95.8	96.0	96.2	95.8		0.038	0.019	0.033	0.030	0.035	0.029	0.011	0.000	0.048	0.050	0.044	0.039	0.029
8	DQ642788 <i>Kotorella pronosoma</i>	97.5	97.2	97.6	97.7	97.3	99.8	95.7		0.025	0.027	0.024	0.027	0.037	0.029	0.029	0.032	0.034	0.034	0.042	0.050
9	FJ572932 <i>Heteronybelinia yamagutii</i>	97.4	97.1	97.4	97.4	96.9	97.4	98.1	97.2		0.028	0.024	0.027	0.012	0.017	0.017	0.035	0.037	0.036	0.041	0.038
10	DQ642790 <i>Nybelinia aequidentata</i>	97.2	97.1	97.2	97.2	96.7	97.0	96.7	96.9	97.1		0.020	0.025	0.037	0.030	0.032	0.035	0.038	0.038	0.038	0.048
11	FJ572930 <i>Nybelinia indica</i>	97.3	97.0	97.4	97.4	96.7	97.2	97.0	97.1	97.4	97.9		0.023	0.034	0.025	0.027	0.028	0.028	0.027	0.037	0.048
12	MK558804 <i>Nybelinia</i> sp. 3	97.2	96.9	97.2	97.0	96.6	97.1	96.5	96.9	97.2	97.5	97.5		0.042	0.030	0.029	0.035	0.036	0.036	0.041	0.052
13	DQ642791 <i>Nybelinia sphyrynae</i>	96.3	96.3	96.2	96.3	96.2	96.2	97.1	96.0	98.8	96.3	96.3	95.7		0.026	0.029	0.047	0.051	0.050	0.051	0.048
14	FJ572931 <i>Heteronybelinia cf. estigmene</i>	96.9	96.5	96.9	96.9	96.4	97.0	98.9	96.8	98.3	96.9	97.3	96.9	97.4		0.008	0.039	0.042	0.035	0.043	0.025
15	FJ572929 <i>Nybelinia surmenicola</i>	96.7	96.3	96.7	96.7	96.3	96.9	100.0	96.8	98.3	96.7	97.1	97.0	97.1	99.2		0.040	0.042	0.035	0.045	0.025
16	FJ572928 <i>Nybelinia africana</i>	96.2	95.8	96.3	96.2	95.6	96.5	95.2	96.4	96.4	96.4	97.1	96.4	95.2	96.0	95.9		0.008	0.041	0.046	0.057
17	FJ572933 <i>Mixonybelinia lepturi</i>	96.1	95.7	96.2	96.1	95.4	96.4	95.0	96.3	96.2	96.2	97.0	96.4	94.8	95.8	95.7	99.2		0.043	0.046	0.063
18	MK558803 <i>Nybelinia</i> sp. 1	96.0	95.5	96.0	96.1	95.1	96.0	94.9	95.9	95.9	95.8	96.7	96.0	94.5	96.0	96.0	95.5	95.3		0.049	0.055
19	AF286975 <i>Nybelinia queenslandensis</i>	93.8	93.2	93.8	93.7	92.7	93.6	94.6	93.6	93.8	94	94.2	94.0	92.4	93.7	93.5	93.5	93.5	92.7		0.060

Remarks

The order Trypanorhyncha (with more than 300 species) is mostly known for its scolex with four eversible armed tentacles, from which oncotaxy is used for taxonomic identification of the adult and the larval stages (Campbell and Beveridge 1994; Beveridge et al. 2014; Zhokov et al 2020). The heteroxenous life cycle of this order consists of a procercoid, plerocercus (or in some cases plerocercoid and merocercoid) followed by the adult stage, in at least three different hosts (Palm and Caira 2008).

The specimens extracted from *C. superciliosus* were postlarvae life stages. They were identified as members of the genus *Nybelinia* based on the fact that the hooks on the bothrial and antibothrial surfaces of the tentacles do not differ significantly in shape. From the genetic divergence, the specimen from the present study is most molecularly similar to *Nybelinia* sp. 4 (99.8% similarity; 3 nt differences) from the dusky flounder (Vidal-Martínez et al. 2019). However, it is unlikely to be the same species, based on the high likelihood that this is not a cosmopolitan species, the impossibility of larval stages being transported from Mexico to the southeast coast of South Africa, as well as the limited host movement between these coastal zones. Morphological descriptions were, however, not available for direct comparisons of these species. Although the 28S gene is useful for placing samples in the correct genus, COI mtDNA is needed for species level identification.

The interspecific divergence of *Nybelinia* sp. (present study) and *Nybelinia africana* Dollfus, 1960 (DQ642786) was 2% (23 nt) (Olson et al. 2010). *Nybelinia africana* was originally described from *Galeoides polydactylus* (Vahl) (lesser African threadfin) by Dollfus (1960), but has since then been reported in a variety of hosts: *Gempylus serpens*, Cuvier (larval) (see Jakob and Palm 2006); *Lamiopsis temminckii* (Müller & Henle) (adult); *Alopias superciliosus* (Lowe) (larval) (see Palm et al. 2009), as well as cephalopod species (Palm et al. 1997a; Tedesco et al. 2020), to name a few. Adult *N. africana* have also been found infecting *Carcharhinus leucas* (Müller & Henle); *Carcharhinus obscurus* (Lesueur); and *Scylliogaleus queketti*, Boulenger, from South Africa (see Palm 1999; Schaeffner and Smit 2019).

Currently, only three species of adult *Nybelinia* have been described from SA elasmobranchs: *N. africana* Dollfus, 1960; *N. schmidtii* Palm, 1999; *N. scoliodoni* (Vijayalakshmi, Vijayalakshmi & Gangdharam, 1996) (see Schaeffner and Smit 2019). Finfish, cephalopods, molluscs and crustaceans are considered preferred prey items among sharks, all of which are potential intermediate / paratenic hosts to elasmobranch cestodes. Unfortunately, not one of these three adult species have been molecularly characterised, thus hindering current studies on the life cycle of *Nybelinia* species.

Six different species of the genus *Nybelinia* have been described from a cephalopod and 16 fish species from Mozambique, specifically the south-west Indian Ocean by Palm et al. (1997). All six species have been reported as interoceanic species (Palm et al. 1997), however, not all have been molecularly characterised and none have been reported infecting Clinidae.

Some fish species are considered paratenic hosts of elasmobranch tapeworms, however, there is currently no data to link the specimen from the present study to a final host. There has been no report of trypanorhynch plerocerci infecting *C. superciliosus* to date, and detailed line-illustrations are needed for further comparison to known species and to confirm identification to lower taxonomic level. Novel sequences of larval cestodes can be used to complete life cycle studies on these parasite species. There are limited molecular studies on the cestodes of elasmobranchs (definitive host) in South Africa, and even more so on larval cestodes of teleost fish (intermediate / paratenic host). However, by obtaining molecular data from larval stages of cestodes, future studies on the life cycles of these species will be possible.

Order: Onchoproteocephalidea Caira, Jensen, Waeschenbach, Olson & Littlewood, 2014
Family: Onchobothriidae Braun, 1900
Genus: *Acanthobothrium* Blanchard, 1848
Species: *Acanthobothrium* sp.

Material examined

Of the 39 individuals collected during the present study (80% prevalence at Chintsa), 21 specimens were used for further investigation of morphological and molecular characterisation (6 whole mounts in Canada balsam; 15 specimens used for molecular extraction). *Acanthobothrium* sp. was found infecting the intestine of *Clinus superciliosus*.

Morphological characteristics

On the living specimens there is a pair of bright red pigmented spots visible posterior to the scolex (Figure 3.18a). No hooks have developed on the scolex yet. The plerocercoids measure 387–642 (524) µm in total body length and 145.9–165.0 (154.7) µm wide at the level of bothridia, tapering posteriorly (Figure 3.18). The scolex 133–170 (146) µm long and 145–165 (154) µm wide, with a large apical sucker and four elongated bothridia (Figures 3.18b and 3.18d). The apical sucker measures 77–97 (84) µm in length and 74–85 (79) µm in width, while the bothridia measures 107–132 (120) µm in length. The bothridia are sessile anteriorly with free posterior ends, divided into an apical pad and posterior loculus. The tegument is characterised by filitriches; however, SEM is needed to further characterise the various types of filitriches on the bothridial and neck surfaces as well as the surface of the strobila.



Figure 3. 18: Photomicrographs of live and fixed *Acanthobothrium* sp. plerocercoid ex *Clinus superciliosus*: a) Freshly preserved whole specimen; b) scolex and apical sucker of stained *Acanthobothrium* sp.; c) stained whole mount with apical sucker and elongated bothridia; d) elongated bothridia with filitriches visible; e) *Acanthobothrium* sp. in glycerol.

Phylogenetic analysis

The 28S rDNA of 13 morphologically similar pooled specimens were amplified by PCR using methods described by Scholz et al. (2013). The resulting four sequences were 1276–1287 bp in length, with no divergence among them. Novel sequences aligned with 24 cestode taxa with a total length of 1342 bp, including *Litobothrium janovyi* Olson & Caira, 2001, as outgroup (Waeschenbach et al. 2007) (Table A. 10). The BLAST results indicated the highest identity similarity (95.8%) with *Acanthobothrium* sp. 6A (see Jensen and Bullard, 2010) (GQ470110) that is 99.9% similar to *Acanthobothrium brevissime* Holland and Wilson (2009) (FJ357445) infecting *Ensis minor* (razor clam) from Mexico (Table 3.10). The overall genetic divergence between the species in the Onchoproteocephalidea 28S dataset was 0–18.6% (0–157 nt). Bayesian inference (BI) and ML criteria was used for phylogenetic analysis, applying the best substitutional model (AIC model: GTR+I+G). Computational resources CIPRES and PhyML was used for BI and ML analyses, respectively. The phylogenetic tree was constructed in FigTree v1.4.4 (Rambaut 2009) and concatenated as BI / ML (Figure 3.19).

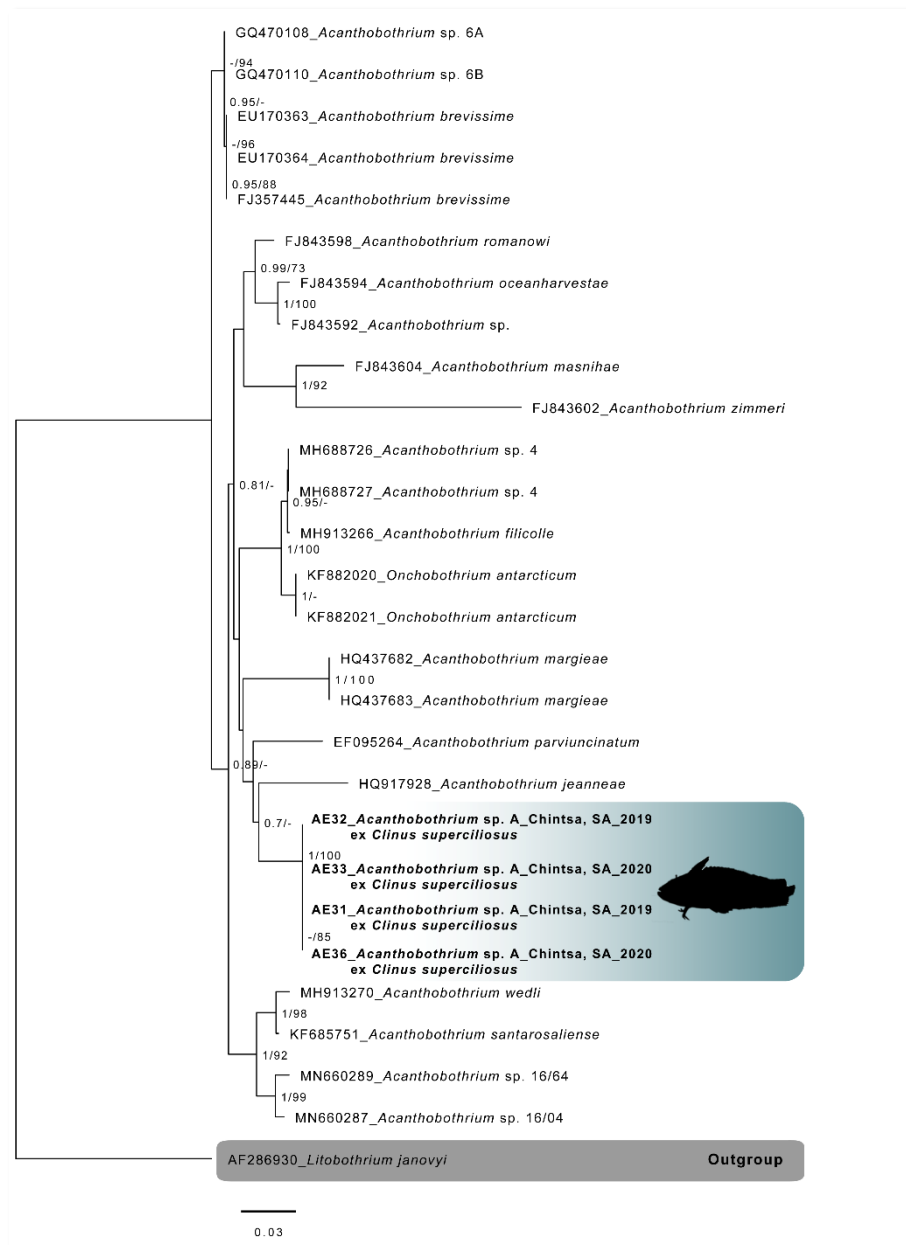


Figure 3. 19: Concatenated Bayesian inference (BI) and Maximum likelihood (ML) phylogenetic tree for representatives of Onchobothriidae spp. on 28S rDNA sequences. Novel sequences are highlighted in bold, and can be seen in the coloured box; the outgroup is displayed in the grey box. Nodal support for BI > 0.7 and ML > 70 is indicated (BI/ML); scale bar indicates the expected number of substitutions per site.

Table 3. 10: Evolutionary distances between species of Onchoproteocephalidea isolated from the 28S rDNA gene region, including phylogenetic analysis, expressed as percent similarity (below diagonal) and p-distances (above diagonal). Newly generated sequence is indicated in the shaded row.

	Onchoproteocephalidea	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	
1	AE31_Acanthobothrium sp.		0.000	0.000	0.000	0.041	0.042	0.044	0.043	0.045	0.045	0.046	0.046	0.046	0.048	0.048	0.048	0.050	0.050	0.051	0.052	0.054	0.053	0.063	0.063	0.069	0.114	0.051	0.133	
2	AE36_Acanthobothrium sp.	100.0		0.000	0.000	0.042	0.042	0.044	0.043	0.045	0.045	0.046	0.046	0.046	0.048	0.048	0.048	0.050	0.050	0.051	0.052	0.054	0.053	0.063	0.063	0.069	0.114	0.051	0.133	
3	AE33_Acanthobothrium sp.	100.0	100.0		0.000	0.042	0.042	0.044	0.043	0.045	0.045	0.045	0.046	0.046	0.048	0.048	0.048	0.050	0.049	0.051	0.052	0.054	0.053	0.063	0.063	0.068	0.113	0.051	0.133	
4	AE32_Acanthobothrium sp.	100.0	100.0	100.0		0.041	0.042	0.044	0.043	0.045	0.045	0.045	0.046	0.046	0.047	0.047	0.047	0.050	0.049	0.051	0.052	0.054	0.053	0.062	0.062	0.068	0.113	0.051	0.133	
5	GQ470108_Acanthobothrium sp. 6A	95.8	95.8	95.8	95.8		0.000	0.035	0.040	0.035	0.037	0.034	0.039	0.039	0.043	0.043	0.041	0.049	0.041	0.037	0.001	0.052	0.001	0.053	0.053	0.058	0.109	0.001	0.121	
6	GQ470110_Acanthobothrium sp. 6B	95.8	95.8	95.8	95.8	100.0		0.035	0.040	0.035	0.038	0.034	0.039	0.039	0.043	0.043	0.041	0.049	0.041	0.037	0.001	0.052	0.001	0.053	0.053	0.058	0.109	0.001	0.121	
7	FJ843598_Acanthobothrium romanowi	95.6	95.6	95.6	95.6	96.4	96.4		0.044	0.026	0.041	0.021	0.041	0.041	0.044	0.044	0.043	0.050	0.043	0.043	0.046	0.059	0.047	0.050	0.050	0.051	0.103	0.046	0.122	
8	MH913270_Acanthobothrium wedli	95.6	95.6	95.6	95.6	96.0	96.0	95.5		0.041	0.008	0.043	0.043	0.043	0.047	0.047	0.044	0.046	0.030	0.029	0.055	0.065	0.056	0.064	0.064	0.060	0.103	0.054	0.128	
9	FJ843594_Acanthobothrium oceanharvestae	95.5	95.5	95.5	95.5	96.4	96.4	97.4	95.8		0.038	0.007	0.043	0.043	0.044	0.044	0.044	0.052	0.043	0.039	0.043	0.061	0.044	0.055	0.055	0.057	0.100	0.043	0.127	
10	KF685751_Acanthobothrium santarosaliense	95.4	95.4	95.4	95.4	96.3	96.2	95.9	99.2	96.2		0.038	0.045	0.045	0.050	0.050	0.046	0.040	0.026	0.023	0.052	0.058	0.053	0.062	0.062	0.054	0.100	0.051	0.129	
11	FJ843592_Acanthobothrium sp.	95.4	95.4	95.5	95.5	96.6	96.6	97.9	95.6	99.3	96.2		0.042	0.042	0.044	0.044	0.044	0.049	0.041	0.037	0.041	0.058	0.041	0.054	0.054	0.056	0.102	0.040	0.124	
12	MH688727_Acanthobothrium sp. 4	95.2	95.2	95.2	95.3	95.9	95.9	95.7	95.5	95.6	95.3	95.6		0.000	0.011	0.011	0.002	0.053	0.046	0.046	0.043	0.061	0.044	0.059	0.059	0.069	0.111	0.043	0.135	
13	MH688726_Acanthobothrium sp. 4	95.2	95.2	95.2	95.3	95.9	95.9	95.7	95.5	95.6	95.3	95.6	100.0		0.011	0.011	0.002	0.053	0.046	0.046	0.043	0.061	0.044	0.059	0.059	0.069	0.111	0.043	0.135	
14	KF882020_Onchobothrium antarcticum	95.1	95.1	95.1	95.1	95.5	95.5	95.5	95.1	95.5	94.8	95.4	98.9	98.9		0.000	0.011	0.056	0.051	0.051	0.049	0.066	0.050	0.062	0.062	0.068	0.111	0.048	0.135	
15	KF882021_Onchobothrium antarcticum	95.1	95.1	95.1	95.1	95.5	95.5	95.5	95.1	95.5	94.8	95.4	98.9	98.9	100.0		0.011	0.056	0.051	0.051	0.049	0.066	0.050	0.062	0.062	0.068	0.111	0.048	0.135	
16	MH913266_Acanthobothrium filicollae	95.1	95.1	95.1	95.1	95.7	95.7	95.6	95.4	95.4	95.1	95.5	99.8	99.8	98.9	98.9		0.054	0.047	0.047	0.045	0.062	0.046	0.061	0.061	0.071	0.112	0.044	0.137	
17	EF095264_Acanthobothrium parviuncinatum	94.9	94.9	94.9	95.0	95.0	95.0	94.9	95.2	94.7	95.8	95.0	94.7	94.7	94.3	94.3	94.5		0.050	0.051	0.066	0.067	0.067	0.059	0.059	0.073	0.107	0.065	0.137	
18	MN660289_Acanthobothrium sp. 16/64	94.8	94.8	94.8	94.9	95.8	95.8	95.5	96.9	95.5	97.3	95.7	95.0	95.0	94.5	94.5	94.9	94.7		0.012	0.056	0.063	0.057	0.067	0.067	0.063	0.106	0.055	0.136	
19	MN660287_Acanthobothrium sp. 16/04	94.7	94.7	94.7	94.7	96.0	96.0	95.5	96.8	95.9	97.4	96.1	95.1	95.1	94.5	94.5	94.9	94.6	98.7		0.051	0.063	0.052	0.066	0.066	0.060	0.103	0.050	0.132	
20	FJ357445_Acanthobothrium brevissime	94.7	94.7	94.7	94.7	99.9	99.9	95.2	94.5	95.5	94.8	95.8	95.4	95.4	94.8	94.8	95.3	93.2	94.1	94.4		0.062	0.000	0.069	0.069	0.076	0.136	0.000	0.166	
21	HQ917928_Acanthobothrium jeanneae	94.6	94.6	94.6	94.6	94.7	94.7	94.1	93.4	93.9	94.1	94.2	93.8	93.8	93.2	93.2	93.6	93.2	93.5	93.5	93.7		0.063	0.074	0.074	0.078	0.124	0.061	0.154	
22	EU170364_Acanthobothrium brevissime	94.4	94.4	94.4	94.4	99.7	99.7	95.0	94.3	95.3	94.6	95.6	95.2	95.2	94.6	94.6	95.0	92.9	93.9	94.2	99.9	93.4		0.070	0.070	0.077	0.139	0.000	0.170	
23	HQ437683_Acanthobothrium margieae	93.7	93.7	93.8	93.8	94.6	94.6	95.0	93.5	94.5	93.8	94.6	93.9	93.9	93.7	93.7	93.8	94.1	93.1	93.1	93.0	92.6	92.7		0.000	0.075	0.120	0.068	0.137	
24	HQ437682_Acanthobothrium margieae	93.7	93.7	93.7	93.7	94.6	94.6	94.9	93.5	94.5	93.7	94.5	93.9	93.9	93.6	93.6	93.7	94.0	93.1	93.1	93.0	92.6	92.7	99.9		0.075	0.120	0.068	0.137	
25	FJ843604_Acanthobothrium masniae	93.0	93.0	93.0	93.0	94.0	94.0	94.8	93.8	94.1	94.3	94.3	92.8	92.8	92.9	92.9	92.7	92.5	93.3	93.6	92.2	92.0	91.9	92.3	92.3		0.092	0.075	0.142	
26	FJ843602_Acanthobothrium zimmeri	87.8	87.8	87.9	87.9	88.2	88.1	88.9	88.8	89.1	89.1	89.0	88.0	88.0	87.9	87.9	87.8	88.5	88.4	88.7	86.0	86.8	85.6	87.2	87.1	89.8		0.134	0.172	
27	EU170363_Acanthobothrium brevissime	81.4	81.4	81.4	81.4	85.8	85.8	81.9	81.3	82.2	81.5	82.4	82.1	82.1	81.6	81.6	81.9	80.2	81.0	81.2	99.9	80.6	99.9	80.0	80.0	79.4	74.1		0.164	
28	AF286930_Litobothrium janovyi	85.1	85.1	85.0	85.1	86.2	86.2	86.2	85.5	85.7	85.4	85.9	84.8	84.8	84.8	84.8	84.6	84.7	84.6	85.1	81.8	83.0	81.3	84.7	84.6	84.1	80.6	70.6		

Remarks

A total of 210 valid species of *Acanthobothrium* are recognised globally, with the majority of species yet to be discovered (Caira and Jensen 2017; Tedesco et al. 2020; van der Spuy et al. 2022). The largest gaps in the distribution of this family are the coast of South America, Africa and the Arabian Peninsula (Caira and Jensen 2017). The biodiversity rich coast of Africa is considered one of the potential hot spots for undescribed *Acanthobothrium* species, with a total of 204 elasmobranchs (definitive hosts) (Ebert and van Hees 2015) and only 19 (9%) of these that have been examined for cestodes (Schaeffner and Smit 2019; van der Spuy et al. 2022). The life cycles of these heteroxenous parasites are still understudied with many intermediate hosts unknown. This genus is characterised by a scolex with four bothridia (van der Spuy et al. 2020).

Compared to sequences available on GenBank, specimens of *Acanthobothrium* from the present study are 95.8% (53 nt) similar to *Acanthobothrium* sp. 6A; 6B (see Jensen and Bullard 2010) (GQ470108; GQ470110) from Mexico (USA); however, based on the phylogenetic tree, they are more similar to *A. jeanneae*. The general morphology of *Acanthobothrium* sp. 16/64; 16/04 (MN660289; MN660287) collected in the Central Mediterranean from *Octopus vulgaris* (Tedesco et al. 2020), closely resembles the specimen from the present study. This species of octopus is very common within South African waters, however, there is no evidence that both lineages are conspecifics.

There are currently only seven species of adult *Acanthobothrium* known from South Africa, all of which have been described in the past two years from *Raja straeleni* Poll and *Rostroraja alba* (Lacépède) (see van der Spuy et al. 2020, 2022). As with many cestode species, limited molecular data is available for comparison (only 7.6% of the 210 known species) (van der Spuy et al. 2022), and to assist in elucidating life cycles. These elasmobranch hosts feed on cephalopods, finfish and benthic crustaceans, which are all possible intermediate hosts to elasmobranch cestodes. Further morphological characterisation (SEM) and comparative sequences for different gene regions (particularly mitochondrial markers) are needed to confirm species identification of specimens of *Acanthobothrium* collected in the present study.

3.3.2.3 Characterisation of Acanthocephalans

Phylum:	Acanthocephala Koelreuter, 1771
Class:	Palaeacanthocephala Meyer, 1931
Order:	Polymorphida Petrochenko, 1956
Family:	Polymorphidae Meyer, 1931
Genus:	<i>Corynosoma</i> Lühe, 1904
Species:	<i>Corynosoma</i> sp.

Material examined

Cystacanths were collected in the present study (Langebaan marina (5.3% prevalence), Tsitsikamma (55% prevalence) and Chintsa (55% prevalence)) and some individuals were used for subsequent investigations (two excysted, and one encysted specimen as whole temporary

mounts in lactophenol for DIC imaging). Cystacanths were collected from the mesentery in the body cavity of *Clinus superciliosus*.

Morphological characteristics

Cystacanths in cyst measure 2.1 mm in length and 1.1 mm in width (Figure 3.20a), while excysted specimens measure 2.7 mm long and 1.0 mm wide (Figure 3.20b). The trunk length is 2.1 mm long, and the proboscis length (PL) ranging from 0.61–0.83 (0.72) mm ($n = 3$), making up a ratio of 1 : 3.8 TL / PL. The maximum width of the proboscis was 0.25–0.31 (0.28) mm, while the width at the base was 0.14–0.22 (0.19) mm (Figure 3.18).

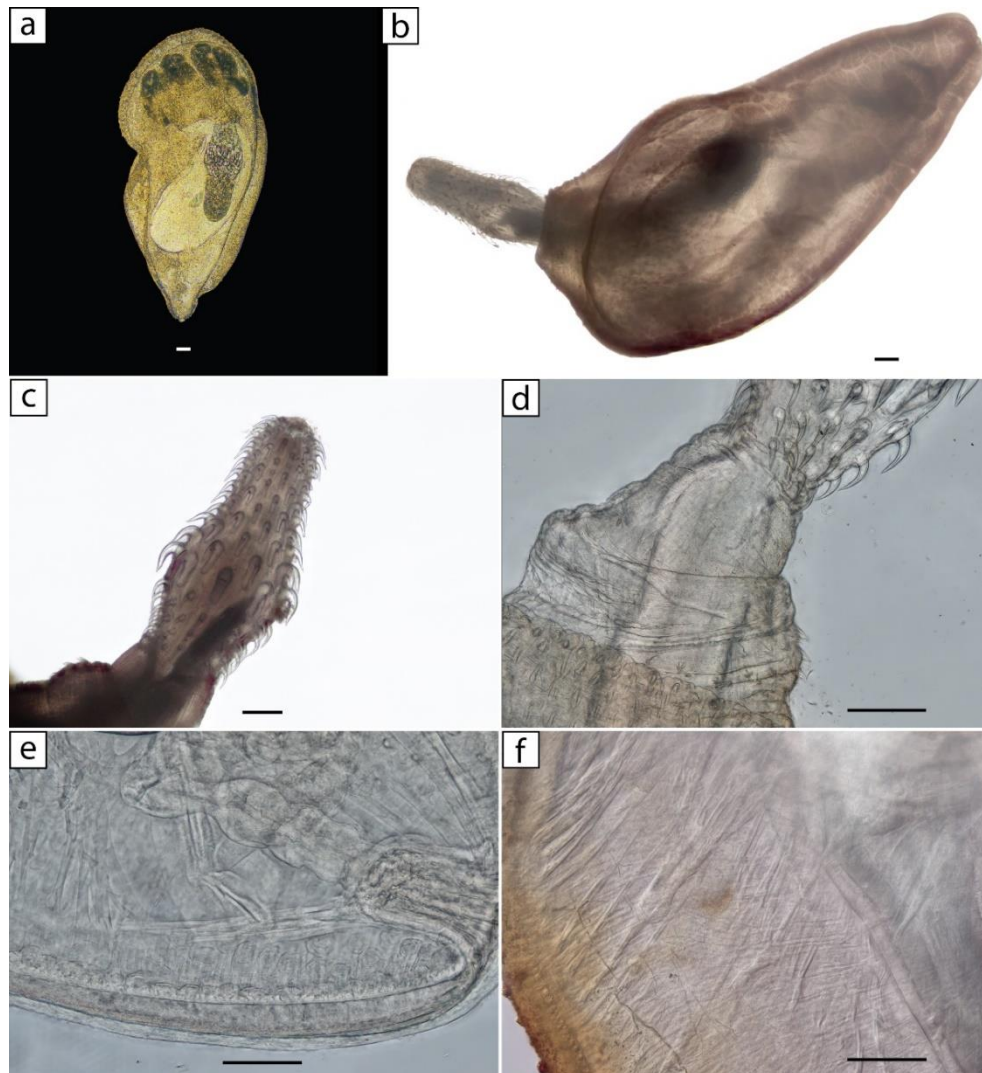


Figure 3. 20: a) Cystacanth extracted from *Clinus superciliosus* at the Tsitsikamma locality in 2019; b) Z-dimensional stacked and merged photomicrograph of excysted *Corynosoma* sp. taken using a Nikon Eclipse light microscope; c) proboscis showing the arrangement of the hooks; d) neck region of *Corynosoma* with trunk spines; e) posterior region; d) muscle fibres from the proboscis receptacle.

Remarks

Palaeacanthocephala is the largest and most diversity rich class of Acanthocephala infecting all vertebrates (Smales 2015). The family Polymorphidae Meyer, 1931 consists of 12 genera, with aquatic birds and mammals as definitive hosts (Smales 2015). Larvae of *Corynosoma* species have been reported from *Thyrstites atun* (Euphrasén) (snoek) from South Africa's west and south

coasts (Nunkoo et al. 2016; Halajian et al. 2018). Various researchers have documented morphological similarities between species of this genus (Johnston and Edmonds 1953; Sardella et al. 2005; Lisitsyna et al. 2019), where species differentiation of *Corynosoma australe* Johnston, 1937 and *Corynosoma obtusens* Lincicome, 1943 was based on the location of the spines on the ventral surface of the female body (Lisitsyna et al. 2019). Other differentiating structures include the length of the trunk and proboscis (Sinisalo and Valtonen 2003). According to various authors (Lisitsyna et al. 2019 and references within), these species have been reported to infect amphipod crustaceans as intermediate hosts, and various marine fish species as paratenic hosts (this refers to a host not needed for the development of the parasite, but essential in maintaining the parasite's life cycle by enabling the appropriate food-web linkages). Definitive hosts of the so far described 43 *Corynosoma* species (Amin 2013), are mammals and piscivorous birds. Additional studies supported the findings that morphological characteristics alone cannot be used for species differentiation, but an integrated approach of both morphological and molecular techniques is needed for precise and efficient identification (Lisitsyna et al. 2019). Further morphological characterisation of the cystacanth, as well as comparative sequences are needed to confirm the identification to a lower taxonomic level.

Order: Echinorhynchida Southwell & Macfie, 1925
Family: Arhythmacanthidae Yamaguti, 1935
Genus: Arhythmacanthidae gen. sp. 1

Material examined

Only 2.2% prevalence was noted for this species, from Langebaan marina and Chintsa (2019). The morphological description is based on two lactophenol cleared adult male specimens collected from the intestine. The measured trunk length does not include the proboscis, neck and bursa.

Morphological characteristics

Arhythmacanthidae gen. sp. 1 had a total length of 1.4 mm, and maximum width of 0.2 mm at the level of the testis. The trunk length is 1.3 mm, where the anterior end of the trunk at the junction near the neck is covered in rows of uniform spines (Figure 3.21a and 3.21b), and no spines were observed on the posterior end of the trunk (Figure 3.21 g). The proboscis is cylindrical to slightly claviform with an abrupt change from apical armature to subapical armature; 3–4 large hooks with roots not visible (apical), and 3 small hooks (subapical). The total length of the extended proboscis (n = 2) ranged from 118.2–250.6 (184.4) μm with a maximum width of 63.2–150.7 (106.9) μm , and 53.3–119.1 (86.2) μm wide at the base of the neck. Lemnisci of medium length, longer than the proboscis receptacle but not to the level of the testes, and two testes of oval shape, anterior testis 127.6 x 102.6 μm and posterior testis 130.7 x 108.5 μm (Figure 3.21d and 3.21e). Three cement glands are visible posterior to the testes (Figure 3.21f) and S  fftigen's pouch is pyriform (pear-shaped) (Figure 3.21 d).



Figure 3. 21: a) Photomicrograph of live *Arhythmacanthidae* gen. sp. 1 collected from *Clinus superciliosus* at Chintsa (2020); b) proboscis; c) spines on the trunk; d) Z-dimensional stacked and merged photomicrograph of adult male *Arhythmacanthidae* gen. sp. 1; e) male testis; f) cement glands; g) posterior end, taken using Nikon Eclipse light microscope.

Genus: **Arhythmacanthidae gen. sp. 2**

Material examined

These specimens were collected from *Hermanus* with a 5.4% prevalence in the intestine, where identification was based on examination of four adult specimens of *Arhythmacanthidae*. Three male and one female specimen was cleared in lactophenol for DIC imaging.

Morphological characteristics

Arhythmacanthidae gen. sp. 2 measured 3 mm long and 0.59 mm wide at the level of the anterior testis, with a trunk length of 2.7 mm (Figure 3.22a). The proboscis (n = 3) is claviform with an unarmed basal neck when fully everted, the former measuring 211.9–284 (243.4) μm long and 221.6–276.3 (240.9) μm at the maximum width, while the width of the proboscis at the base of the neck measured 180.8–241.6 (208.8) μm wide (Figure 3.22b). Two oval shaped testes were observed (Figure 3.22a) with the anterior testis measuring between 310.9–386.9 x 280–336.1 μm (n = 3) and the posterior testis measuring 287.7–383 long x 263.9–320.8 μm wide (n = 2). Three cement glands were observed (Figure 3.22a). The everted bursa was hemispherical in shape with circumbursal sensory receptors (Figure 3.33c). One female was observed bearing eggs of 54.2–58.2 x 18.1–21.6 μm (n = 3) in size.

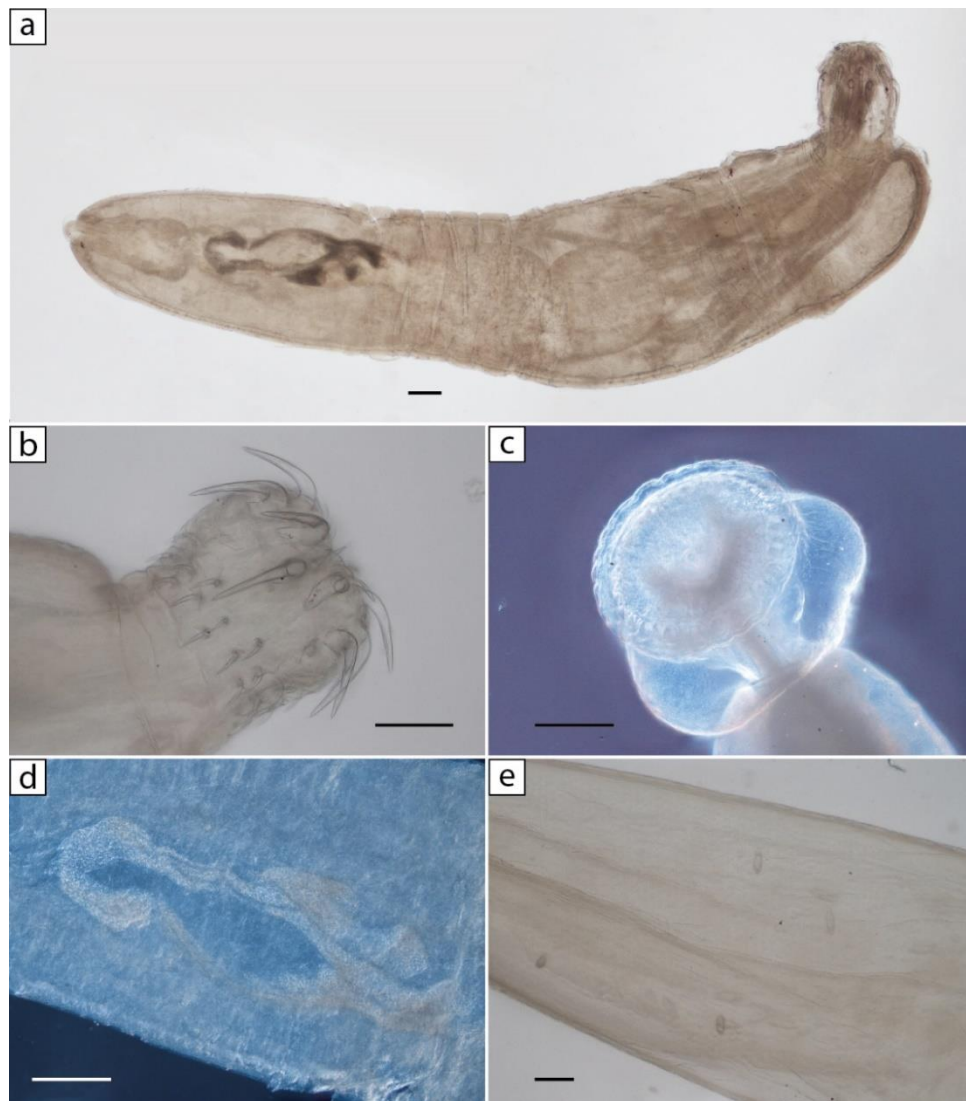


Figure 3. 22: Nikon Eclipse photomicrograph of: a) adult *Arhythmacanthidae* gen. sp. 2 from *Clinus superciliosus*; Z-dimensional stacked and merged photomicrograph of adult male *Arhythmacanthidae* gen. sp. 2; b) the proboscis; c) bursa; d) cement glands; e) female *Arhythmacanthidae* gen. sp. 2 with eggs.

Remarks

This family of Acanthocephala are small to medium sized worms, with a short proboscis. The hooks are arranged in longitudinal rows with an abrupt transformation from apical to subapical hooks (Smales 2015). Seven genera have been described from this family with both freshwater and marine fish as definitive hosts (Smales 2015). Species of the genus *Acanthocephaloides* have only been reported from *Gobius bucchichi* Steindachner from the Mediterranean Sea. Based on the checklist of acanthocephalan parasites from South Africa by Halajian et al. (2018), no species of *Acanthocephaloides* have been reported from South Africa marine fish species, however, Bray et al. (1988) reported *Acanthocephaloides cyrusi* ex *Pegusa nasuta* (*Solea bleekeri*) (Pallas) and *Pomadasys commersoni* (Lacépède) from Lake St Lucia, KwaZulu-Natal.

Two morphotypes of this family were collected during the present survey. They differ from each other in the following characters: the overall size of the specimens was distinctly different even though both specimens were fully developed; the arrangement of the hooks on proboscis; the spines on the trunk; and the Säftigen's pouch differing in shape. The arrangement of the hooks on the proboscis is one of the distinguishing factors between genera of this family (Smales and Weaver 2015), where *Arhythmacanthidae* gen. sp. 1 has proboscis hooks increasing in size towards the anterior end, and *Arhythmacanthidae* gen. sp. 2 has proboscis hooks in longitudinal rows alternating between 2 and 3 hooks, at least 1 large, per row.

More specimens and further morphological characterisation and comparison, as well as comparative sequences are needed to confirm the identification to a lower taxonomic level. This is believed to be the first host and distribution record of *Acanthocephala* parasitising the family Clinidae, more specifically *Clinus superciliosus*.

Family: Rhadinorhynchidae Lühe, 1912
Genus: Rhadinorhynchidae gen. sp.

Material examined

One morphotype from the family Rhadinorhynchidae was observed only once in the field (Figure 3.23) and no further classification could be performed. The specimen of unknown sex, was collected from Chintsa during the 2019 survey, in the intestine of *Clinus superciliosus*.

Morphological characteristics

Limited morphological features are visible from the single photomicrograph taken in the field. The specimen has a long, cylindrical proboscis, curved ventrally and tapered to the anterior end. The trunk is orange in colour and segmented towards the posterior end.



Figure 3. 23: Photomicrograph of live *Rhadinorhynchidae* gen. sp. collected from Chintsa 2019.

Remarks

Seven species of Rhadinorhynchidae have been reported infecting marine fish in Africa along the west and south coasts: *Rhadinorhynchus africanus* (Golvan, Houin & Deltour, 1963); *R. atheri* (Farooqi, 1981); *R. cadenati* (Golvan & Houin, 1964) Golvan, 1969; *R. camerounensis* Golvan, 1969; *R. saltatrix* Troncy & Vassiliades, 1973; *R. capensis* Bray, 1974; and *R. lintoni* Cable & Linderorth, 1963 (see Lisitsyna et al. 2019a). No host records on the family Clinidae have been reported. This genus is known for its long trunk with a single field of 19–26 irregular circular rows of spines. The proboscis is long and cylindrical, curved ventrally and wider anterior than posterior, with 9 or 10 longitudinal rows of 28–32 hooks each (Lisitsyna et al. 2019a). There are four cement glands, in two pairs of differing lengths, with the copulatory bursa hemispherical (Lisitsyna et al. 2019a). The genital pore is subterminal in both sexes (Lisitsyna et al. 2019a).

Only a single specimen was collected during all of the surveys, and no further analyses could be performed to determine the identification. Further surveys for more specimens are needed in order to complete morphological characterisation and comparison, as well as comparative sequences to confirm the identification to a lower taxonomic level.

3.3.2.4 Characterisation of Leeches

Phylum:	Annelida Lamarck, 1802
Class:	Clitellata Michaelson, 1919
Order:	Rhynchobdellida Blanchard, 1894
Family:	Piscicolidae Johnston, 1865
Genus:	<i>Pterobdella</i> Kaburaki, 1921
Species:	<i>Pterobdella arugamensis</i> (de Silva, 1963)

Material examined

This taxon was recorded from the fins of *Clinus superciliosus* at Tsitsikamma (25% prevalence) and Chintsa (15% prevalence), South Africa. The morphological description and molecular analysis are based on five specimens (four specimens were used for whole temporary mounts in glycerol for DIC and one of the four was also used for SEM; one specimen was used for molecular characterisation).

Morphological characterisation

Specimens measured a total length (TL) of 1.9–6 (3.6) mm, width (W) 0.3–0.8 (0.54) mm and ratio (TL : W) 1 : 6.6 (Figure 3.24d and 3.24e). The body of the specimen was divided into a subcylindrical trachelosome and longer and wider urosome. The anterior sucker had a diameter of 157–253 (210.5) µm, while the posterior sucker measured 241–518 (408) µm. The ratio of posterior to anterior suckers was 1 : 2 (Figures 3.24a; 3.24c; 3.24f; 3.24g; 3.24h). Body pigmentation was noted as cross bars anteriorly; with nephridiopores on the lateral line; lacking branching digitiform or leaf-like branchiae (Figure 3.24). Body without tubercles, tapering towards the anterior sucker.

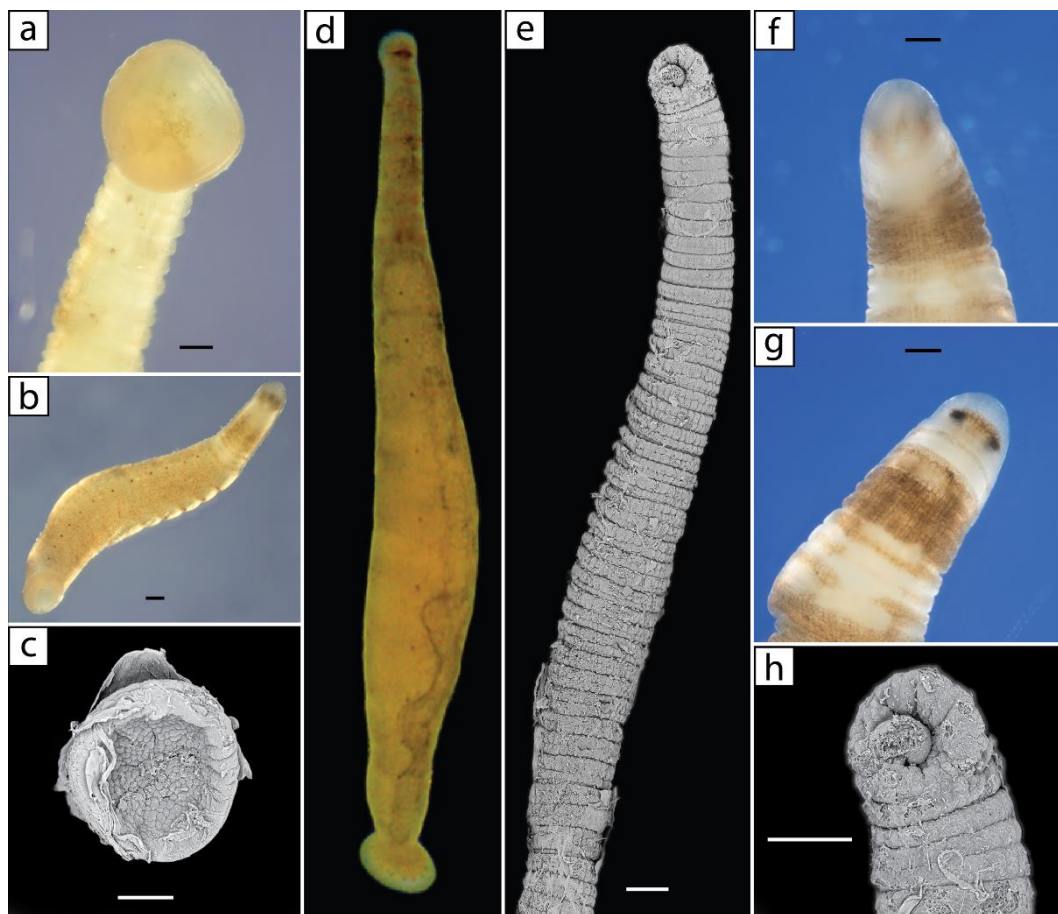


Figure 3. 24: Scanning electron microscopy (SEM) and differential interference contrast (DIC) photomicrographs of *Pterobdella arugamensis* collected from *Tsitsikamma*: a) ventral view of posterior sucker; b) full ventral view; c) SEM photomicrograph of posterior sucker; d) full habitus, DIC photomicrograph; e) SEM photomicrograph of anterior end of *P. arugamensis*; f) ventral view of anterior sucker; g) dorsal view of anterior sucker, with eyespots and body pigmentation; h) SEM photomicrograph of ventral view of anterior sucker.

Genus: **Piscicolidae gen. sp.**

Material examined

Based on *Piscicolidae* gen. sp., $n = 2$ (one specimen was used for SEM; one specimen was used for molecular extraction). No voucher was maintained for DIC photographs as the entire specimen was used for SEM and molecular analysis, respectively. This specimen was recorded infecting the fins of *Clinus superciliosus* at Langebaan marina (10.5% prevalence) on the west coast of South Africa.

Morphological characterisation

Piscicolidae gen. sp. with 3.2–4.9 (4.1) mm TL and 0.4–0.5 (0.47) mm W and ratio (TL : W) 1 : 8.7 (Figure 3.25a and 3.25b). The body of the specimen was divided into an indistinct trachelosome and urosome. The anterior sucker measured 322–537 (430) μm , while the posterior sucker measured 492–671 (582) μm . The ratio of posterior to anterior suckers was 1 : 1.4 (Figures 3.25a and 3.25d). No pigmentation was noted; lacking branching digitiform or leaf-like branchiae, and tubercles (Figure 3.25).

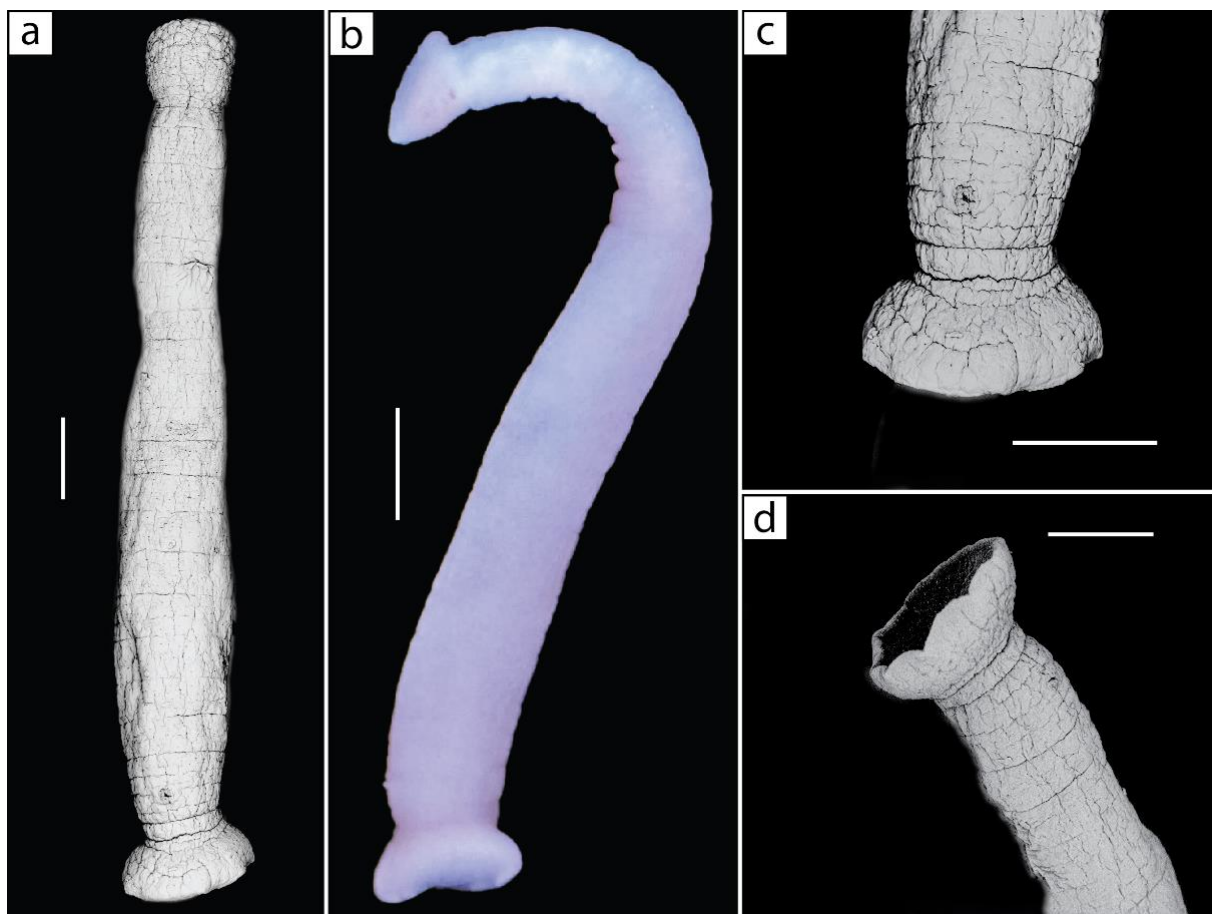


Figure 3. 25: Scanning electron microscopy (SEM) and differential interference contrast (DIC) photomicrographs of *Piscicolidae* gen. sp. from Langebaan, a) full SEM photomicrograph of leech specimen; b) full DIC photomicrograph of leech specimen, c) posterior sucker, and d) anterior sucker.

Phylogenetic analysis

Two morphologically different specimens were cut in half for DNA extraction. Extracted samples were then subjected to multiple primer sets based on previous studies to amplify *Trypanosoma* (18S rRNA) (Maslov et al. 1996; McInnes et al. 2009; Botero et al. 2013; Hayes et al. 2014), *Haemogregarina* (18S rRNA) (Kvičerová et al. 2008) (results not shown) and Annelida (COI) (Ravi and Yahaya 2017; Utevsky et al. 2021). The resulting COI sequences for Annelida (604–652 bp) were verified to belong in the family Piscicolidae using BLAST, and assembled and aligned using the MUSCLE alignment tool in Geneious. The final alignment consisted of 31 sequences with a total length of 631 bp (Table A. 11; Table 3.11). Invertebrate mitochondrial translation frame 1 was used to remove any stop codons from the alignment. The best AIC nucleotide substitution model was GTR+I+G. Maximum likelihood (ML) (Figure 3.26) analysis was performed to infer phylogenetic relationships between known species and newly generated sequences using Geneious and MEGA7 (Tamura et al. 2021). It was not possible, based on the phylogenetic analysis, to identify the collected specimens to a lower taxonomic level due to inconsistent topography within the BI and ML trees.

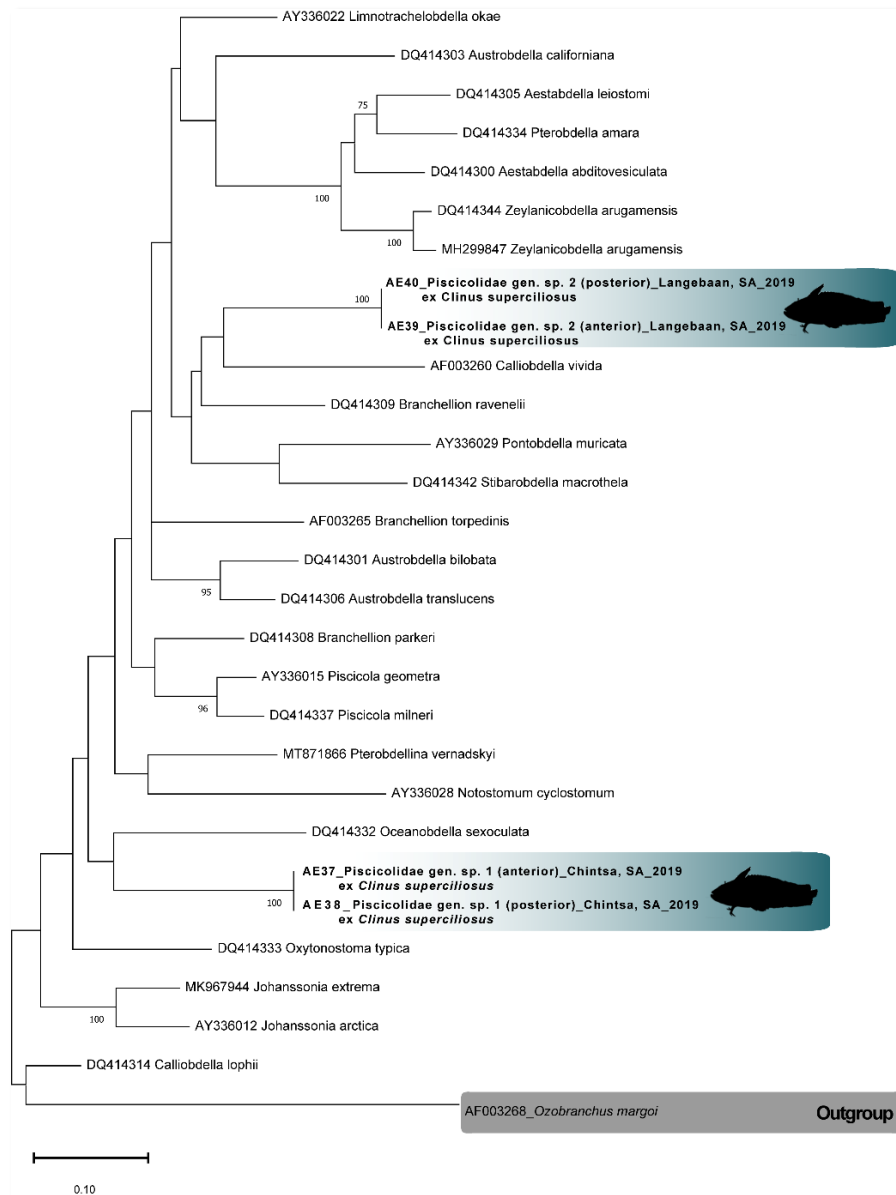


Figure 3. 26: Maximum likelihood (ML) phylogenetic tree for representatives of Piscicolidae on COI mtDNA sequences. Novel sequences are highlighted in bold, and can be seen in the coloured box; the outgroup is displayed in the grey box. Nodal support for ML > 70 is indicated; scale bar indicates the expected number of substitutions per site.

Table 3. 11: Evolutionary distances between species of *Piscicolidae* isolated from the COI mtDNA gene region, including phylogenetic analysis, expressed as percent similarity (below the diagonal) and *p*-distances (above the diagonal). Newly generated sequence is indicated in the shaded row.

Piscicolidae species	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
1 AE37 <i>Pterobdella arugamensis</i> (anterior)		0.000	0.197	0.193	0.141	0.150	0.146	0.146	0.149	0.152	0.152	0.156	0.157	0.157	0.161	0.162	0.167	0.170	0.173	0.173	0.175	0.178	0.178	0.183	0.185	0.183	0.186	0.196
2 AE38 <i>Pterobdella arugamensis</i> (posterior)	100.0		0.197	0.193	0.141	0.150	0.146	0.146	0.150	0.153	0.153	0.156	0.158	0.158	0.162	0.162	0.167	0.170	0.174	0.174	0.175	0.178	0.178	0.184	0.185	0.184	0.186	0.196
3 AE39 <i>Piscicolidae</i> gen. sp. (anterior)	80.3	80.3		0.000	0.159	0.164	0.129	0.158	0.169	0.154	0.138	0.189	0.147	0.144	0.149	0.141	0.169	0.143	0.159	0.149	0.159	0.158	0.177	0.179	0.174	0.156	0.166	0.169
4 AE40 <i>Piscicolidae</i> gen. sp. (posterior)	80.7	80.7	100.0		0.157	0.158	0.131	0.157	0.167	0.149	0.140	0.181	0.146	0.144	0.147	0.138	0.161	0.140	0.164	0.144	0.155	0.152	0.177	0.173	0.177	0.154	0.164	0.161
5 DQ414314 <i>Calliobdella lophii</i>	85.9	85.9	84.1	84.3		0.139	0.132	0.120	0.153	0.129	0.126	0.146	0.113	0.131	0.128	0.126	0.149	0.158	0.162	0.168	0.173	0.150	0.156	0.171	0.174	0.179	0.180	0.155
6 AY336015 <i>Piscicola geometra</i>	85.0	85.0	83.6	84.2	86.1		0.102	0.153	0.123	0.057	0.129	0.164	0.140	0.121	0.131	0.127	0.153	0.135	0.170	0.142	0.142	0.156	0.167	0.149	0.159	0.159	0.150	0.145
7 DQ414308 <i>Branchellion parkeri</i>	85.4	85.4	87.1	86.9	86.8	89.8		0.147	0.126	0.114	0.123	0.155	0.134	0.111	0.129	0.111	0.146	0.108	0.161	0.126	0.155	0.144	0.132	0.148	0.156	0.167	0.159	0.155
8 DQ414333 <i>Oxytonostoma typica</i>	85.4	85.4	84.2	84.3	88.0	84.7	85.3		0.144	0.149	0.146	0.155	0.141	0.144	0.144	0.158	0.151	0.158	0.170	0.179	0.168	0.157	0.163	0.172	0.174	0.165	0.171	0.163
9 MT871866 <i>Pterobdellina vernadskyi</i>	85.1	85.0	83.1	83.3	84.7	87.7	87.4	85.6		0.129	0.137	0.158	0.156	0.133	0.145	0.134	0.146	0.140	0.164	0.153	0.150	0.159	0.158	0.175	0.155	0.175	0.153	0.152
10 DQ414337 <i>Piscicola milneri</i>	84.8	84.7	84.6	85.1	87.1	94.3	88.6	85.1	87.1		0.131	0.159	0.129	0.131	0.125	0.128	0.144	0.135	0.156	0.143	0.146	0.156	0.161	0.139	0.159	0.163	0.150	0.134
11 DQ414301 <i>Austrobdella bilobata</i>	84.8	84.7	86.2	86.0	87.4	87.1	87.7	85.4	86.3	86.9		0.152	0.138	0.135	0.131	0.078	0.149	0.129	0.159	0.135	0.143	0.149	0.170	0.148	0.167	0.175	0.150	0.147
12 DQ414332 <i>Oceanobdella sexoculata</i>	84.4	84.4	81.1	81.9	85.4	83.6	84.5	84.5	84.2	84.1	84.8		0.150	0.152	0.153	0.152	0.146	0.164	0.198	0.168	0.188	0.168	0.155	0.179	0.183	0.178	0.189	0.194
13 MK967944 <i>Johanssonia extrema</i>	84.3	84.2	85.3	85.4	88.7	86.0	86.6	85.9	84.4	87.1	86.2	85.0		0.133	0.077	0.131	0.140	0.147	0.182	0.167	0.176	0.167	0.175	0.183	0.177	0.175	0.180	0.164
14 AY336022 <i>Limnotrachelobdella okae</i>	84.3	84.2	85.6	85.6	86.9	87.9	88.9	85.6	86.7	86.9	86.5	84.8	86.7		0.128	0.121	0.140	0.132	0.162	0.138	0.155	0.150	0.156	0.147	0.156	0.158	0.156	0.146
15 AY336012 <i>Johanssonia arctica</i>	83.8	83.7	85.0	85.2	87.1	86.8	87.0	85.5	85.4	87.4	86.8	84.6	92.2	87.1		0.134	0.142	0.137	0.186	0.155	0.166	0.158	0.174	0.168	0.183	0.169	0.172	0.166
16 DQ414306 <i>Austrobdella translucens</i>	83.8	83.8	85.9	86.2	87.4	87.3	88.9	84.2	86.6	87.2	92.2	84.8	86.9	87.9	86.5		0.140	0.134	0.168	0.140	0.161	0.152	0.150	0.163	0.162	0.164	0.164	0.152
17 DQ414303 <i>Austrobdella californiana</i>	83.3	83.3	83.1	83.9	85.1	84.7	85.4	84.9	85.4	85.6	85.1	85.4	86.0	86.0	85.7	86.0		0.157	0.164	0.153	0.161	0.158	0.179	0.179	0.156	0.160	0.162	0.190
18 AF003265 <i>Branchellion torpedinis</i>	83.0	83.0	85.7	86.0	84.2	86.5	89.2	84.2	86.0	86.5	87.1	83.6	85.3	86.8	86.2	86.6	84.3		0.183	0.143	0.172	0.143	0.155	0.160	0.174	0.166	0.187	0.163
19 DQ414300 <i>Aestabdelta abditovesiculata</i>	82.7	82.6	84.1	83.6	83.8	83.0	83.9	83.0	83.6	84.4	84.1	80.2	81.8	83.8	81.3	83.2	83.6	81.7		0.179	0.098	0.197	0.220	0.199	0.093	0.101	0.096	0.189
20 DQ414309 <i>Branchellion ravenelii</i>	82.7	82.6	85.1	85.6	83.2	85.8	87.4	82.1	84.7	85.7	86.5	83.2	83.3	86.2	84.4	86.0	84.7	85.7	82.1		0.153	0.158	0.181	0.159	0.173	0.176	0.164	0.152
21 DQ414344 <i>Pterobdella arugamensis</i>	82.5	82.5	84.1	84.5	82.7	85.8	84.5	83.2	85.0	85.4	85.7	81.2	82.4	84.5	83.3	83.9	83.9	82.8	90.2	84.7		0.176	0.211	0.195	0.110	0.108	0.030	0.164
22 DQ414342 <i>Stibarobdella macrothela</i>	82.2	82.2	84.2	84.8	85.0	84.4	85.6	84.3	84.1	84.4	85.1	83.2	83.3	85.0	84.1	84.8	84.2	85.7	80.3	84.2	82.4		0.164	0.144	0.188	0.179	0.183	0.171
23 AY336028 <i>Notostomum cyclostomum</i>	82.2	82.2	82.3	82.3	84.4	83.3	86.8	83.7	84.2	83.9	83.0	84.5	82.5	84.4	82.6	85.0	82.1	84.5	78.0	81.9	78.9	83.6		0.179	0.200	0.196	0.212	0.200
24 AY336029 <i>Pontobdella muricata</i>	81.5	81.5	81.9	82.5	82.7	84.9	84.9	82.7	82.2	85.9	84.9	81.9	81.4	85.2	82.9	83.5	82.0	83.8	79.9	83.9	80.4	85.5	81.9		0.188	0.187	0.201	0.184
25 DQ414305 <i>Aestabdelta leiostomi</i>	81.5	81.5	82.6	82.3	82.6	84.1	84.4	82.6	84.5	84.1	83.3	81.7	82.3	84.4	81.6	83.8	84.4	82.6	90.7	82.7	89.0	81.2	80.0	81.0		0.086	0.111	0.197
26 DQ414334 <i>Pterobdella amara</i>	81.6	81.6	84.3	84.6	82.0	84.0	83.2	83.4	82.5	83.7	82.5	82.1	82.5	84.1	83.0	83.5	84.0	83.3	89.8	82.3	89.1	82.0	80.3	81.1	91.3		0.104	0.183
27 MH299847 <i>Pterobdella arugamensis</i>	81.4	81.4	83.4	83.6	82.0	85.0	84.1	82.9	84.7	85.0	85.0	81.1	82.0	84.4	82.7	83.6	83.8	81.3	90.4	83.6	97.0	81.7	78.8	79.8	88.9	89.5		0.171
28 AF003260 <i>Calliobdella vivida</i>	80.4	80.4	83.1	83.9	84.5	85.5	84.5	83.7	84.8	86.6	85.3	80.6	83.6	85.4	83.3	84.8	81.0	83.7	81.1	84.8	83.6	82.9	80.0	81.4	80.3	81.6	82.9	

Remarks

Marine leeches parasitising elasmobranch and teleost fish are known from the family Piscicolidae (Burreson 2020). The taxonomy of this family is based on diverse characters of the coelomic system and genital anatomy (Utevsky et al. 2019). The specimen from the present study can be distinguished from the most prominent marine leech subfamily, Pontobdellinae, by the lack of tubercles on their body, their size difference and the fact that they do not parasitise sharks and rays (Utevsky et al. 2007).

To date only a single species, *Pterobdella arugamensis* (de Silva, 1963) was identified parasitising *C. superciliosus* (Hayes et al. 2014). However, it was recently suggested by Burreson (2020) that the genus *Zeylanicobdella* from Australia should be synonymised with *Pterobdella* (de Silva, 1963), and this synonymy was recently accepted by Utevsky et al. (2021). Since *Z. arugamensis* has been reported from various host species around the world, this placement was also adopted here (from here on referred to as *Pterobdella arugamensis*, for the South Africa species). *Aestabdella*, *Pterobdella*, and *Zeylanicobdella* are grouped together by synapomorphies (characteristics inherited from their common ancestor), including two pairs of mycetomes and an open, spacious coelomic system (Burreson 2020). The latter are unknown from any other genus of piscicolid leeches. Based on a recently published key to marine leech species of Australia / New Zealand (Burreson 2020), the characteristics defining *P. arugamensis* are as follows: body < 35.0 mm total length, lacking branching digitiform or leaf-like branchiae, tubercles absent; urosome elongate, cylindrical to sub-cylindrical; caudal sucker eccentrically attached with urosome tapering towards caudal sucker; pigmentation of dark brown to black longitudinal stripes; trachelosome with four longitudinal stripes broken segmentally by unpigmented areas, and oral sucker with black band through paired eyespots (Burreson 2020). This description is consistent with *Pterobdella arugamensis* from Tsitsikamma and Chintsa on the southeast coast of South Africa, however, differs from the specimen collected at Langebaan marina on the west coast of South Africa.

Both morphotypes from the present study lack tubercles of any form (wart-like, branching digitiform, leaf-like) on the lateral sides, while *Pterobdella arugamensis* has black spots on each lateral side, with two distinct eye spots, some brown longitudinal pigmentation across the trachelosome, and a segmented urosome, Piscicolidae gen. sp. lacks both spots and brown pigmentation, with indistinct segmentation on the urosome. The anterior sucker of Piscicolidae gen. sp. (mean = 430 µm) is double the size of *Pterobdella arugamensis* (mean = 210 µm).

The specimens collected from Chintsa and Langebaan marina differ genetically with 19.3–19.7%. The newly generated sequence for *P. arugamensis* differs with 17.5% from sequences of the same species (Ravi and Yahaya 2017) available on GenBank. According to Tseng et al. (2017), genetic divergence for freshwater and medical leeches is considered > 2%, while species of *Ozobranchus branchiatus* were reported to differ 2.6% between monophyletic groups from Taiwan and the Atlantic. Based on these molecular results it is thus most likely that what is

currently morphologically considered as *P. arugamensis* are in fact a species complex, however this needs further investigation.

Due to the low nodal support and inconsistent topography of the BI and ML phylogenetic trees, more genetic markers with less variability and details regarding the internal anatomy of the specimens collected from *C. superciliosus* are needed to confirm their taxonomic placement.

3.3.2.5 Characterisation of various nematodes

Phylum: Nematoda Cobb, 1932
Class: Chromadorea Inglis, 1983
Order: Rhabditida Chitwood, 1933
Family: Anisakidae Railliet & Henry, 1912
Genus: *Anisakis* Type II

Material examined

Larval prevalence of this species was 5.4%, infecting the intestine, as well as found within cysts attached to the outside of the gallbladder at Langebaan marina. A single specimen was examined using light microscopy, following lactophenol clearing and identification to family or genus level was based on the morphology of the lips, tail, and oesophagus.

Morphological characteristics

Anisakis Type II measured 103.98 mm long and 4.97 mm wide. Due to the developmental stage of the specimen, no other measurements were taken. Anisakidae can be identified to genus level, even in the larval stage, by typical characteristics of anterior and posterior regions (Hien et al. 2021). They are then further grouped into Type I and Type II based on the total length of the ventriculus and the presence or absence of a tail spine, called the mucron (Hien et al. 2021). The specimen collected lacks a mucron on its tail and is thus classified as *Anisakis* Type II. Prominent ridges are visible on the outer surface of the body.

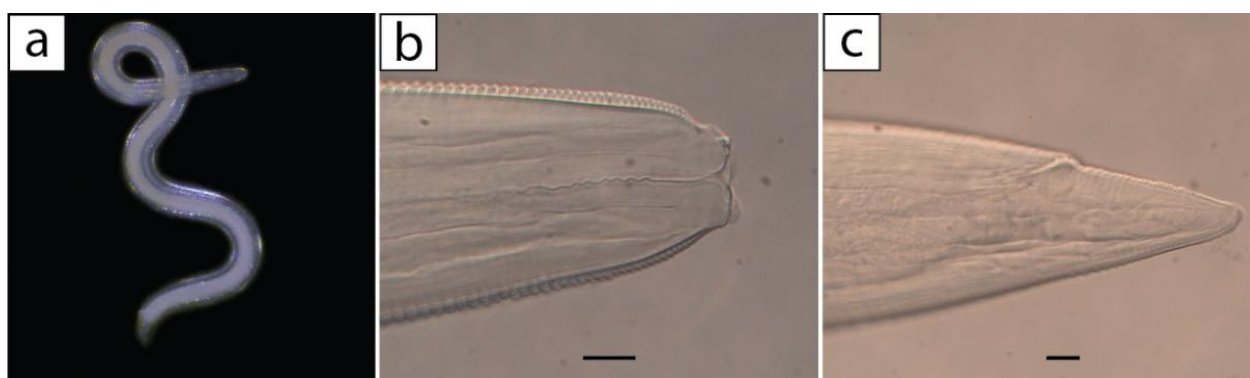


Figure 3. 27: a) Photomicrograph of live *Anisakis* Type II collected from *Clinus superciliosus*; b) head region c) tail region.

Remarks

Anisakis is among the World Health Organization's (WHO) list of parasites infecting fish associated with consumption (Aibinu et al. 2019). This group of parasites is known to cause severe pathologies in humans (Pampiglione et al. 2002; Aibinu et al. 2019). Three species are

responsible for infections in humans: *Anisakis simplex sensu stricto* Davey, 1971; *A. pegreffii* Campana-Rouget & Biocca, 1955 and *A. physeteris* (Baylis, 1923) (see Mattiucci and Nascetti 2008; Aibinu et al. 2019). Species of Anisakidae have been reported from South African freshwater crocodiles (Ángeles-Hernández et al. 2020), with earlier reports of *A. simplex* from mackerel and hake (Hecht 1976; Krzeptowski 1980; Botha 1986). Marine fish species form an important part of the Anisakidae life cycle, as they act as a paratenic host together with cephalopods, while marine mammals and birds are known definitive hosts (Ángeles-Hernández et al. 2020). Humans are also reported as part of the life cycle as accidental hosts (Mattiucci et al. 2018; Martinez-Rojas et al. 2021).

The morphology of this family is not fully known, due to the different developmental stages, as well as the shared characters of different genera (Ángeles-Hernández et al. 2020). Molecular identification remains the most efficient tool for identification of the genera that make up the Anisakidae family (Berland 1961; Ángeles-Hernández et al. 2020). Characteristics include the presence of the cuticular tooth, ventrolateral lips, the position and shape of extractor pore, and the pointed shape of the tail, among others (Garbin et al. 2013). Species of *Anisakis* have been known to cause parasitic castration during the early stages of seasonal development, with intensities of more than 65% of the total gonad mass (Hecht 1976, 1990; Reed 2015). Further morphological characterisation and comparisons, including SEM, as well as comparative sequences, are needed to confirm the identification to a lower taxonomic level.

Family: **Cucullanidae Cobbold, 1864**
Genus: ***Cucullanus* Müller, 1777**
Species: ***Cucullanus* sp.**

Material examined

The morphological description is based on one whole specimen, and the posterior ends of one male, and one female in a temporary lactophenol mount. This species was also found only infecting the intestine of fish from Langebaan marina (7.5%).

Morphological characteristics

Cucullanus sp. (based on two specimen) measured 65.21 mm long and 3.93 mm wide, with an oesophagus length of 719.2 µm. The maximum width at the anterior end of the oesophagus measured 159.7 µm, while the maximum posterior width measured 107.8 µm. The ratio of body length to oesophagus length was 1 : 90.8, and the length of the oesophagus and maximum width ratio was 1 : 4.5. The excretory gland is situated 567.9 µm from the head, towards the posterior end of the oesophagus. The female tail (Figure 3.28e) measures 255.6 µm in length and 96 µm wide at the anus. The male tail (Figure 3.28f) measures 170 µm from the anus, and 93.4 µm wide.

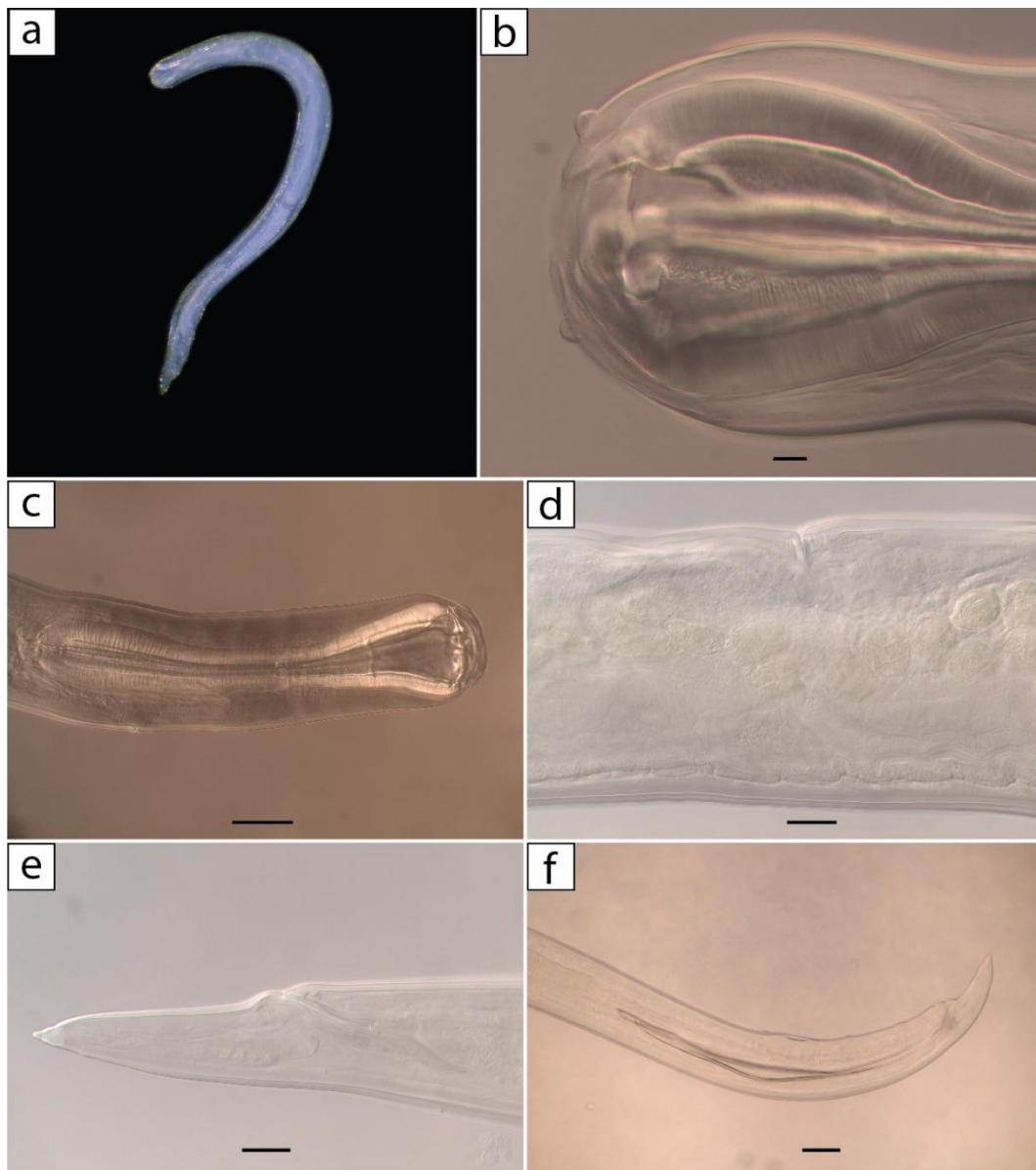


Figure 3. 28: a) Photomicrograph of live *Cucullanus* sp. collected from *Clinus superciliosus*; b) and c) head region (male); d) mid-section (female); e) female tail; and f) male tail regions.

Remarks

There are various taxonomic problems regarding *Cucullanus* species, due to their rather uniform morphology, with limited proper descriptions available for comparison (Moravec and Justine 2018, 2020). Parasites of this genus are consequently grouped according to their hosts or geographical distribution, infecting freshwater, brackish and marine fish species worldwide (Moravec et al. 1997; Moravec and Justine 2018, 2020). To date, there has been no reports of *Cucullanus* infecting Clinidae, thus this species is most probably new to science.

The oesophagus of members of this genus is muscular and expanded at the anterior end to form a bulbous pseudobuccal capsule and the tails of both genders are conical, sharply pointed at tip (Moravec and Justine 2018). Limited studies on these nematode species have been conducted in South African waters, with experts in the taxonomy of marine nematodes situated outside the borders of South Africa (Smit and Hadfield 2015).

No lower-level identification of the genus could be performed and further morphological characterisation and comparisons, as well as comparative sequences are needed to confirm the identification to a lower taxonomic level.

Family: Rhabdochonidae Travassos, Artigas & Pereira, 1928
Genus: Rhabdochonidae gen. sp.

Material examined

Based on the anterior and posterior ends of one male specimen placed in lactophenol. Only a single specimen was collected from the intestine of a *C. superciliosus* collected at Langebaan marina.

Morphological characteristics

The specimen measured 5.3 μm wide at the mouth, with 96.7 μm from the anterior extremity to the nerve ring of the oesophagus (Figure 3.29a). Detailed measurements of the tail were difficult since the specimen was fixed in a curled formation, however, the tail measured 448.3 μm long, from the anal pore. The right spicule measured 109 μm long, and the left spicule 203.9 μm . Prominent ridges were visible on the outer surface of the body.

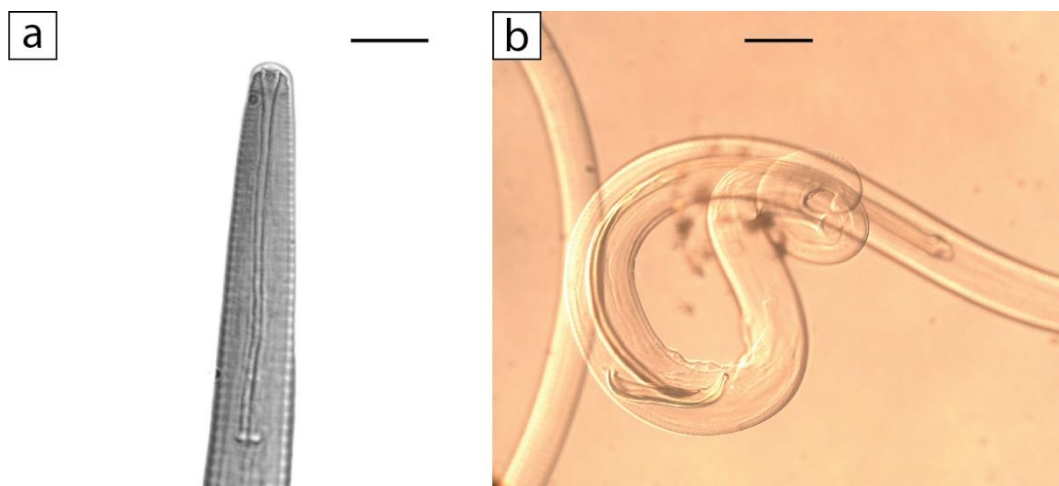


Figure 3. 29: Photomicrograph of *Rhabdochonidae* gen. sp. collected from *Clinus superciliosus*; a) head region d) tail region.

Remarks

The spirurine nematodes represent a diverse group of parasites with a cosmopolitan distribution and have been reported infecting all classes of vertebrates with invertebrates (except copepods) as intermediate hosts (Chabaud 1974; Moravec 2007). Moravec et al. (2001) listed ten Rhabdochonidae genera parasitising fish, namely: *Beaninema* Caspeta-Mandujano, Moravec & Salgado-Maldonado, 2001; *Fellicola* Petter & Køie, 1993; *Hepatinema* Rasheed, 1964; *Heptochona* Rasheed, 1965; *Johnstonmawsonia* Campana-Rouget, 1955; *Megachona* Mejía-Madrid & Pérez-Ponce de León, 2007; *Pancreatonema* McVicar & Gibson, 1975; *Prosungulonema* Roytman, 1963; *Rhabdochona* Railliet, 1916; and *Vasorhabdochona* Martin & Zam, 1967 (see Moravec 2007). Parasites of this family infecting fish are characterised by their

usually bilateral symmetric mouth, with various sclerotised formations as plates, teeth, or spines (Moravec 2007).

Due to a limited number of samples, lower-level identification was not possible, and more samples are needed for further morphological characterisation and comparison, specifically SEM to observe the various features of the mouth, as well as comparative sequences, to confirm the identification to a lower taxonomic level.

Family: **Ascarididae Baird, 1853**

Genus: **Ascarididae gen. sp.**

Material examined

Based on the head region of a single adult specimen placed in lactophenol for DIC examination. As with the previous nematode species, this specimen was only collected from Langebaan marina with the very low abundance of two individuals.

Morphological characteristics

The head width measured 80.7 μm , where one lip is 58.5 μm wide with a height of 40.7 μm . The width at the base of the lips is 83.5 μm (Figure 3.30b). The three prominent lips are visible following the oesophagus, with a cylindrical body. Scanning electron microscopy will be able to shed more light on the appearance of these lips.



Figure 3. 30: Photomicrograph of *Ascarididae* gen. sp. collected from *Clinus superciliosus*: head region showing three prominent lips.

Remarks

This family of nematodes parasitising fish is known by the presence of three prominent lips, external labial papillae and numerous caudal papillae (McClelland 2005; Mašová and Kuchta, 2018). They are found parasitising the intestinal tracts of all classes of vertebrates (marine fish, reptiles, mammals, and birds) (McClelland 2005). Together with spirunids, they make up the majority of marine fauna (McClelland 2005). According to World Register of Marine Species

(WoRMS) (WoRMS 2022) there are only two valid marine species of the family Ascarididae: *Paradujardinia* Travassos, 1933 and *Porrocaecum* Railliet & Henry, 1912.

Further morphological characterisation (such as SEM) and comparisons to known species, as well as comparative sequences are needed to confirm the identification to a lower taxonomic level.

Family: Camallanidae Railliet & Henry, 1915

Genus: Camallanidae gen. sp.

Material examined

Based on the head and tail region of one female specimen in lactophenol. This was the only nematode species that was not collected at Langebaan marina, with a low infection of 1.1% prevalence in *C. superciliosus* from Chintsa.

Morphological characteristics

The body width at the level of the oesophagus was 81.1 μm (Figure 3.31a), with the head width at the base of the buccal capsule measuring 37.3 μm wide. The maximum buccal capsule width was 23.9 μm and the length was 29 μm (Figure 3.31b). The tail of the specimen is tapered with a forked end. The tail width at the fork was 10 μm (Figure 3.31c), however the anal pore was not visible to measure the tail length.



Figure 3. 31: Interference contrast photomicrographs of *Camallanidae* gen. sp.: a) and b) head region; c) tail region.

Remarks

This family of cosmopolitan distributed nematodes are known to infect the digestive tract of marine and freshwater fishes, as well as amphibians, turtles and snakes (Rigby and Rigby 2014; Svitin et al. 2019). However, the nematode fauna of South Africa, especially the marine nematodes, is under-examined (Svitin et al. 2019). Prior to the study by Svitin et al. (2019), no camallanid species have been reported from marine organisms from this region (Smit and Hadfield 2015). *Camallanus sodwanaensis* Svitin, Truter, Kudlai, Smit & du Preez, 2019 from Sodwana Bay, KwaZulu Natal, was reported from the intestine of five species of marine fishes: dusky sweeper *Pempheris adusta* Bleeker; the stocky hawkfish *Cirrhitus pinnulatus* (Förster); the banded grunter *Pomadasys furcatus* (Bloch & Schneider), the jarbua terapon *Terapon jarbua* (Forsskål) and the

largespotted dart *Trachinotus botla* (Shaw). None of these fish belong to the family Clinidae, making this a new host record for camallanid nematodes from South African marine waters.

Identification of the family is based on the buccal capsule, however, additional techniques such as molecular analyses is advised to differentiate the taxa within Camallanidae (Svitin et al. 2019). Spiral ridges in the buccal capsule (as seen in Figure 3.31b) should be considered a generic differentiating factor, dividing species without ridges into a different genus. In addition, the male caudal region is also considered a feature for discerning species, where the left and right spicules are considered. Detailed SEM is needed to find the often-inconspicuous left spicule (Svitin et al. 2019). The female tail differs between life stages in the number of mucrons, thus this feature can only be used as discerning character for gravid females (Moravec et al. 2006; Svitin et al. 2019). Phylogenetic analyses of the 18S rRNA gene have resulted in numerous debates, while other genetic markers are scarce, resulting in various questions on the evolutionary relationships among this family (Svitin et al. 2019).

Detailed morphological characterisation and comparison, as well as comparative molecular sequences are needed to establish whether the specimens from *C. superciliosus* are *Camallanus sodwanaensis* or a new species to science.

3.3.2.6 Characterisation of gnathiid isopods

Phylum:	Arthropoda von Siebold, 1848
Class:	Malacostraca Latreille, 1802
Order:	Isopoda Latreille, 1817
Family:	Gnathiidae Leach, 1814
Genus:	<i>Gnathia</i> Leach, 1814
Species:	<i>Gnathia africana</i> Barnard 1914

Material examined

Based on multiple photographs of live gnathiids (male, female and juvenile) collected for culturing purposes, as well as samples prepared for SEM imaging (n = 1). *Gnathia africana* larvae were collected from the external surface of *Clinus superciliosus*, specifically on the areas around the fins (Langebaan marina, P = 10.5%; Tsitsikamma, P = 75%; Chintsa, P = 60%).

Morphological characteristics

The taxonomy of gnathiid isopods is based on the morphology of the adult male (Smit and Davies 2004). The morphological characters of the males, that moulted from stage 3 praniza larvae collected from *C. superciliosus* in the present study, were consistent with the description of the male *Gnathia africana* (Smit et al. 1999). The most characteristic feature of this species is the medially divided mediofrontal process, prominent para-ocular process, mandibles, unique colour pattern of the organisms, and sensory pits (Figure 3.32).

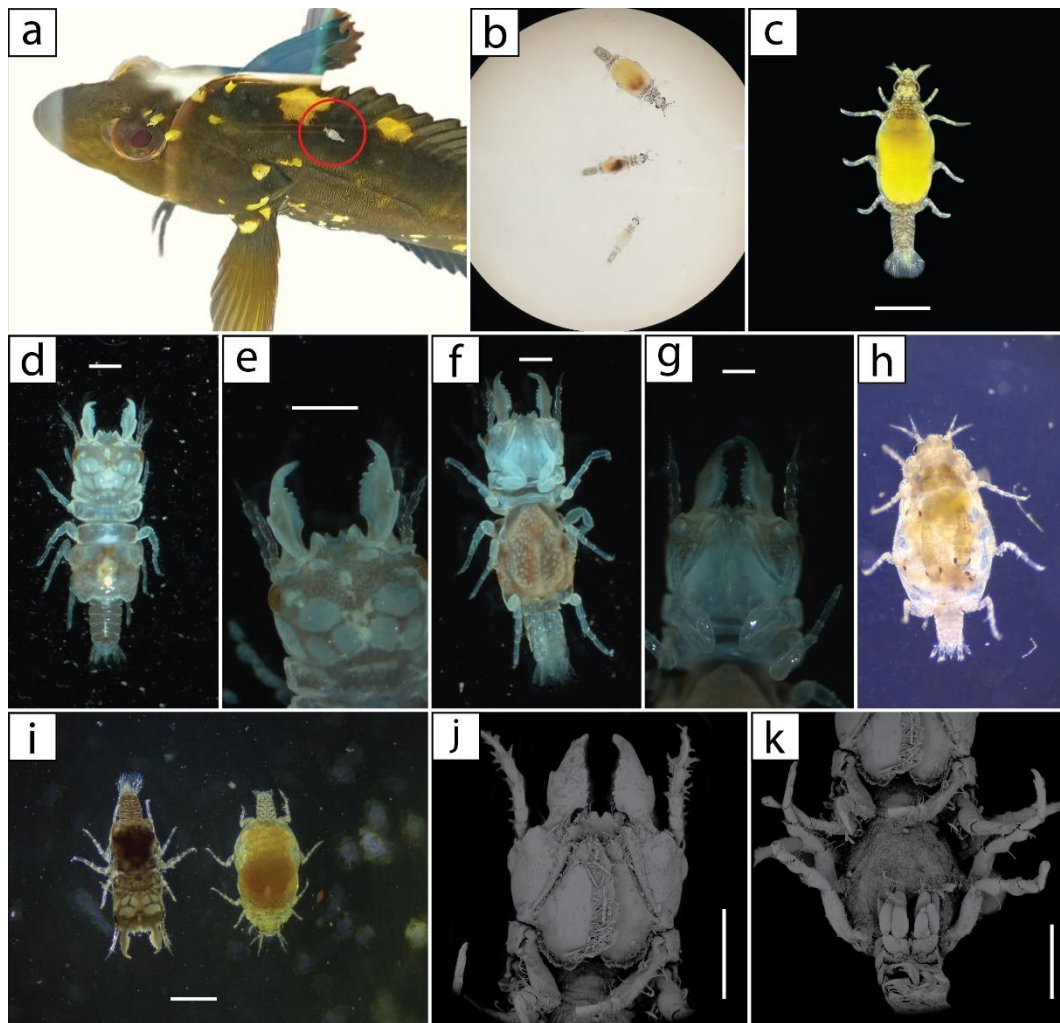


Figure 3.32: a) Field photograph of gnathiid P3 attached to the base of the dorsal fin of *Clinus superciliosus*; b) P1, P2, P3 of *Gnathia africana*; c) P3; d) – g) photomicrographs of live male *Gnathia africana* showing prominent mandibles and colour patterns on the head region; h) female with larvae in brood pouch; i) male and female *Gnathia africana*; scanning electron micrograph of male mandibles j) and pleopods k).

Species: *Gnathia pilosus* Hadfield, Smit & Avenant-Oldewage, 2008

Material examined

Based on photographs of live gnathiids collected for culturing purposes and samples prepared for SEM. *Gnathia pilosus* larvae were found on the fins and skin of *Clinus superciliosus* from Chintsa, Eastern Cape, South Africa (Prevalence = 25%).

Morphological characteristics

Morphological features coincide with the description of the male by Hadfield et al. (2008). *Gnathia pilosus* can be identified by the numerous setae covering the cephalosome and pereon, the short stout mandibles and large eyes (Figure 3.33).

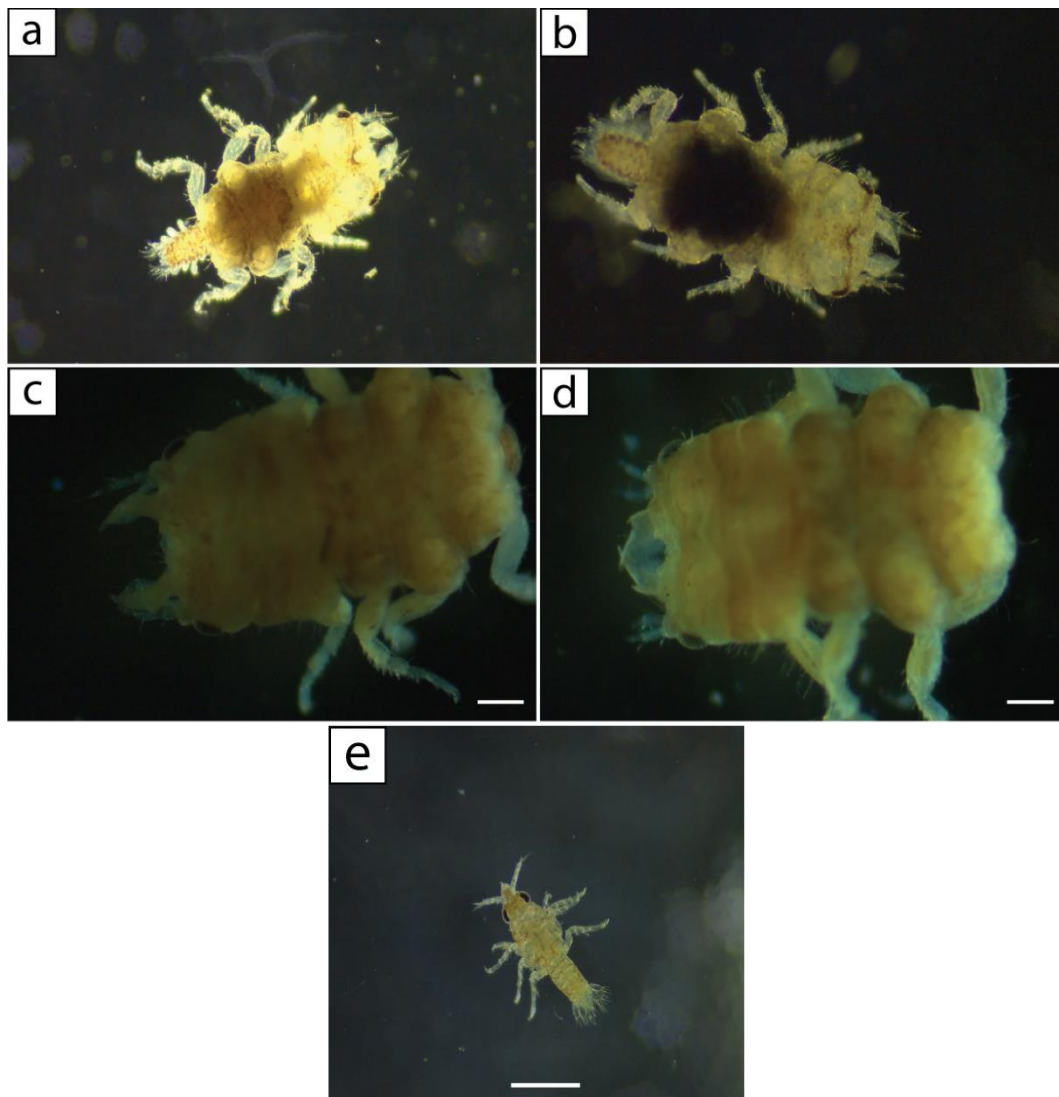


Figure 3.33: Photomicrographs of *Gnathia pilosus* specimens: a) – d) Male *Gnathia pilosus*; e) Z2 larval stage.

Remarks

There are currently 12 genera with 226 species described from the family Gnathiidae Leach, 1814 (see Smit et al. 2019). Most of the knowledge of global gnathiid species is limited to the localities of interest to researchers, rather than their actual distribution (Smit et al. 1999). Eight species have been described with a South African distribution: *Afrignathia multicavea* Hadfield & Smit, 2008; *Caecognathia cryptopais* (Barnard, 1925); *Gnathia. Africana*; *G. disjuncta* Barnard, 1920; *G. nkulu* Smit & Van As, 2000; *G. pantherina* Smit & Basson, 2000; *G. pilosus* Hadfield, Smit & Avenant-Oldewage, 2008; and *G. spongicola* Barnard, 1920. As mentioned above, the identification of this genus is based on the morphology of the male (Hadfield et al. 2019). Gnathiid isopods can be distinguished from other isopods by a pereon with only five pairs of ambulatory pereopods (Ferreira 2011). The genus *Gnathia* can be identified by the frontal processes, frontal border, a broad 2 or 3 articulated pylopod, and non-extended mandibles with a dentate blade (Hadfield et al. 2019). Various studies have focussed on *Gnathia*'s role in food web ecology, potential vectors of blood parasites, and their role in cleaner interactions (Demopoulos and Sikkil 2015; Cook et al. 2015; Artim et al. 2017).

Clinus superciliosus is a known host of *Gnathia africana* and has been reported from temperate southern Africa (Smit et al. 1999). This is the first record of *Gnathia pilosus* infesting *C. superciliosus*. Previous studies on *G. pilosus* reported its distribution on the warmer east coast of South Africa, compared to the other colder west coast species (Hadfield et al. 2009). These authors suggested that future studies are needed to confirm if its distribution overlaps with other African gnathiid species and their suspicions are thus hereby confirmed.

3.3.2.7 Characterisation of copepods

The family Caligidae are described below, however, another family collected from *Clinus superciliosus*, includes the genus *Alella* from the family Lernaeopodidae. A full description, including morphological and molecular characterisation, was done in Chapter 4.

Class:	Hexanauplia Oakley, Wolfe, Lindgren & Zaharof, 2013
Subclass	Copepoda Milne, 1840
Order:	Siphonostomatoida Burmeister, 1835
Family:	Caligidae Burmeister, 1835
Genus:	<i>Caligus</i> Müller, 1785
Species:	<i>Caligus mortis</i> Kensley, 1970

Material examined

Based on the samples collected during the present study from the skin of *Clinus superciliosus* at Langebaan marina (two female specimens), Western Cape, South Africa (one female was placed in lactophenol and used for DIC imaging, and one specimen used for SEM imaging). A 21.1% prevalence was noted for this species.

Morphological characteristics

Based on the description and illustrations of *Caligus mortis* (Kensley 1970; Dojiri 1989; Grobler et al. 2002), the morphological characters are consistent with the description from *Caligus mortis*. The most characteristic features of this species are the sternal furca (Figure 3.34 and 3.35), and the two-segmented exopod of leg 4 (Figure 3.34f and 3.35b).

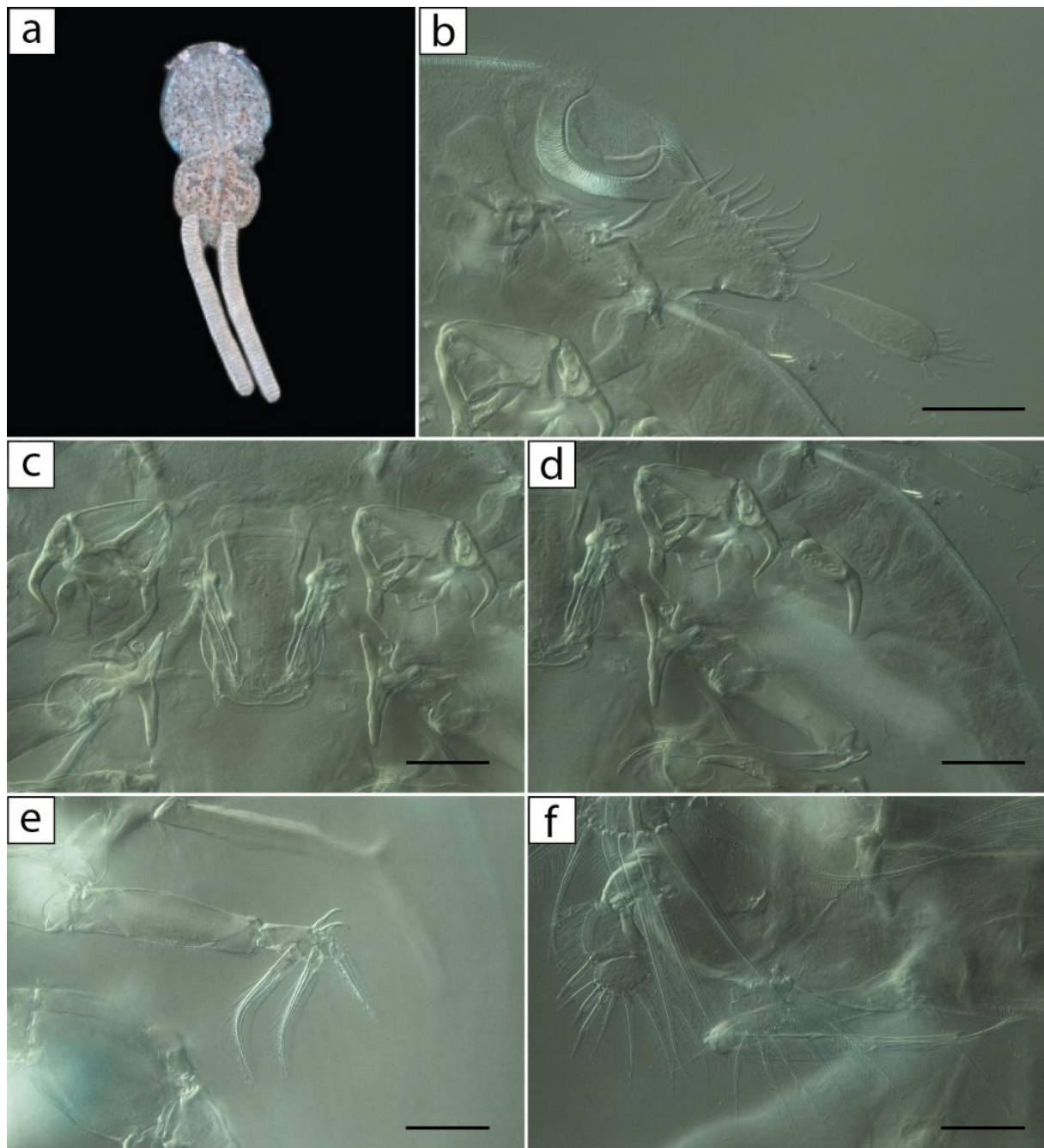


Figure 3. 34: Differential interference contrast photomicrographs of: a) live *Caligus mortis*; b) lunules; c) mouth region; d) from top to bottom antenna, posterior process, maxilla; e) first leg; f) from top to bottom, second, third- and fourth legs.

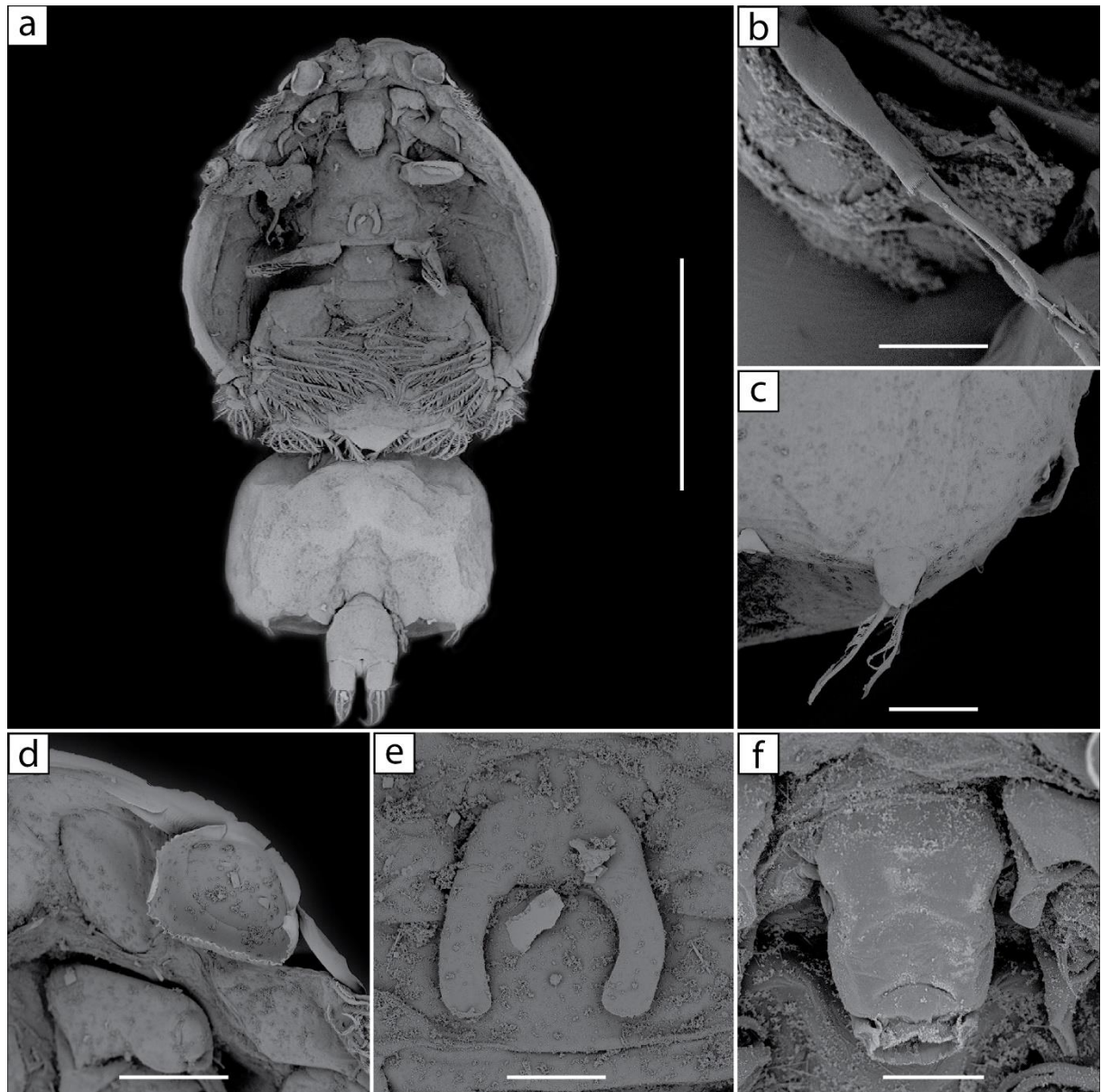


Figure 3. 35: Scanning electron microscope photographs of *Caligus mortis*: a) habitus; b) fourth leg; c) fifth and sixth legs; d) lunule; e) sternal furca; f) mouth cone.

Species: *Caligus* sp.

Material examined

Based on female specimens collected from Chintsa (with a prevalence of 15%) two individuals were examined in detail (one female specimen was placed in lactophenol for observation using DIC imaging; one female specimen was used for SEM imaging). Total body length includes the rami but not the eggs of the female specimen.

Morphological characteristics

The total body measured 2 mm long, and 1 mm wide. Cephalothorax is approximately oval with sinuses present; length / width ratio calculated at 1 : 2. The genital complex is 660.2 µm long and 684.2 µm wide with rounded square posterolateral angles, 3.2 times longer than abdomen. Abdomen with paired caudal rami distally and indistinct anal slit terminally (Figure 3.36a). Each ramus with five setae, two small lateral setae compared to three approximately equal medial setae.

Antennula (Figure 3.36b) is two-segmented with plumose seta along the anteroventral margin, and the antenna (Figure 3.36c) comprised of three segments; the terminal segment forms a short, curved claw. The posterior process is well developed and curved medially at a right angle, with sensory papillae on and surrounding the process. Maxilla two-segmented with apical armature, and the maxilliped subchelate with large proximal segment and distal segment of almost equal size. Sternal furca (Figure 3.37e) with divergent tines (not tapered), each with square rounded tips. Leg 4 (Figure 3.36f and Figure 3.37b) three-segmented with plumose spine between proximal and medial segments; second segment with at least one spine, bearing pectinate membrane at the base; distal segment with blade-like plumose seta and short terminal element. Rudimentary fifth and sixth legs on either side of posterolateral corner of genital complex.

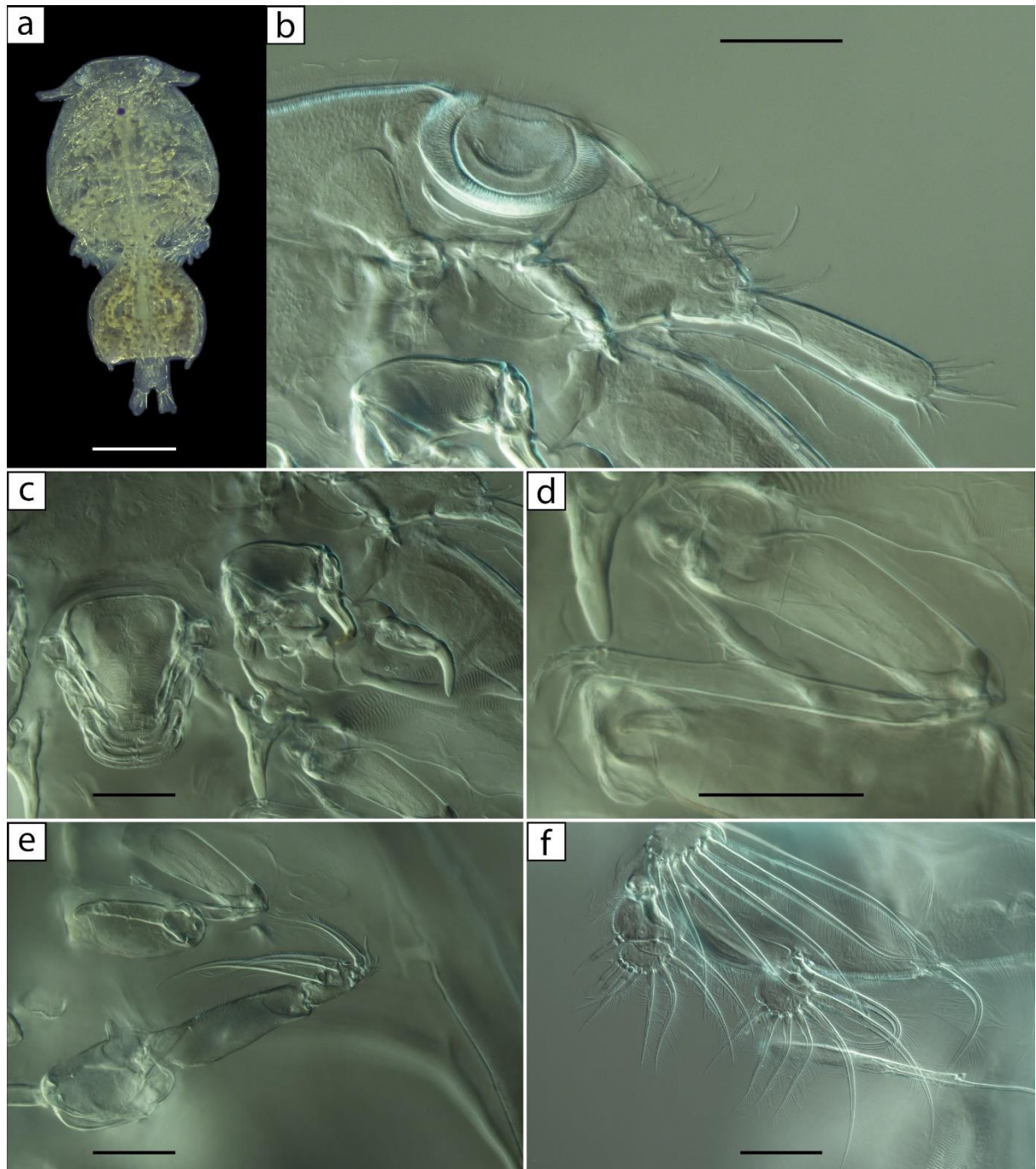


Figure 3.36: Differential interference contrast photomicrographs of: a) live *Caligus* sp.; b) lunules; c) mouth region; d) from top to bottom antenna, posterior process, maxilla; e) first leg; f) from top to bottom, second, third- and fourth legs.

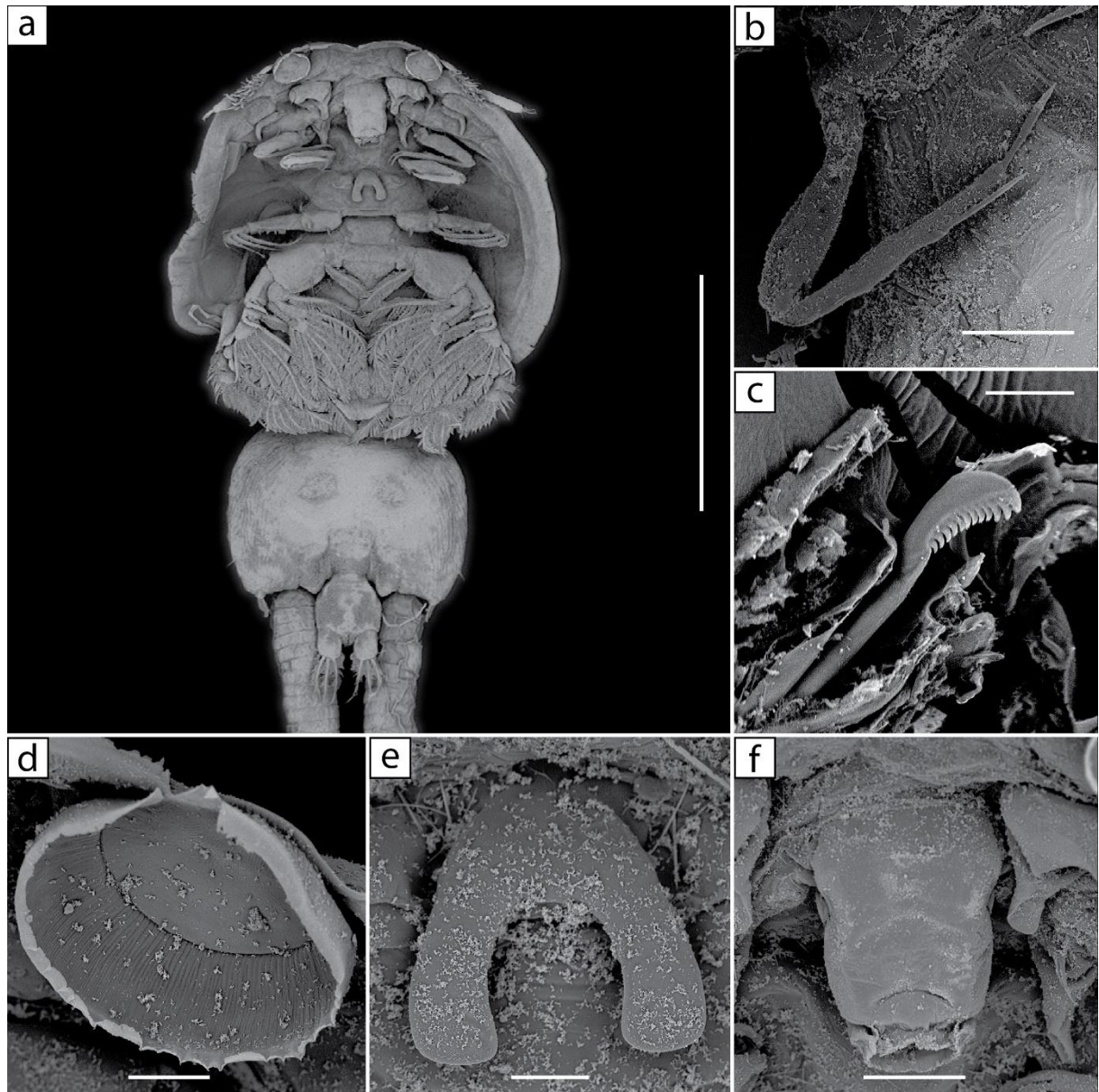


Figure 3. 37: Scanning electron microscope photographs of *Caligus* sp.: a) habitus; b) fourth leg; c) fifth and sixth legs; d) lunule; e) sternal furca; f) mouth cone.

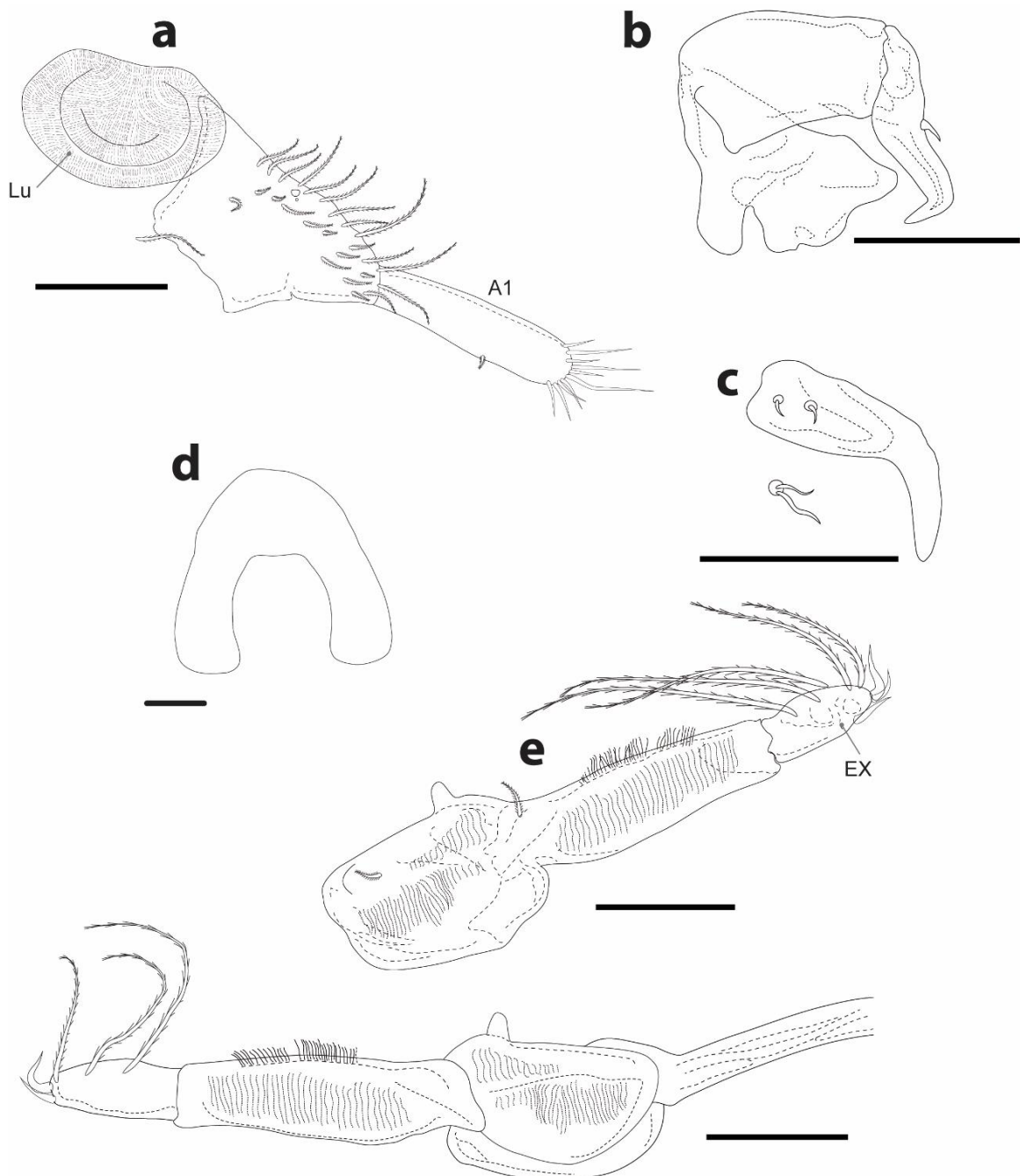


Figure 3.38: Illustrations of *Caligus* sp.: a) lunule; b) antenna; c) posterior process; d) sternal furca; e) and f) first leg.

Phylogenetic characterisation

The two morphologically different species were extracted separately and subjected to amplification using the universal COI primers, LCO4190 and HCO2198 (Folmer et al. 1994; Hayes et al. 2021). The resulting sequences were assembled and aligned in Geneious and verified as species of *Caligus* using BLAST. The sequences were then aligned with published sequences of other caligids (Table 3.12; Table A. 12) using MUSCLE and visualised using FigTree and MEGA. *Lepeophtheirus pollachius* Bassett-Smith, 1896 was selected as the outgroup. Bayesian inference analysis was performed, with the best AIC nucleotide substitution model as GTR+I+G. Invertebrate mitochondrial translation frame 1 was used to remove any stop codons from the alignment, followed by the construction of the phylogenetic tree in FigTree (Figure 3.39).

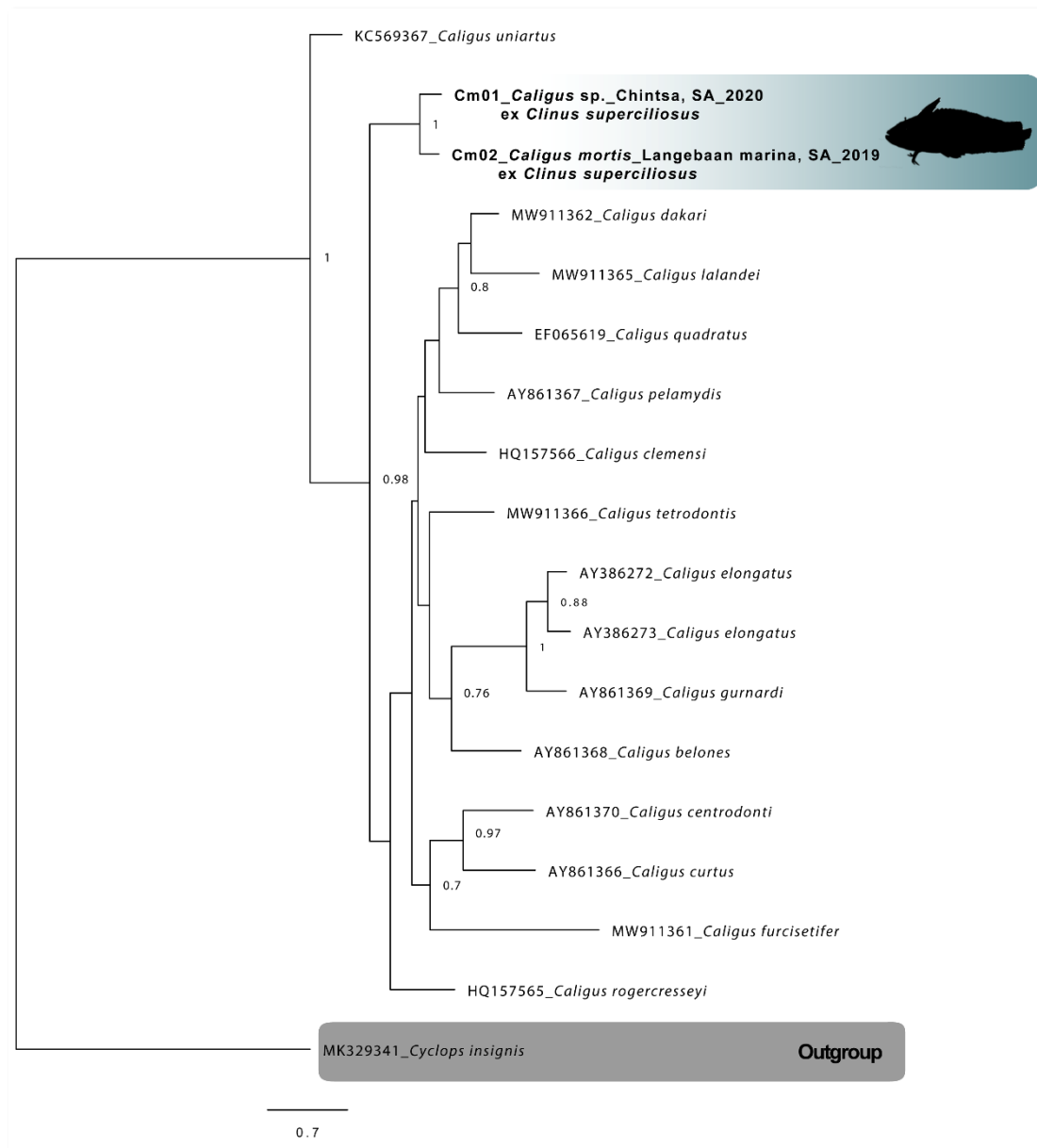


Figure 3. 39: Bayesian inference phylogram based on partial sequences of the COI mtDNA gene for representatives of *Caligus*. Novel sequences are highlighted in bold, and can be seen in the coloured box; the outgroup is displayed in the grey box. Nodal support > 70% is indicated; scale bar indicates the expected number of substitutions per site.

Table 3. 12: Evolutionary distances between species of *Caligus* isolated from the COI mtDNA gene region, including phylogenetic analysis, expressed as percent similarity (below the diagonal) and *p*-distances (above the diagonal). Newly generated sequence is indicated in the shaded row.

	Caligus sequences	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	Cm01_ <i>Caligus</i> sp.		0.108	0.155	0.157	0.157	0.159	0.168	0.167	0.174	0.172	0.179	0.188	0.189	0.191	0.193	0.227	0.239
2	Cm02_ <i>Caligus mortis</i>	89.2		0.172	0.174	0.174	0.178	0.187	0.197	0.170	0.195	0.189	0.206	0.205	0.205	0.203	0.212	0.241
3	MW911362_ <i>Caligus dakari</i>	84.5	82.8		0.146	0.134	0.169	0.166	0.144	0.182	0.168	0.141	0.189	0.170	0.170	0.186	0.199	0.212
4	HQ157566_ <i>Caligus clemensi</i>	84.3	82.6	85.4		0.142	0.172	0.168	0.188	0.186	0.166	0.156	0.174	0.184	0.182	0.189	0.203	0.212
5	EF065619_ <i>Caligus quadratus</i>	84.3	82.6	86.6	85.8		0.170	0.166	0.159	0.182	0.164	0.155	0.186	0.189	0.182	0.197	0.191	0.216
6	AY861370_ <i>Caligus centrodonti</i>	84.1	82.2	83.1	82.8	83.0		0.170	0.165	0.188	0.176	0.181	0.165	0.195	0.176	0.210	0.218	0.220
7	MW911366_ <i>Caligus tetradontis</i>	83.2	81.3	83.4	83.2	83.4	83.0		0.171	0.200	0.179	0.171	0.175	0.195	0.181	0.206	0.197	0.239
8	MW911365_ <i>Caligus lalandei</i>	83.3	80.3	85.6	81.3	84.1	83.5	82.9		0.180	0.180	0.181	0.214	0.191	0.195	0.188	0.208	0.218
9	HQ157565_ <i>Caligus rogercresseyi</i>	82.6	83.0	81.8	81.4	81.8	81.3	80	82.0		0.184	0.185	0.193	0.189	0.195	0.203	0.195	0.239
10	KC569367_ <i>Caligus uniartus</i>	82.4	80.1	82.8	83.0	83.0	82.0	81.7	81.5	81.3		0.171	0.195	0.207	0.178	0.229	0.193	0.226
11	AY861367_ <i>Caligus pelamydis</i>	81.6	80.8	85.5	83.9	84.2	81.5	82.7	81.6	81.1	82.0		0.200	0.198	0.179	0.204	0.195	0.208
12	AY861366_ <i>Caligus curtus</i>	81.3	79.4	81.1	82.6	81.4	83.5	82.5	78.6	80.7	80.1	79.6		0.212	0.216	0.218	0.205	0.229
13	AY386272_ <i>Caligus elongatus</i>	81.1	79.5	83.0	81.6	81.1	80.5	80.5	80.9	81.1	79.0	79.8	78.8		0.182	0.119	0.152	0.225
14	AY861368_ <i>Caligus belones</i>	80.9	79.5	83.0	81.8	81.8	82.4	81.9	80.5	80.5	81.8	81.8	78.4	81.8		0.195	0.197	0.233
15	AY386273_ <i>Caligus elongatus</i>	80.7	79.7	81.4	81.1	80.3	79.0	79.4	81.3	79.7	76.7	79.2	78.2	88.1	80.5		0.161	0.239
16	AY861369_ <i>Caligus gurnardi</i>	77.3	78.8	80.1	79.7	80.9	78.2	80.3	79.2	80.5	80.3	80.2	79.5	84.8	80.3	83.9		0.231
17	MW911361_ <i>Caligus furcisetifer</i>	76.1	75.9	78.8	78.8	78.4	78.0	76.1	78.2	76.1	77.0	79.0	77.1	77.5	76.7	76.1	76.9	

Remarks

Members of the family Caligidae (comprising 31 genera with 513 valid species) are commonly referred to as sea lice (Boxshall and Hayes 2019; Hayes et al. 2021). Nine of the 31 genera have previously been reported from South African elasmobranchs and actinopterygians (Hayes et al. 2021). The genus *Caligus* covers more than half of these species (270 species), predominantly reported from teleost fish, with a pan-global distribution (Boxshall and Hayes 2019). Species from this genus, have been reported as pests of finfish in marine aquaculture facilities, causing osmoregulatory failure, anaemia, and ulcers (Hutson et al. 2007; Johnson et al. 2004, 2019; Hayes et al. 2021). Generally, the life cycle of caligids consists of two nauplius stages, one infective copepodid stage, four parasitic chalimus stages, two parasitic preadult stages and then the parasitic adult stage (Dippenaar et al. 2010). However, researchers found that the life cycles differ between genera (for example between *Caligus* and *Lepeophtheirus*), while only 3.8% of the 450 species' life cycles have been described in the past (Venmathi Maran et al. 2013; Williams and Bunkley-Williams 2019).

Caligus mortis has previously been reported infecting *C. superciliosus* from various localities (Namibia, Saldanha Bay; De Hoop; Jeffreys Bay) along the southern Africa coast (Dippenaar 2004). This study adds Hermanus as a new locality within the distribution of *Caligus mortis*. The present study also reports a new species of *Caligus* infecting *C. superciliosus* from Chintsa on the east coast of South Africa. The new species differs from *Caligus mortis* based on the sternal furca, the shape of the mandibles, angle of the antenna, and the shape and size of the maxilla and maxillipeds.

Published sequences available on GenBank, including species reported from South Africa, were used to indicate the evolutionary distances of newly generated sequences, however, based on the genetic divergence of 10.8% between the novel two species, and the 3% divergence threshold for species delineation (Herbert et al. 2003; Hayes et al. 2021), it can be deduced that the two specimens collected during the present study, are different species. Based on the genetic distances of the novel sequences and the references sequences, this is also the first sequence report of *Caligus mortis*, with 84.5% similarity to the closest related species, *C. dakari*, reported from the Japanese meagre, *Argyrosomus japonicus* (Temminck & Schlegel) collected at Witsand, South Africa (Hayes et al. 2021).

With no other reports of *Caligus* infecting *C. superciliosus* on the east coast of South Africa, and based on the morphological and molecular differences found during the present study, it can be inferred that the specimen collected at Chintsa is new to science. A detailed morphological study of *Caligus* sp. will be performed as part of a species description publication, including the results from DIC and SEM imaging, as well as a phylogenetic analysis using more genetic markers as in Hayes et al. (2021).

3.3.3 Summary list

From literature and the present study, it is evident that *Clinus superciliosus* is host to a conceivable 43 species of protozoan and metazoan parasites:

- At least five morphologically and molecularly distinguishable Myxozoa including, *Myxobolus* sp., *Henneguya* sp., *Ceratomyxa* spp., *Sphaeromyxa clini*, and *Myxidium* sp., as well as an additional molecularly identified species, *Kudoa thyrsites*;
- intermediate host to four metacercariae digenean species: *Cardiocephaloides physalis*, *Cardiocephaloides* sp., *Dollfustrema* sp. and *Stephanostomum* sp.;
- definitive host to ten digenean species: *Coitocaecum capense*, *Coitocaecum* sp. 1, *Coitocaecum* sp. 2, *Coitocaecum* sp. 3, *Helicometra* sp. 1, *Helicometra* sp. 2, Hemiuridae gen. sp. 1, Hemiuridae gen. sp. 2, *Proctoeces* sp. and Zoogonidae gen. sp.;
- intermediate host to one Acanthocephala cystacanth *Corynosoma* sp.;
- definitive host to three Acanthocephala species: Arhythmacanthidae gen. sp. 1, Arhythmacanthidae gen. sp. 2 and Rhadinorhynchidae gen. sp.;
- host to two species of Annelida (leech);
- intermediate host to at least one plerocercus *Nybelinia* sp. and one plerocercoid *Acanthobothrium* sp.;
- five morphologically different species of adult and larval Nematoda: *Anisakis* Type II, *Cucullanus* sp., Rhabdochoniidae gen. sp., Ascarididae gen. sp., and Camallanidae gen. sp.;
- at least three species of copepods of the family Siphonostomatoida; and
- two species of gnathiid isopods.

Only two metazoan species previously reported to parasitise *Clinus superciliosus* were not collected during the present study: the digenean *Helicometra fasciata* (Rudolphi, 1819) Odhner, 1902 and the cymothoid isopod *Elthusa xena* van der Wal, Smit & Hadfield, 2019, while members of three genera of protozoans (*Trypanosoma*, *Haemogregarina* and *Trichodina*) were not considered during the present study. Additionally, the validity of *Caligus labracis* Scott, 1902 as parasite to *C. superciliosus* has been in question for some time (Dippenaar 2004), and the present study concluded that this is not a valid report, due to a lack of evidence by original and subsequent reports (Barnard 1955; Kabata 1979). The majority of the species collected during the present study represent new host records, with some species most probably new to science (Table 3.13; also see Chapter 4). Although the present study yielded significant sampling and identifications, this might only be an estimation of the parasites of *C. superciliosus* from its South African distribution, and even more species will arise from the Namibian distribution.

Table 3. 13: Summary table indicating novel contribution with regard to characterising the parasite biodiversity of *Clinus superciliosus* during the present study. HR – new host record; n. sp. – possibly new species; DIC – differential interference contrast; SEM – scanning electron microscopy.

Parasite taxa	HR	n. sp.	Live observation	DIC	SEM	DNA
Myxozoa						
<i>Myxidium</i> sp.						
<i>Henneguya clini</i>						
<i>Myxobolus</i> sp.						
<i>Ceratomyxa</i> spp.						
<i>Sphaeromyxa clini</i>						
<i>Kudoa thyrssites</i>						
Digenea						
<i>Coitocaecum capense</i>						
<i>Coitocaecum</i> sp. 1						
<i>Coitocaecum</i> sp. 2						
<i>Coitocaecum</i> sp. 3						
<i>Helicometra</i> sp. 1						
<i>Helicometra</i> sp. 2						
<i>Proctoeces</i> sp.						
<i>Zoogonidae</i> gen. sp.						
<i>Cardiocephaloides</i> sp.						
<i>Cardiocephaloides physalis</i>						
<i>Hemiuridae</i> gen. sp. 1						
<i>Hemiuridae</i> gen. sp. 2						
<i>Stephanostomum</i> sp.						
<i>Dollfustrema</i> sp.						
Monogenea						
<i>Gyrodactylus</i> sp.						
Cestoda						
<i>Nybelinia</i> sp.						
<i>Acanthobothrium</i> sp.						
Acanthocephala						
<i>Polymorphidae</i> sp.						
<i>Arhythmacanthidae</i> sp. 1						
<i>Arhythmacanthidae</i> sp. 2						
<i>Rhadinorhynchidae</i> sp.						
Annelida						
<i>Pterobdella arugamensis</i>						
<i>Piscicolidae</i> gen. sp.						
Nematoda						
<i>Anisakis</i> Type II						
<i>Cucullanus</i> sp.						
<i>Rhabdochonidae</i> sp.						
<i>Ascarididae</i> sp.						
<i>Camallanidae</i> sp.						
Isopoda						
<i>Gnathia africana</i>						
<i>Gnathia pilosus</i>						
Caligidae						
<i>Caligus mortis</i>						
<i>Caligus</i> sp.						
Lernaeopodidae						
<i>Alella</i> sp. A						

3.4 Conclusion

Considering the unique habitats of the South African coast and its biodiversity, if each marine species from South Africa hosted just one species of parasite, then it is likely that the majority of parasites have not yet been discovered or described (Smit and Hadfield 2015). Through the provision of new host records, new distribution records and new species to science, surveying this single host species from various localities has contributed greatly to the knowledge of parasites of South Africa. The present study highlights the need for more explorative investigations on the diversity of marine ichthyoparasites, targeting specific host species instead of specific parasite species. In addition, the integrative approach of molecular and morphological methods to elucidate parasite communities provides data of good quality for ongoing taxonomic research and discovery.

The number of hosts involved in a parasite life cycle can also be used to assess the biotic interaction of ecosystems. Contaminated systems have been previously reported to harbour more parasites with a single intermediate host or monoxenous life cycles than uncontaminated systems (MacKenzie et al. 1995; Anderson and Sukhdeo 2013; Schwelm et al. 2021a). In order to understand and predict anthropogenic pressures on ecosystems, a thorough understanding of parasite communities and their ecological role within an ecosystem is required. This is frequently ignored during environmental assessments and the conservation of parasites is as important as any free-living species.

Prior to this study, some of the parasite phyla had not yet been reported infecting *Clinus superciliosus*. While other phyla were identified as being the most prevalent species infecting this host, they did not even account for half of the species found in the present study. Previously, most of the Digenea species were reported from Sparidae (n = 12), however, Clinidae have now surpassed this number with a total of at least 14 species. More explorative studies are still needed to confirm species identification, as well as elucidate zoogeography of the parasites of *C. superciliosus*. With multiple factors influencing species distribution, the warming of marine waters will also alter parasite distribution.

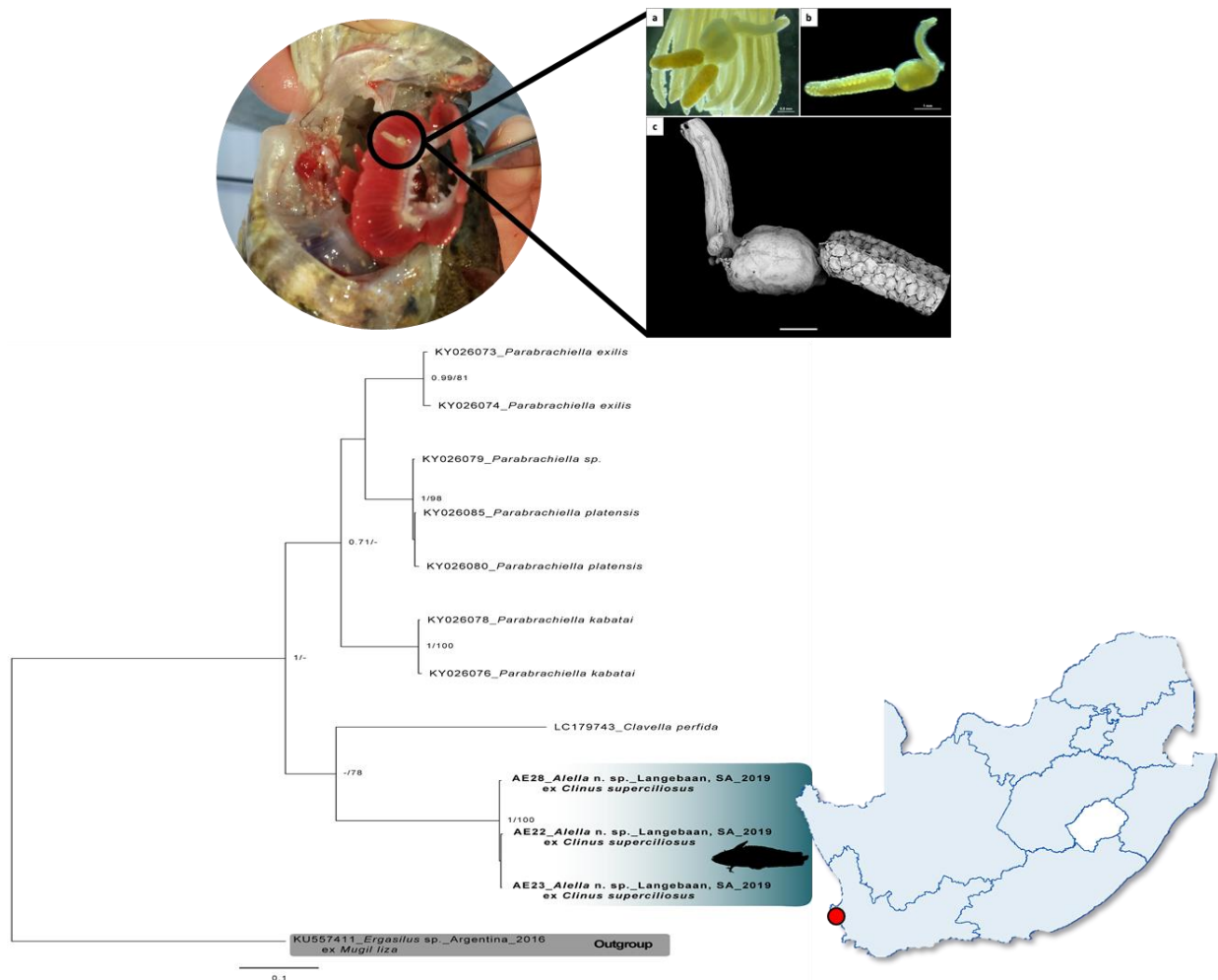
The present study demonstrates, through multiple species examples, the importance of molecular characterisation as an integrative approach to taxonomic descriptions (Blasco-Costa et al. 2016). This has proven to be an efficient tool to advance our knowledge of parasite species identification and geographic distribution ranges, even based on larval stages that are difficult to identify morphologically. Larval species sequenced during the present study will be published on GenBank for future researchers to possibly link adult stages and complete life cycle studies, for example cestodes, nematodes and acanthocephalans.

CHAPTER 4

Morphological and molecular characterisation of *Alella* sp. A (Copepoda: Siphonostomatoida: Lernaeopodidae) parasitising the southern African endemic intertidal klipfish, *Clinus superciliosus*

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* This PhD is disclaimed for purposes of Zoological Nomenclature in accordance with the International Code of Zoological Nomenclature, Fourth Edition Articles 8.2 and 8.3 (ICZN 1999). No new names or nomenclatural changes are available from statements in this PhD thesis.

4.1 Introduction

Copepods of the highly modified family Lernaepodidae Milne Edwards 1840, are parasitic on predominantly marine elasmobranch and teleost fish, as well as some freshwater fishes (Kabata 1979; Piasecki et al. 2010). These parasites are occasionally referred to as “gill maggots”, derived from the most common site of infection within the gills of fish and the movement of the parasite’s bodies when irritated (Ho 1977). Approximately 44 genera and 334 valid species are known globally from this family, and many genera have been synonymised or transferred in the past (Piasecki et al. 2010; Lebepe and Dippenaar 2016a; Boxshall and Hayes 2019). The taxonomy of the lernaepodid-group has been under contention from as early as the 19th century, impacting on its taxonomic arrangement and exacerbated by deficient information of its morphology (Kabata 1979; Ohtsuka et al. 2020). It was further suggested that many species are morphologically closely related to each other with different degrees of overlap (Piasecki et al. 2010). Piasecki et al. (2010) and Montes et al. (2017) reported that most species of lernaepodids exhibit narrow host specificity, as well as specific attachment sites on their fish hosts. Only female lernaepodids are adapted to a parasitic lifestyle, using a chitinous attachment organ (bulla), while the much smaller male lives as a temporary associate on the body of its partner.

To date, only 13 of the 48 known Lernaepodidae genera have been recorded from South Africa (Dippenaar 2004; 2016), mainly infecting chondrichthyans [see Schaeffner and Smit (2019) for full list of chondrichthyan lernaepodids from South Africa]. In comparison, little is known about parasites of actinopterygians (ray-finned fishes), with existing records only from sparid fishes.

As part of a study on the parasite diversity of the endemic intertidal clinid, *Clinus superciliosus* (Linnaeus, 1758), lernaepodids were collected from the gills of specimens sampled in the Langebaan marina, Saldanha Bay, along the west coast of South Africa. Using morphological and molecular techniques, this species was identified as new to science and herein described as a new species of *Alella*.

4.2 Materials and methods

4.2.1 Field collection

Over a three-year period (2018–2020), a total of 111 *Clinus superciliosus* (total length (TL) = 120–240 mm (174.7 mm)) were sampled along the South African coast [Langebaan marina (n = 19); Cape Town harbour (n = 16); Simons Town marina (n = 12); Hermanus (n = 20); Tsitsikamma (n = 20); Chintsa (n = 20)] (Figure 4.1). Fish were examined for any parasitic infections according to standard protocols (Klimpel et al. 2019) and the site of infection and number of parasites were noted per fish. Only the Langebaan marina specimens were infested with lernaepodids on the gill rakers. Copepod specimens were fixed and preserved in 70% ethanol until further examination in the laboratory. An additional 42 species of other parasitic groups (protozoan and metazoan) were also reported during these surveys, of which only 12 have been reported from literature prior

to this study (see Chapter 3). Fish were identified using Smith Sea Fishes (Smith and Heemstra 2012), with fish taxonomy and host nomenclature following FishBase (Froese and Pauly 2022) and Eschmeyer's Catalog of Fishes (Fricke et al. 2022).

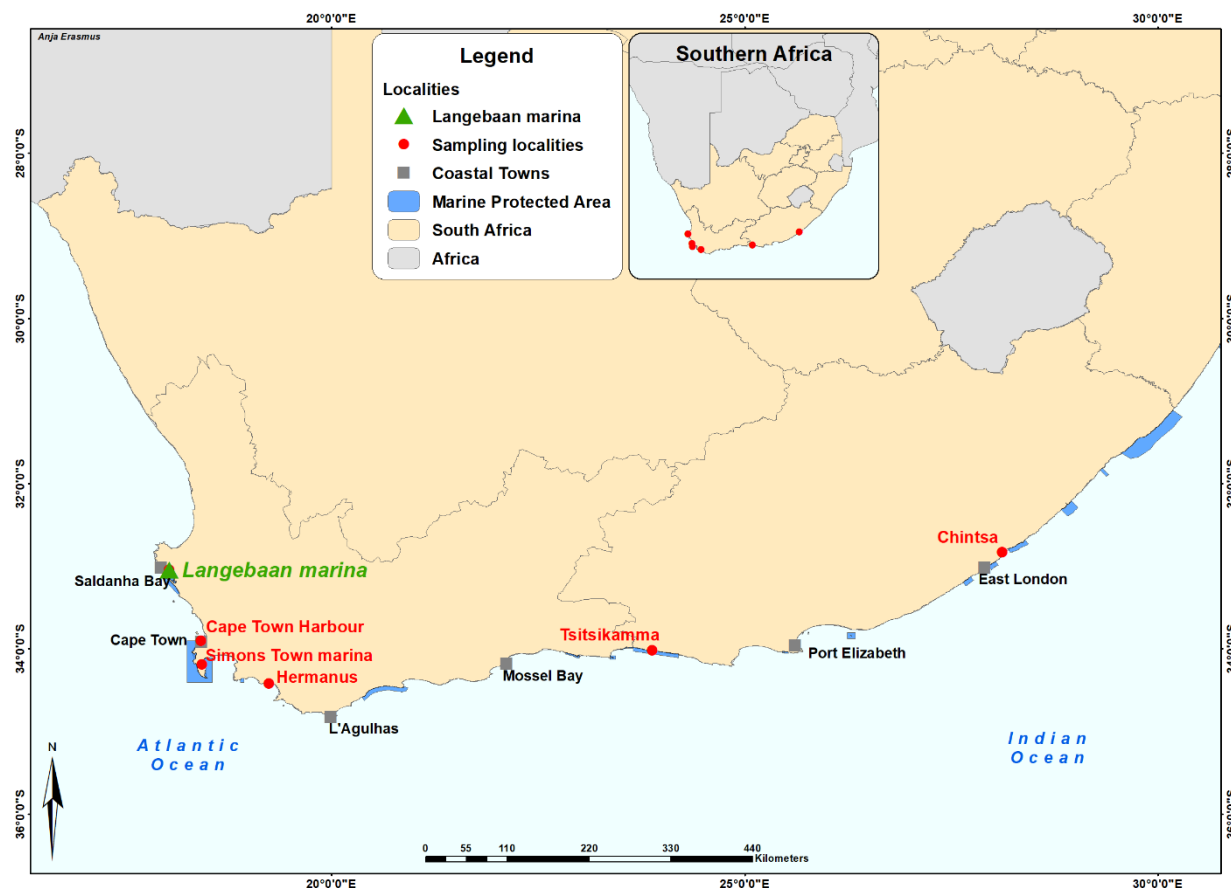


Figure 4. 1: Map indicating the localities in South Africa sampled for parasites infecting *Clinus superciliosus* in red, and the single locality where lernaepodids were collected is indicated in green.

4.2.2 Morphological methods

A dissection microscope (Zeiss Stemi 305 compact stereo microscope with an AxioCam camera and ZEN lite imaging software (Zeiss, Oberkochen, Germany)) was used to study the macro-morphology. Bright field and phase-contrast microscopy (Nikon Eclipse Ni, Nikon Instruments, Tokyo, Japan) were used to further study the organisms in the laboratory, where the z-dimensional stacking (Z-stack) function was applied for differential interference contrast (DIC) micrographs of different structures. Preserved specimens were cleared in lactic acid, stained with lignin pink, and appendages were dissected off to aid in morphological identification.

Three specimens were prepared for scanning electron microscopy (SEM) by dehydrating each in a graded ethanol series followed by a hexamethyldisilane (HMDS) series, placed on aluminium stubs using carbon tape, and sputter coated in gold/palladium (SPI-module, SPI Supplies, Pennsylvania, USA). Specimens were examined and photographed using a Phenom Pro Desktop SEM (ThermoScientific, Waltham, Massachusetts, USA).

Illustrations were made using z-stack micrographs, Adobe Illustrator CC v25.4.1, Adobe Photoshop CC v25.0 and a WACOM Intuos Pro v6.3.44-1 (Saitama, Japan) drawing tablet with a grip pen. Species description was prepared in DELTA (DEscriptive Language for TAXonomy) (available at <https://www.delta-intkey.com/>) based on a comparative Lernaepodidae character set developed in the present study. Measurements (TL – total length; L – length; W – width) and ratios were done using ImageJ software v1.53j (Schindelin et al. 2012) (available at <https://imagej.net/software/fiji/>), and based on the maximum values of each measured structure, while all proportional values were rounded to one decimal place. In the case where no measurements were given for comparative species, measurements were obtained from illustrations. Terminology for morphological features follow Kabata (1979) and Boxshall and Halsey (2004), and synonyms of the terms used in other descriptions with their respective definitions are listed in Appendix Table B. 1.

4.2.3 Molecular analyses

DNA extraction of an excised piece of the egg sacs from the lernaepodid specimens (n = 3) was done using a modified protocol for the PCR BIO Rapid Extract PCR Kit (PCRBiosystems, Analytical Solutions, Randburg, South Africa), where 10 µl of lysis buffer and 5 µl of protease buffer was added to the sample, and was diluted with 450 µl molecular grade water. The barcode region of the mitochondrial DNA cytochrome c oxidase subunit 1 (mtDNA COI) complex was amplified using universal primers LCO4190 (forward) (5'-GGT CAA CAA ATC ATA AAG ATA TTG G-3') and HCO2198 (reverse) (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al. 1994; Hayes et al. 2021), with the following PCR conditions: 5 min initial denature at 95 °C, followed by 35 cycles of amplification of 30 s at 95 °C, 30 s at 47 °C and 1 min at 72 °C, and final 7 min extension at 72 °C. The ribosomal genes 28S rRNA and 18S rRNA were amplified with the primers 28SF (forward) (5'-ACA ACT GTG ATG CCC TTA G-3') and 28SR (reverse) (5'-TGG TCC GTG TTT CAA GAC G-3') as in Santacruz et al. (2020), as well as 18S-E (forward) (5'-CCG AAT TCG TCG ACA ACC TGG TTG ATC CTG CCA GT-3') and NEM18SR (reverse) (5'-GGG CGG TAT CTG ATC GCC-3') as in Neethling and Avenant-Oldewage (2020), respectively. The PCR conditions for 28S rRNA were as follows: 2 min initial denature at 94 °C, followed by 35 cycles of amplification of 30 s at 94 °C, 30 s at 50 °C and 40 s at 72 °C, and final 7 min extension at 72 °C. For 18S rRNA the PCR conditions varied from 28S: 5 min initial denature at 94 °C, followed by 30 cycles of amplification of 30 s at 94 °C, 30 s at 56 °C and 2 min at 72 °C, and final 10 min extension at 72 °C. The amplified PCR products were visualised using agarose gel electrophoresis and sent for cleaning, purification and sequencing to Inqaba Biotechnical Industries (Pty) Ltd. (Pretoria, South Africa). Forward and reverse sequences were assembled, aligned and edited with Geneious 11.1.4 (Biomatters, Auckland, New Zealand).

4.2.4 Phylogenetic analysis

In total nine novel sequences were generated from three lernaepodid specimens (three sequences per gene for COI, 28S and 18S). The consensus sequences were compared to available sequences on BLAST tools (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) to confirm that the specimens belong to the family Lernaepodidae. Only sequences associated with peer-reviewed publications with per ident > 70% from the BLAST results were used for the phylogenetic comparison (Appendix Table B. 2). The selected outgroups (*Ergasilus* spp. von Nordmann, 1832) for the three alignments were based on the *Parabrachiella* Wilson, 1915 phylogenetic results from Montes et al. (2017). Due to the limited comparable sequences available for the 18S and 28S gene regions, a phylogenetic tree was only constructed for the COI region. The COI alignment was constructed using MUSCLE with the default parameters as in Geneious (Edgar 2004), whereafter the extremes were trimmed to an alignment of 654 bp. The invertebrate mitochondrial translation frame 1 was used to remove any stop codons from the alignment. A model test was performed using jModelTest 2.1.4 to determine the best nucleotide substitution model, based on the Akaike information criterion (AIC) (Guindon and Gascuel 2003; Darriba et al. 2012). The best AIC substitution model for COI mtDNA was GTR+G. A Bayesian Inference (BI) tree was constructed using MrBayes parameters in CIPRES Science Gateway v3.3 (available at <https://www.phylo.org/>) and a maximum likelihood (ML) tree was constructed using PhyML v3.0 (available at <http://www.atgc-montpellier.fr/phyml/>) (Guindon et al. 2010; Miller et al. 2010). Nodal support for ML analyses was estimated at 100 bootstrap repetitions. The mtDNA COI phylogenetic tree was edited and visualised in FigTree v1.4.3 software (Rambaut 2012). Pairwise genetic distance matrices (percentage differences and pairwise distances) were determined using Geneious and MEGA7 (Tamura et al. 2021).

4.3 Results

Genus: *Alella* Leigh-Sharpe, 1925

Type species: Alella pagelli (Krøyer, 1863) (type by subsequent designation)

4.3.1 Diagnosis

Utilising keys in Kabata (1979) and Boxshall and Halsey (2004), the current specimens conformed to the generic characteristics of *Alella* (Figure 4.2). The genus *Alella* Leigh-Sharpe 1925, has a Type C body plan, a cylindrical cephalothorax longer than the trunk and short fused maxilla with aliform expansions at the base (Kabata 1979).

4.3.2 Remarks

Originally *Alella* consisted of eight species, however, according to the World Register of Marine Species (WoRMS 2022) only three of these are currently considered as valid: *Alella canthari* (Heller, 1865); *Alella macrotrachelus* (Brian, 1906); and *Alella pagelli* (Krøyer, 1863). The other

five species were placed into synonymy by Dippenaar (2016): *Alella ditrematis* (Yamaguti, 1939), *A. gibbosa* van Niekerk & Olivier 1995 and *A. pterobrachiata* (Kabata, 1968) have been synonymised with *A. pagelli* based on morphological similarities; *A. berecynthia* Leigh-Sharpe 1936 is the junior synonym for *Clavellomimus berecynthia* (Leigh-Sharpe, 1936); and *A. tarakihi* Hewitt & Blackwell, 1987 the junior synonym for *Clavellotis tarakihi* (Hewitt & Blackwell, 1987) (Dippenaar 2016a). Although Dippenaar (2016) also synonymised *Alella macrotrachelus* with *A. pagelli*, Piasecki et al. (2016) challenged this decision and proposed that *A. macrotrachelus* should remain a separate species until further evidence can be supplied to support the synonymy of these two species. Thus, this species is still considered valid until more studies (particularly molecular work) can provide more information on this matter.

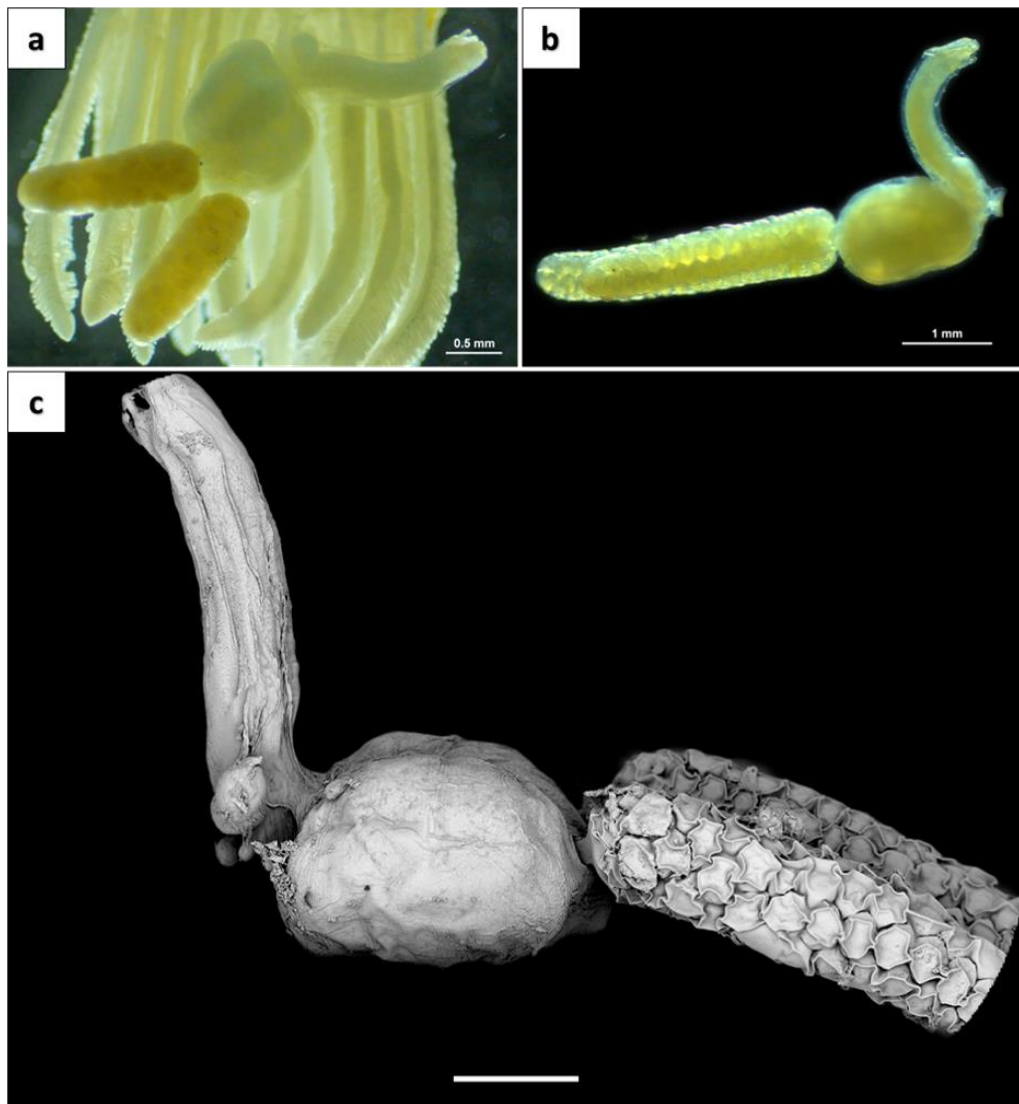


Figure 4. 2: Photographs of *Alella* sp. A a) dorsal view of lernaepodid attached to the gill rakers; b) lateral view of lernaepodid cephalothorax, trunk and egg sacs visible. Cephalothorax cylindrical and longer than $\frac{1}{4}$ of the trunk, arising from the anterior end of the trunk (Structural type C (Kabata, 1979)), with aliform expansions at bases of second maxillae.

***Alella* sp. A.**

Type Host: *Clinus superciliosus* (Linnaeus, 1758)

Type Locality: Langebaan marina, Saldanha Bay, Western Cape South Africa (33° 2' 44.4582" S; 18° 2' 19.0602" E), collected in August 2019.

Type Material: Holotype female with male attached, paratypes 3 female deposited in the collections of the National Museum, Bloemfontein. The remaining specimens are held in the parasite collection of the Water Research Group (WRG) at the North-West University.

ZooBank number: To be added

Genbank accession numbers: (18S). to be added; (28S). to be added; (COI). To be added

4.3.3 Description

Adult female (Figures 4.2, 4.3, 4.4) *Cephalothorax* (Figures 4.2, 4.3) cylindrical, longer than the trunk, arising from the anterior end of the trunk. *Dorsal shield* (Figures 4.2b, 4.4a, 4.5a) with indistinct posterior margin. *Aliform expansions and excretory ducts* (Figures 4.2, 4.3a, 4.4h, 4.5c-d) present. *Trunk* (Figures 4.2, 4.3a) unsegmented, oval; anal slit indistinct. *Genital process* (Figures 4.2a, 4.2c, 4.3a; 4.4i; 4.5e) present, ventral to egg sacs, conical. *Posterior process* absent. *Caudal rami* absent. *Egg sacs* (Figures 4.2; 4.3a) longer than trunk, length variable; eggs spherical, multiseriate (see Table 4.1 for morphometrics).

Antennula (Figure 4.3c) indistinctly segmented, tapered (basal part wider); armed with a whip posteroventrally, stout protrusion (possibly tubercle 1) between whip and apical elements, apical armature with 5 elements (as per Kabata 1979): digitiform seta 4 (S4) on medial side; flagelliform seta 6 (S6) on lateral side; elongated setae 5 (S5) in centre; tubercle 3 (T3) between S6 and S5; tubercle 2 (T2) between S5 and S4. *Antenna* (Figure 4.3d) biramous; exopod 1-segmented, bulbous with rounded tip and sensory pit, prominent, agglomerated denticles on outer margin, armed with stout protrusion on lateral aspect near base; endopod 1-segmented, basal segment with armature, terminal end with prominent denticles, 3–4 elements. *Mandible* (Figure 4.5b) primitive, with 6 teeth; anteriorly with 2 teeth of similar size, middle tooth largest, followed by 3 smaller teeth posteriorly. *Maxillula* (Figure 4.3e) with endite/inner lobe and palp/outer lobe; inner lobe with 2 terminal papillae, 1 small seta at base of inner papillae; outer lobe smaller than endite, with 2 setae reaching half the length of papillae. *Second maxillae* (Figure 4.3f) short, not completely fused, bilobed anteriorly. *Bulla* (Figure 4.3f) sub-spherical cup, not separable into manubrium and anchor. *Maxilliped* (Figure 4.3g) connected at the base; broad corpus and myxa with setae; corpus with short setae posterolaterally; myxa with short setae medially. *Subchela* (Figure 4.3h) with 4 basal setae, barb slender, reaching to half the length of the claw, slightly curved with sharpish tip.

Male (Figures 4.4e-f, 4.6) exhibits complete sexual dimorphism. Body not divisible into cephalothorax and trunk, with rounded posterior extremity. *Uropods* absent. *Posterior process* absent. *Genital process* absent externally.

Table 4. 1: Morphometric data (in mm) for *Alella* sp. A and other species used for morphological and molecular comparison.

Species	<i>Alella</i> sp. A		<i>Alella</i> pagelli		<i>Clavella</i> adunca		<i>Proclavellodes</i> pillai	
Host	<i>Clinus superciliosus</i> (Linnaeus)		<i>Rhabdosargus sarba</i> (Forsskål) <i>R. holubi</i> (Steindachner) <i>Acanthopagrus berda</i> (Forsskål) <i>R. globiceps</i> (Valenciennes)		<i>Boreogadus saida</i> (Lepechin, 1774)		<i>Gazza minuta</i> (Bloch, 1797)	
Family	Clinidae		Sparidae		Gadidae		Leiognathidae	
Attachment site	Gill rakers		Gill filaments		External – Fins and skin		Gill rakers	
Locality	Langebaan marina, WC, South Africa		St. Lucia estuarine system, KZN; Langebaan, WC, South Africa		Barents Sea		Trivandrum, South India	
Source	(Present study)		Dippenaar 2016a		Gjøsæter 1987		Kabata 1967	
	range (n = 5)	mean	range (n = 24–28)	mean	range (n = 20–26)	mean	range (n = 4)	mean
Total body L	4.5–7.8	6.2						
Cephalothorax L	1.8–2.4	2.1	-	2.7*	3.4–6.0	4.3	1.4–1.8	1.6
Cephalothorax W	0.4–0.4	0.4	-	0.4*	-	-	0.16–0.2	0.2
Cephalothorax W/L	-	1:0.2	-	1:0.2	-	-	-	1:0.1
Trunk L	1.1–1.7	1.4	0.8–2.0	1.4	2.9–5.3	3.8	1.08–1.4	1.2
Trunk W	1–1.3	1.1	-	0.9*	2.5–3.6	3	0.4–0.6	0.5
Trunk W/L	-	1:0.8	-	1:0.7	-	1:0.8	-	1:0.4
Trunk L/ cephalothorax L	-	1:0.7	-	1:0.5	-	1:0.9	-	1:0.8
Egg sacs L	1.6–3.8	2.5	0.9–2.5	1.7	3.9–6.8	5.3	1.5–1.6	1.5
Egg sacs W	0.5–0.6	0.5	-	0.4*	-	-	0.2	0.2
Male body L (n = 2)	489–645.9 µm	567.5 µm	230–244 µm	237 µm	-	-	-	-

*Measurements are based on illustration from Dippenaar (2016), since no measurements were provided in the description

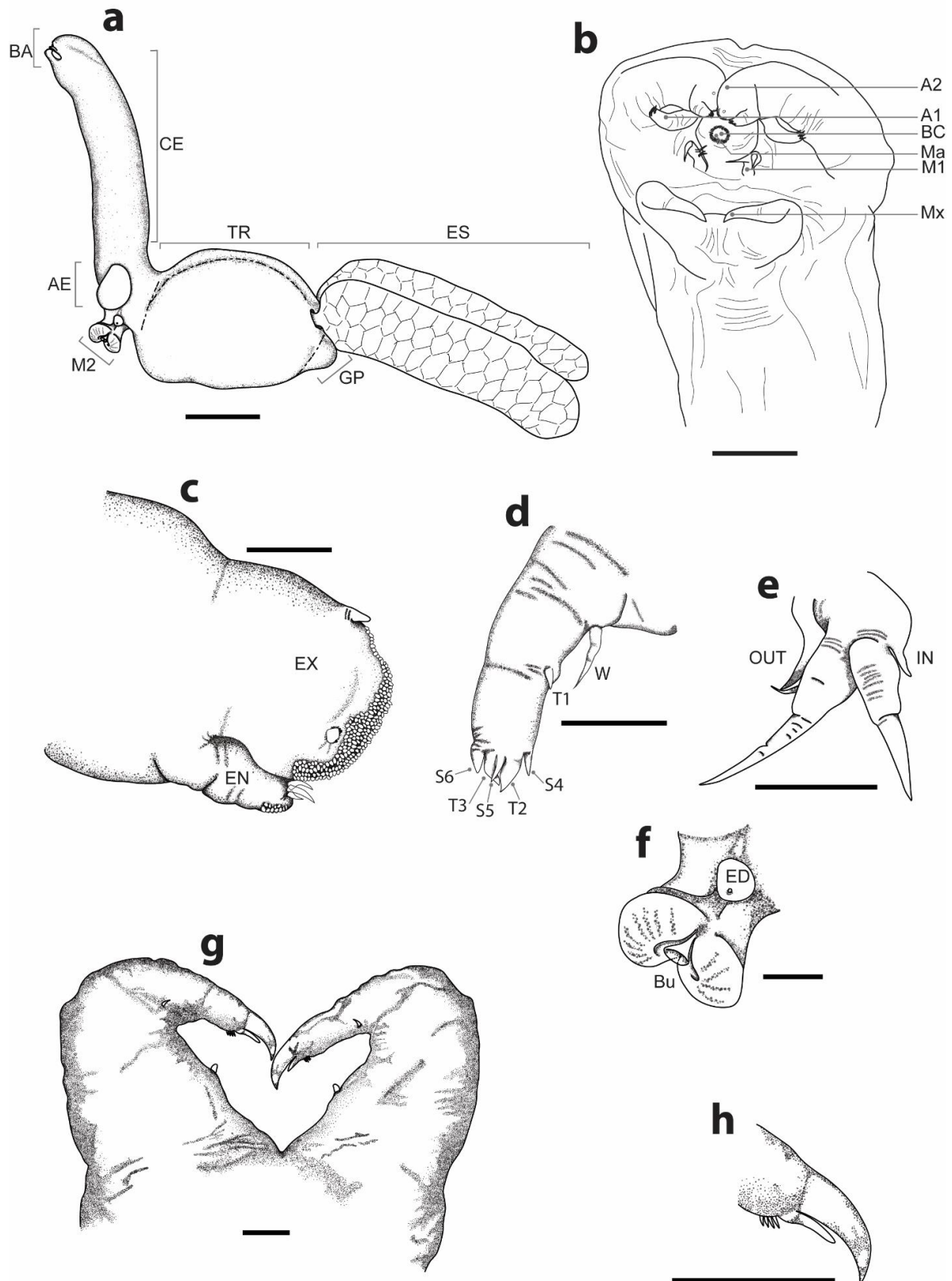


Figure 4. 3: *Alella* sp. A, adult female ex *Clinus superciliosus* (L.). a) habitus, lateral view; b) buccal apparatus, anteriorly; c) antenna; d) antennula; e) maxillula; f) maxilla with excretory duct (ED); g) maxillipeds; h) distal part of maxilliped, subchela and claw. A1 – antennula; A2 – antenna; AE – aliform expansions; BA – buccal apparatus; BC – buccal cone; Bu – bulla; CE – cephalothorax; ED – excretory ducts; EN – endopod; ES – egg sacs; EX – exopod; GP – genital process; IN – Inner lobe (endite); M1 – maxillula; M2 – second maxilla; Ma – mandibles; MX – maxillipeds; OUT – outer lobe (palp); S4 – digitiform seta 4; S5 – intermediate seta 5; S6 – flagelliform seta 6; T2 – tubercle 2; T3 – tubercle 3; TR – trunk; W – whip. Scale bars: A, B, F 100 μ m; C, D, E, G, H 20 μ m.

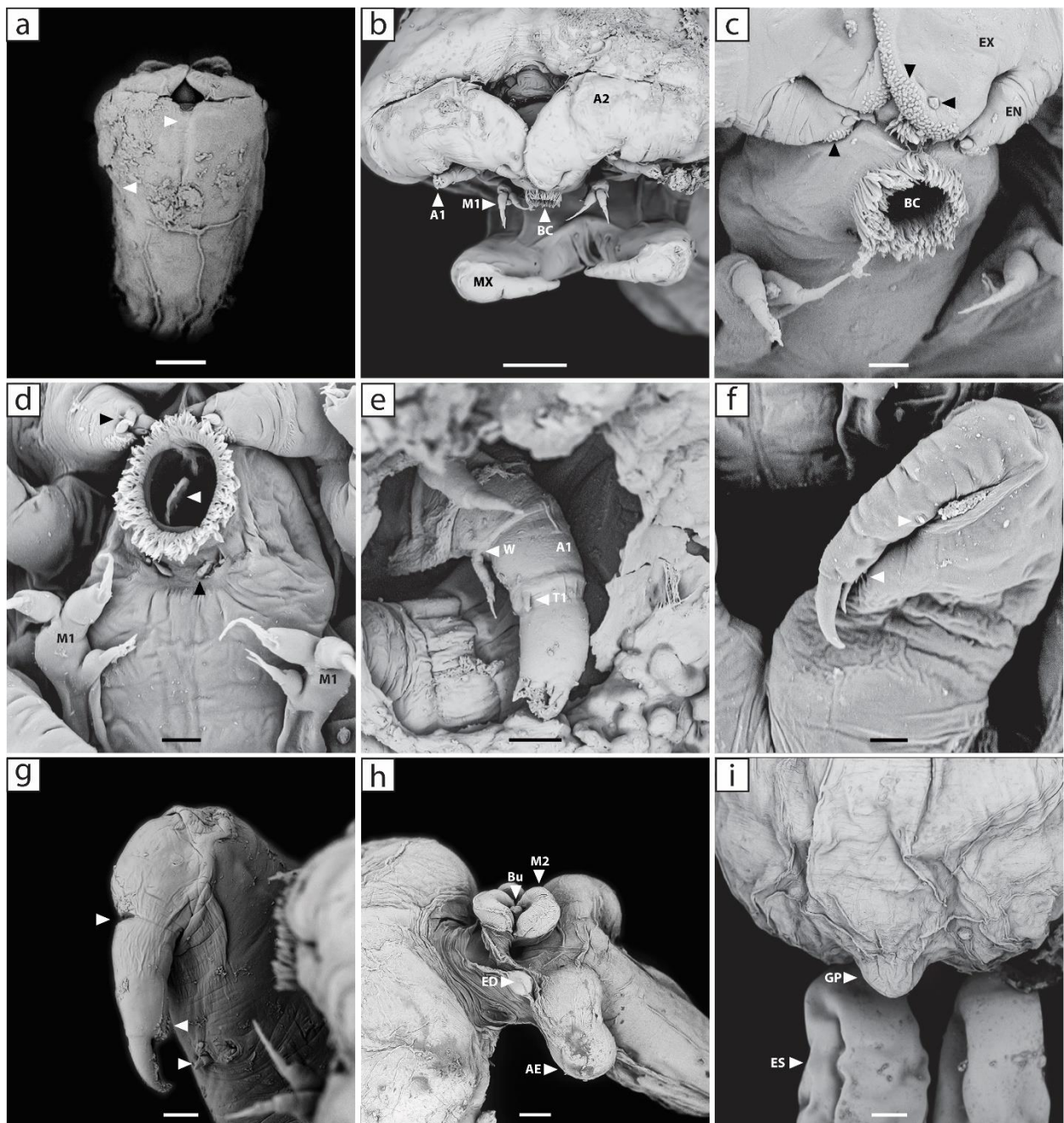


Figure 4. 4: Scanning electron photomicrographs of characteristic structures of *Alella* sp. A: a) Anterodorsal view of anterior end of cephalothorax; b) anterior view of cephalothorax; c) endo- and exopod of antenna showing sensory pit and setae, buccal cone and maxillula; d) ventral view of mouthparts and buccal cone; e) antennula; f) maxilliped subchela with prominent denticles on inner margin; g) dorsal view of maxillipeds; h) lateral view of cephalothorax with second maxilla, aliform expansions and excretory ducts; i) posterior end of trunk with genital process and egg sacs. A1 – antennula; A2 – antenna; BC – buccal cone; ED – excretory ducts; EN – endopod; ES – egg sacs; EX – exopod; GP – genital process; LS – lateral swellings (aliform expansions); M1 – maxillula; M2 – maxilla; MX – maxillipeds; W – whip. Scale bars: a, h, i 100 μ m; b 50 μ m; and c, d, e, f, g 10 μ m

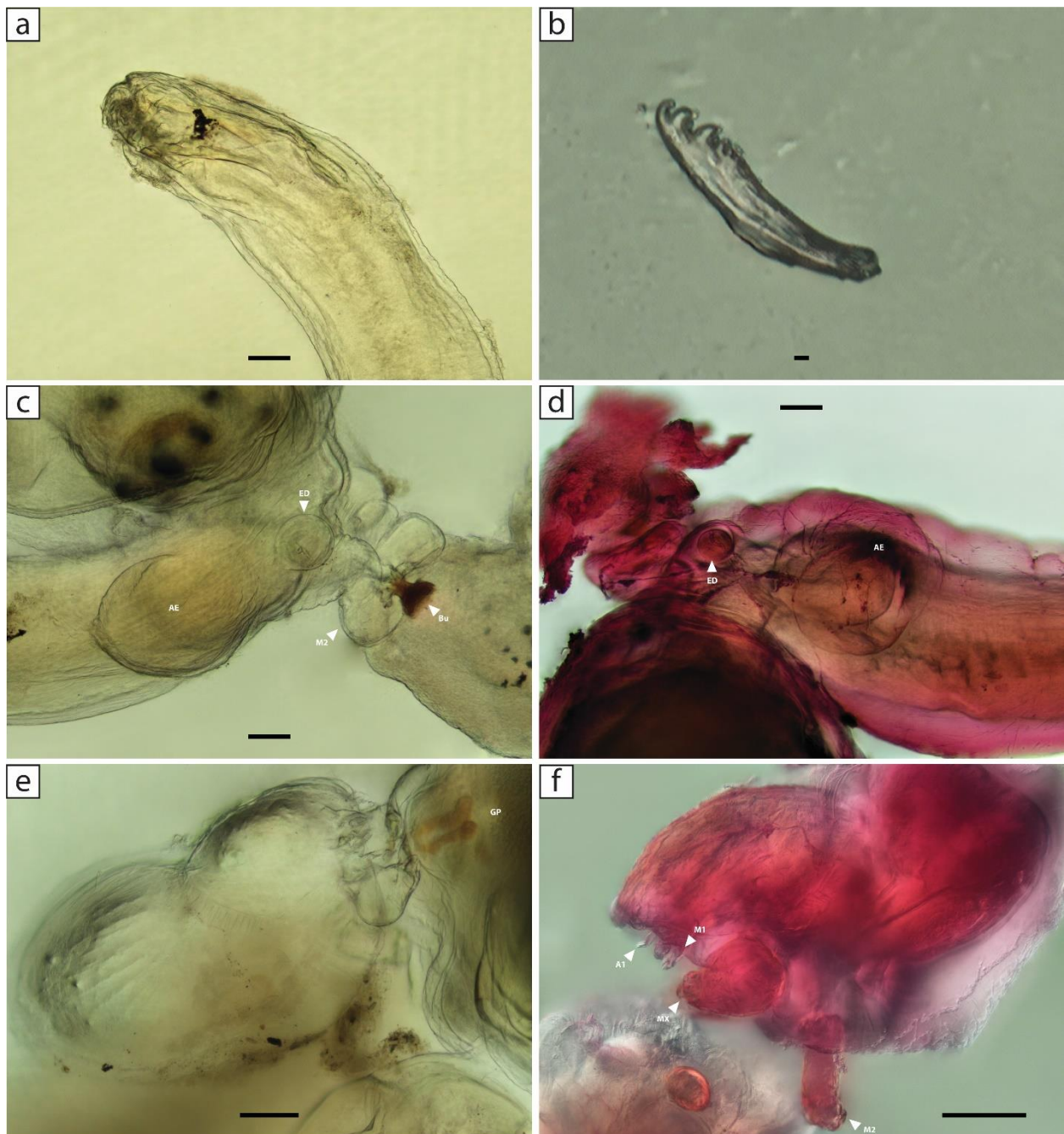


Figure 4. 5: Bright field and phase-contrast photomicrographs with the z-dimensional stacking function applied to view *Alella* sp. A: a) anterodorsal end of cephalothorax showing dorsal shield (arrow); b) mandible dissected from mouth cone; c) second maxilla with bulla visible inside the gill filament; d) stained aliform expansions with excretory duct, lateral view; e) male as seen attached to female genital process; f) stained male with appendages visible. Abbreviations: ♀ – female; ♂ – male; AE – aliform expansions; Bu – bulla; ED – excretory ducts; GP – genital process; M2 – maxilla. Scale bars: a, c, d, e, f 100 μ m; and b 10 μ m

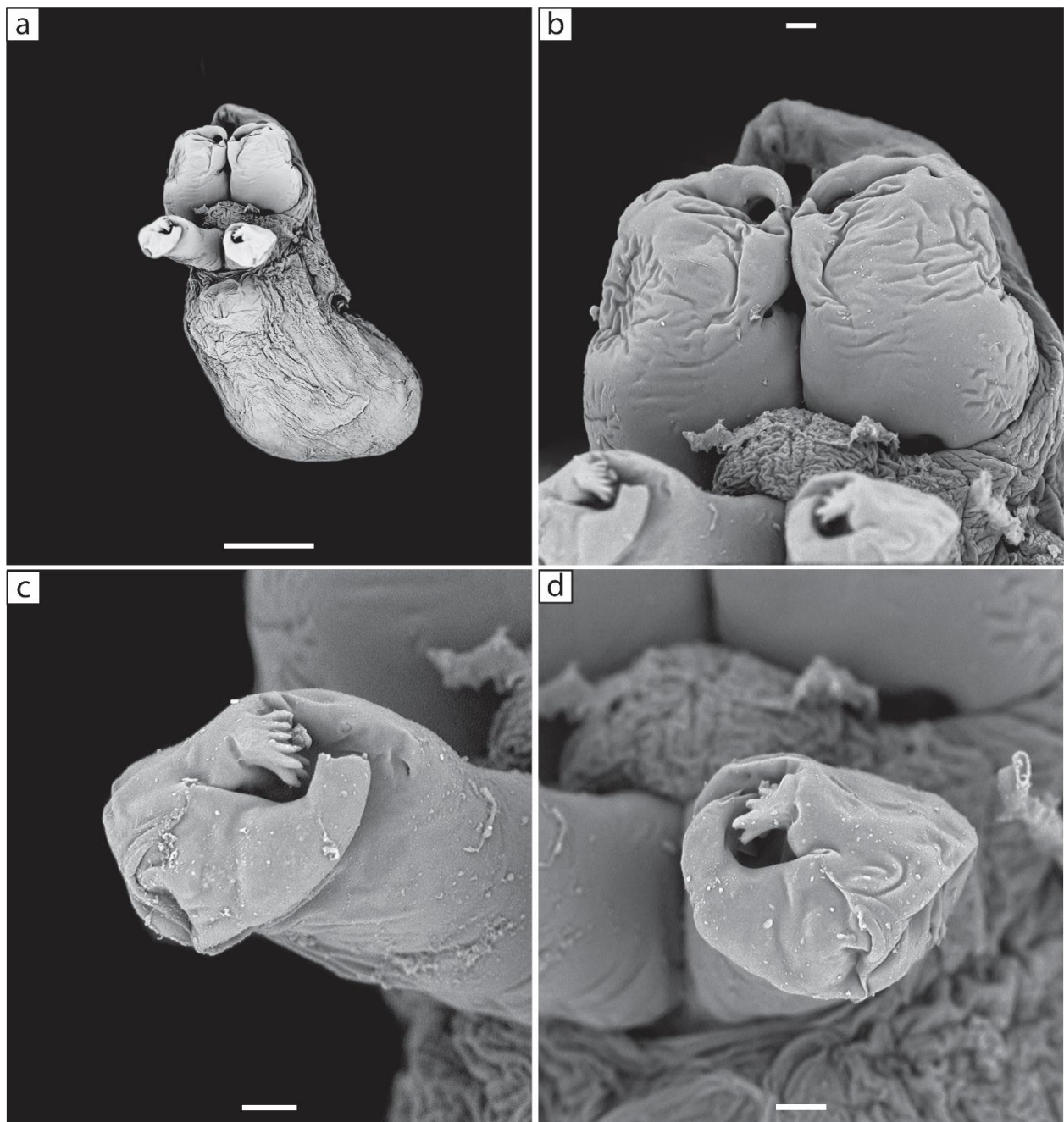


Figure 4. 6: Scanning electron photomicrographs of the male *Alella* sp. A: a) habitus of male *Alella* sp. A; b) maxillipeds; c) left second maxilla, with distal end broken; d) right second maxilla. Scale bars: a 100 μ m; and b, c, d 10 μ m

4.3.4 Remarks

Alella sp. A is characterised by prominent agglomerated denticles on the female antenna, the arrangement of the apical armature of the antennula, as well as the anteriorly bilobed maxilla, the six teeth of the mandible, and small size and sub-spherical cup shape of the bulla. Also, the presence of the distinct excretory ducts visible between the maxilla and the aliform expansions.

This species of *Alella* is morphologically distinguishable from other similar species through a combination of the following characteristics: i) antenna; ii) antennula; iii) mandible; iv) maxillula; v) maxilla; vi) bulla. *Alella* sp. A can easily be distinguished from the other three known species

of *Alella* based on the unique prominent agglomerated denticles around the outer margin of the antenna exopod, and tubercle 1 of the apical armature of the antennula located closer to the whip than the apical armature. The latter characteristic might have been modified during the course of ontogeny. The apical armature of the antennula are all elements relatively equal in size compared to the distinct size differences described in Kabata (1979). Based on the antennula description in Kabata (1979), the apical armature conforms to the *Charopinus*-branch that includes the structural type C body plan for lernaeopodids such as the genus *Alella*. *Alella pagelli*, a species described from sparid fish from South Africa, is morphologically the most similar species, however, based on the recent redescription of this species by Dippenaar (2016) it differs as follows: the distinctly prominent agglomerated denticles on the antenna; the unusual arrangement of the apical armature on the antennula; the mandible of *A. pagelli* has 8 teeth, compared to 6 teeth in *Alella* sp. A; the maxillula of the latter with additional setae; and no mention of excretory ducts at the base of the maxilla and aliform expansions, as well as the different host species. *Alella* sp. A also lacks the prominent collar around the manubrium as in *Clavella adunca* (Strøm, 1762), and the shape of the bulla differs from another morphologically similar species, *Proclavellodes pillaii* Kabata, 1967, confirming its placement within the genus.

Molecular analysis: Comparison of the partial 18S rRNA sequences from the present study (Table 4.2), to the limited GenBank sequences demonstrated genetic similarity of 98.2% (9 nt) to the closest relative (*Clavellisa emarginata* (Krøyer, 1837)), while for the partial 28S rRNA (Table 4.3) the highest similarity was found to be 73.9% (171 nt) for *Salmincola edwardsii* (Olsson, 1869), a freshwater copepod. The species divergence for COI (Table 4.4) between the novel sequences and the GenBank obtained sequences was 79.3% (130 nt), with sequences of *Parabrachiella kabatai* (Luque & Farfan, 1991) as the most closely related. These alignments did not include any of the unpublished sequences, nor any sequences from retracted publications available on Genbank (Table B. 2). As a result of low support due to the limited number of published sequences, phylogenetic trees for 18S and 28S were not constructed.

Table 4. 2: Genetic matrix for 18S rRNA sequences of Alella sp. A and other members of the family Lernaeopodidae, indicating percentage similarity below the diagonal division and pairwise distances above the division.

	Lernaeopodidae species	1	2	3	4	5	6	7
1	AE22_ <i>Alella</i> n. sp.		0.000	0.000	0.010	0.010	0.022	0.093
2	AE23_ <i>Alella</i> n. sp.	100.0		0.000	0.010	0.010	0.022	0.090
3	AE28_ <i>Alella</i> n. sp.	100.0	100.0		0.010	0.010	0.022	0.092
4	KR349122_ <i>Clavellisa emarginata</i>	98.2	98.3	98.3		0.010	0.019	0.081
5	AY627027_ <i>Parabrachiella baponosa</i>	98.5	98.6	98.6	98.5		0.019	0.075
6	AY627028_ <i>Clavella addunca</i> [sic]	96.7	96.8	96.7	97.0	97.5		0.077
7	DQ107578_ <i>Ergasilus yaluzangbus</i>	89.9	90.2	90.0	90.4	91.6	91.1	

Table 4. 3: Genetic matrix for 28S rRNA sequences of *Alella* sp. A and other members of the family *Lernaeopodidae*, indicating percentage similarity below the diagonal division and pairwise distances above the division.

	Lernaeopodidae species	1	2	3	4	5	6
1	AE22_ <i>Alella</i> n. sp.		0.000	0.000	0.178	0.178	0.267
2	AE23_ <i>Alella</i> n. sp.	100.0		0.000	0.173	0.173	0.261
3	AE28_ <i>Alella</i> n. sp.	100.0	100.0		0.173	0.173	0.261
4	KY113081_ <i>Salmincola edwardsii</i>	73.9	74.2	74.1		0.015	0.274
5	KY113083_ <i>Salmincola californiensis</i>	74.0	74.2	74.2	97.3		0.279
6	DQ107540_ <i>Ergasilus yaluzangbus</i>	66.6	67.0	66.9	68.9	68.2	

Table 4. 4: Genetic matrix for COI mtDNA sequences of *Alella* sp. A and other members of the family *Lernaeopodidae*, indicating percentage similarity below the diagonal division and pairwise distances above the division.

	Lernaeopodidae species	1	2	3	4	5	6	7	8	9	10	11	12
1	AE22_ <i>Alella</i> n. sp.		0.002	0.003	0.207	0.203	0.205	0.206	0.208	0.208	0.214	0.235	0.321
2	AE23_ <i>Alella</i> n. sp.	99.8		0.002	0.206	0.202	0.203	0.205	0.209	0.209	0.213	0.237	0.319
3	AE28_ <i>Alella</i> n. sp.	99.7	99.8		0.204	0.200	0.202	0.203	0.209	0.209	0.211	0.235	0.318
4	KY026078_ <i>Parabrachiella kabatai</i>	79.3	79.4	79.6		0.123	0.123	0.124	0.132	0.136	0.003	0.217	0.309
5	KY026079_ <i>Parabrachiella</i> sp.	79.7	79.8	80.0	87.7		0.002	0.006	0.107	0.109	0.125	0.217	0.290
6	KY026085_ <i>Parabrachiella platensis</i>	79.5	79.7	79.8	87.7	99.8		0.005	0.109	0.110	0.125	0.216	0.291
7	KY026080_ <i>Parabrachiella platensis</i>	79.4	79.5	79.7	87.6	99.4	99.5		0.110	0.112	0.127	0.214	0.291
8	KY026073_ <i>Parabrachiella exilis</i>	79.2	79.1	79.1	86.8	89.3	89.1	89.0		0.011	0.141	0.220	0.298
9	KY026074_ <i>Parabrachiella exilis</i>	79.2	79.1	79.1	86.4	89.1	89.0	88.8	98.9		0.145	0.220	0.295
10	KY026076_ <i>Parabrachiella kabatai</i>	78.6	78.7	78.9	99.7	87.5	87.5	87.3	85.9	85.5		0.222	0.305
11	LC179743_ <i>Clavella perfida</i>	76.5	76.3	76.5	78.3	78.3	78.4	78.6	78.0	78.0	77.8		0.329
12	KU557411_ <i>Ergasilus</i> sp.	67.0	67.1	67.3	68.1	70.0	69.9	69.9	69.3	69.6	68.5	66.2	

The alignment for mtDNA COI gene consisted of nine sequences obtained from GenBank, including the outgroup. Overall, the BI and ML analyses recovered similar topography, with relative support at the majority of the nodes (Figure 4.7). Due to the limited number of published sequences, a lower resolution in the deeper nodes was obtained for the family.

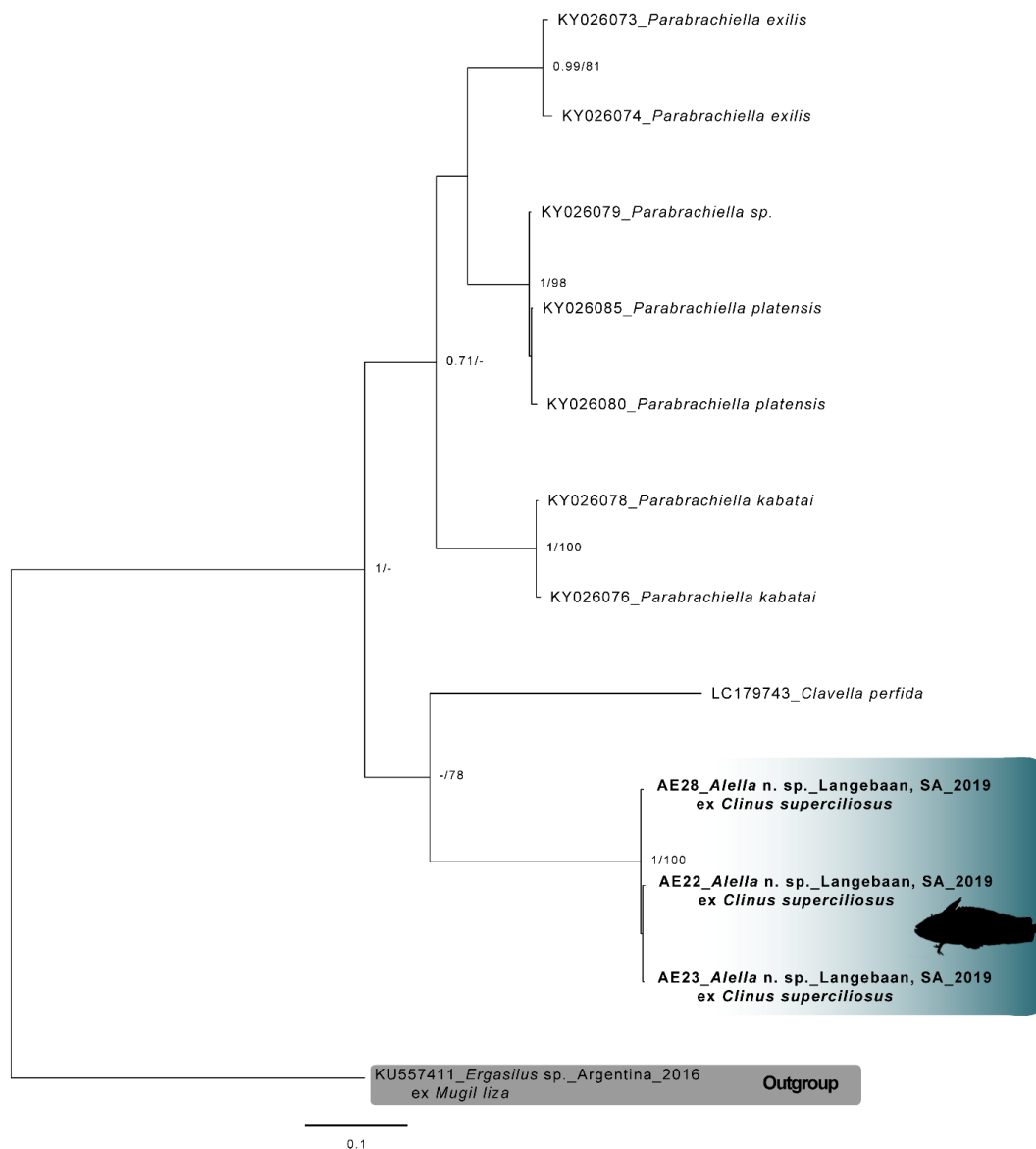


Figure 4. 7: Bayesian inference and maximum likelihood tree (BI/ML) of members of the family Lernaepodidae, based on COI mtDNA sequences. Bootstrap support is displayed at the nodes only if > 0.7 or > 70% for BI and ML respectively. Novel sequence are displayed in the coloured box, while the outgroup (*Ergasilus* sp.) is displayed in the grey box. The scale bar represents the number of nucleotide substitutions per site.

The phylogenetic analysis confirms the morphological classification of *Alella* as most closely related to *Clavella* Oken, 1815 but not the same genus, with a bootstrap support value of 72%. The ingroup sequence divergence for COI was high, with an estimated 20.6% variation between *Alella* sp. A and *Parabrachiella kabatai*, and 23.5% variation between the novel sequences and *Clavella perfida* Wilson, 1915. Monophyly of the genus *Alella* could not be determined since no other sequences from this genus were available for genetic comparison.

4.4 Discussion

To date all species of *Alella* have been reported from Sparidae (Dippenaar 2016a). *Alella pagelli* has been reported from *Rhabdosargus sarba*, *R. holubi*, *R. globiceps* and *Acanthopagrus berda*, *A. canthari* is known from *Pachymetopon blochii*, while *A. macrotrachelus* has been reported to also infest *Spicara melanurus* (Valenciennes) (as in Kabata, 1979), *Acanthopagrus butcheri* (Munro), *A. berda*, *A. latus* (Houttuyn) (as in Byrnes, 1988), and *A. schlegeri* (Bleeker) (as in Kawatow, 1980; Ho 1983). These host species are distributed across the world's oceans, as well as within South Africa waters. The description of *Alella* sp. A from clinid fishes is thus the first non-sparid host record for a species of *Alella* as well as the first record of clinids as hosts of Lernaepodidae copepods.

We agree with Piasecki et al. (2016) that basic morphological methods cannot be used to distinguish between host specific and closely related lernaepodids, and that molecular tools are needed for a comprehensive revision of Lernaepodidae. At this stage, however, too few sequences are available to make a definitive decision based on molecular differentiation. While the phylogenetic relationships of Lernaepodidae are not well resolved it requires more representatives of the species and genera to provide a robust classification. Due to the limited number of published sequences, lower resolution on the deeper nodes were obtained for the phylogeny of the family, which again highlights the need for more representative sequences for the genera and the family. It is important to keep in mind that as more taxa are added into the phylogenetic tree the relationships will change. To indicate distinct copepod genera, the authors accepted a range of 9–24% sequence divergence for COI (Bucklin et al 1999; Bucklin et al 2003; Mangena et al 2014). Interspecific sequence divergence was reported as > 15% (Lefébure et al. 2006; Øines and Heuch 2007; Dippenaar et al. 2010; Mangena et al. 2014), while intraspecific divergence was set at 0–4% (Bucklin et al. 2003; Øines and Heuch 2007; Dippenaar et al. 2010; Mangena et al. 2014).

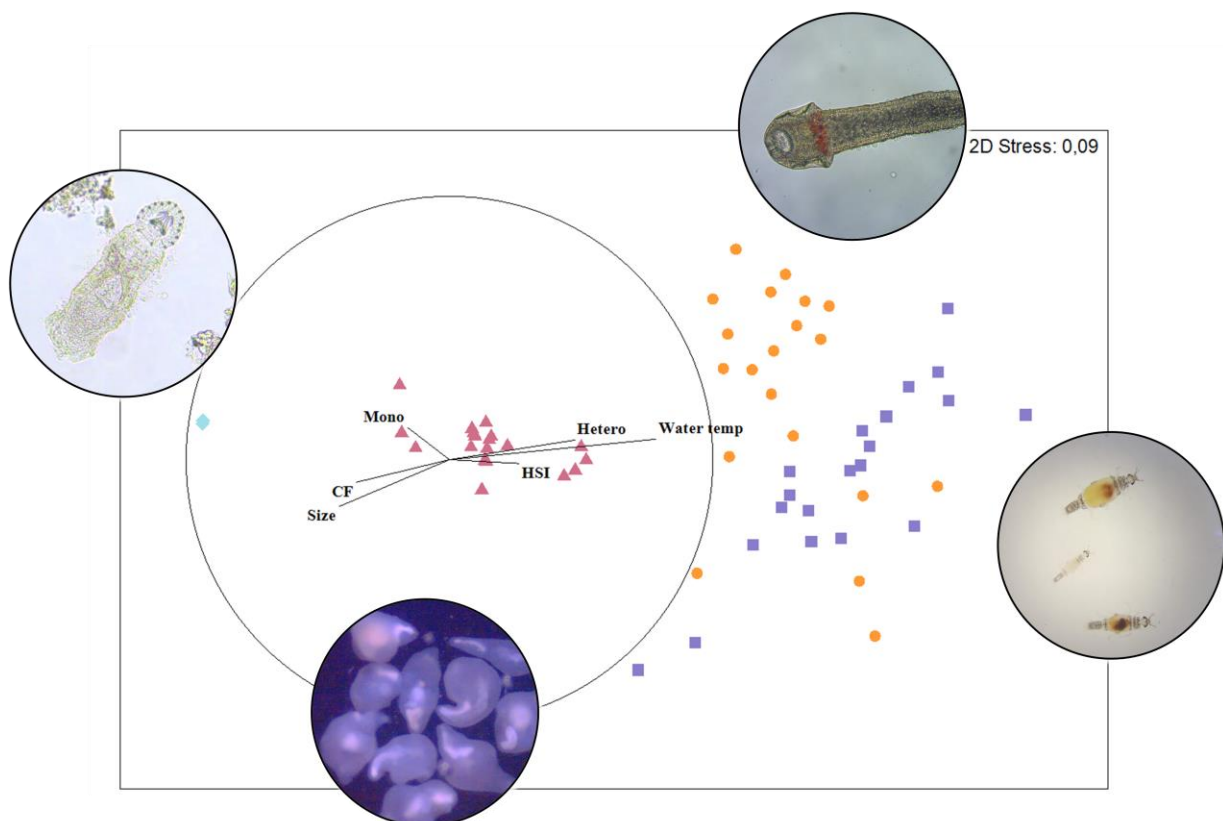
The present study provides the first partial gene sequences of three different genes for the genus *Alella*. This genus is related to *Clavella*, forming a clade as sister taxa, therefore confirming that these two genera are also morphologically closely related. Although limited sequences are currently available, our results confirm that *Alella* and *Clavella* are separate genera. Future studies on this copepod family, should involve an integrated approach to taxonomy, where species are described and compared based on multiple morphological techniques, as well as molecular data. The present study is also the first to use DELTA for the species description and we here propose that, to avoid the use of multiple terms for the same morphological characters, that future studies on Lernaepodidae should consider using this method of species description as well.

CHAPTER 5

Metazoan parasite diversity of the endemic South African intertidal klipfish, *Clinus superciliosus*: correlates of parasite community structure

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5.1 Introduction

Contributing to the global biodiversity, parasites comprise an estimated 40% of all animal species (Dobson et al. 2008; Wood et al. 2014; Wood and Johnson 2015). Parasites are intimately involved in ecosystem functions and play important ecological roles in host populations, ecosystems, and energy flow (Lafferty et al. 2008; Wood and Johnson 2015). They are considered to be significant drivers of biodiversity, where each host is regarded as an individual habitat that parasites can colonise (Hudson et al. 2006). Nonetheless, records of parasites are often limited to taxonomic descriptions and general surveys of parasite fauna (Reed 2015), and geographic distributions and ecological roles are less frequently focussed on (Torchin and Kuris 2005). However, considering how little we know about the influence parasites have on communities and ecosystems, numerous parasite ecologists have been interested in establishing which ecological factors influence parasite community composition and structure (Hoberg and Adams 2000; Wood and Johnson 2015; Benhamou et al. 2017). This parasite community composition can be used as an environmental health indicator by providing information on the local food web assemblages, revealing potential disturbances that affect them, shedding light on dietary shifts, and indicating dissimilarities in infracommunities to discriminate between host populations (MacKenzie and Abaunza 2005; Constenla et al. 2015; Münster et al. 2015; Pérez-i-García et al. 2015; Dallarés et al. 2016). The presence of each parasite species can indicate information on its particular life cycle, as well as biological and ecological traits that, in turn, provide a more comprehensive perspective on ecosystems (Marcogliese 2005; Pérez-del-Olmo et al. 2007b; Dallarés et al. 2016). As an example, parasites can infer host endemism and invasion status, or provide information on host migration (Torchin and Kuris 2005). Parasites can also interfere with host behaviour, where some parasite species hinder the host's ability to see or move, which can result in the host being up to 10–30 times more susceptible to predation (Hudson et al. 1992; Hudson et al. 2006; Lafferty 2008). In addition to the influence that parasites have on their host, their behaviour modulates changes on other components within the ecological community (Wood et al. 2007). However, parasites can also have a positive impact as they contribute to the species richness of an ecosystem and can help stabilise a community structure following events damaging ecosystem health by increasing resilience and launching the restoration success of the ecosystem (Huspeni and Lafferty 2004; Hudson et al. 2006). More recently, there has been strong evidence to support the idea that parasites can reduce environmental toxins, especially metals, within their fish hosts, and thus play a positive role in host health (Sures 2004; Sures et al. 2017, Erasmus et al. 2020a). Various studies even suggest that ecosystems with a large number of parasite species can be assumed to be a healthy ecosystem (Hudson et al. 2006; Vidal-Martínez et al. 2019).

The diversity of parasites is dependent on various biotic and abiotic factors, including host susceptibility and sensitivity, host fitness, parasite life cycle, as well as pollution or climatic events, eutrophication, conservation, and fishing status of the habitat (Lafferty 2008; Vidal-Martínez et al.

2019; Martins et al. 2021). Depending on the parasite life cycle and their intermediate and definitive hosts, distribution ranges will also be altered as a result. While some parasites are not directly affected by environmental factors (pollution, eutrophication, etc.), intermediate and definitive hosts can be and will, in turn, cause reduced transmission or completion of its life cycle (Hogue and Swig 2007; Lafferty 2008; Vidal-Martínez et al. 2019). Parasite groups respond differently to environmental variables depending on their life cycle (Martins et al. 2021). It is thus important to consider the whole ecosystem when studying parasite distribution patterns. Various studies have also reported latitudinal and longitudinal variation in species richness of endo- and ectoparasites (Torchin and Kuris 2005; Thieltges et al. 2009a, 2009b; Martins et al. 2021). Since parasites form part of food webs, they are sensitive to changes in their environment, that being the fish (endoparasites) or the ecosystem (ectoparasites) (Lafferty 2008; Paula et al. 2021). Changes in water temperature, for example, can influence gnathiid isopod survival and result in significant ecological effects on parasite-host interactions of cleaner fish where gnathiids serve as a major food source (Shodipo et al. 2020). Parasites can alter the behaviour and mobility of their host, as in the case of *Anilocra haemuli* Bunkley-Williams & Williams, 1981 infecting French grunts where infected fish have reduced movement compared to uninfected fish, which indirectly influences energy flow between connected ecosystems (Welicky and Sikkell 2019). It is also suggested that parasites are the links between predator-prey interactions by making prey more susceptible for predation (Lafferty 2008; Reisinger et al. 2015). These parasites are ideal models for evolutionary and ecological processes (Dallas et al. 2019; Grutter et al. 2020; Grunberg 2021). More complex food webs reported supports a more diverse parasite community within complex community networks (Hudson et al. 2006; Lafferty 2008).

Parasite records along the South African coastline are infrequent, where most consist of single taxonomic records or parasites associated with marine hosts from single sampling localities (Reed 2015; Smit and Hadfield 2015). This is also true for *Clinus superciliosus* (Linnaeus, 1758), a klipfish endemic to southern Africa, for which a number of protozoan and metazoan parasite records do exist (15 species), but reports tend to be limited to taxa specific papers (see Vermaak et al. 2021 and references therein). *Clinus superciliosus* has a distribution range from Namibia on the west coast of Africa to the Kei River mouth in the Eastern Cape, South Africa (Smit et al. 2003; von der Heyden et al. 2011; Holleman et al. 2012; Smith and Heemstra 2012). This fish species has a range of feeding habits representing a wide variety of trophic groups including Mollusca, Amphipoda, Isopoda, Decapoda, Echinodermata and small fish species (Bennett et al. 1983; Holleman et al. 2012). The species has also been reported to contribute to about 40–70% of the total biomass of rocky shore fish (Bennett 1984; Prochazka and Griffiths 1992). The above characteristics makes *C. superciliosus* a great study organism for determining the ecological factors that influence parasite community composition and structure of an inter- and infratidal fish.

The aim of this section of the present study was to gain information on which factors influence parasite community composition within this species by examining the metazoan parasite

community structure at various localities within the distribution range of *C. superciliosus*. To achieve this, this research aimed to: i) collect, identify and enumerate metazoan parasites of *C. superciliosus* from four different localities; ii) calculate and determine significant differences between selected diversity indices for each locality; and iii) complete a multivariate differentiation of the community composition taking into consideration spatial variability, habitat variability, parasite life cycle, as well as fish host size, gender, and condition factors.

5.2 Material and Methods

5.2.1 Study area and sampling procedure

A total of 75 fish were collected at four localities (Figure 5.1) [Langebaan marina (33°02'44.5"S; 18°02'19.1"E), $n = 19$; Cape Town harbour (33°54'29.1"S; 18°25'05.8"E), $n = 16$; Tsitsikamma (within the Garden Route National Park) (34°01'15.2"S; 23°52'43.2"E), $n = 20$; and Chintsa (32°50'11.5"S; 28°07'01.2"E), $n = 20$] along the coast of South Africa within the distribution range of *C. superciliosus*.

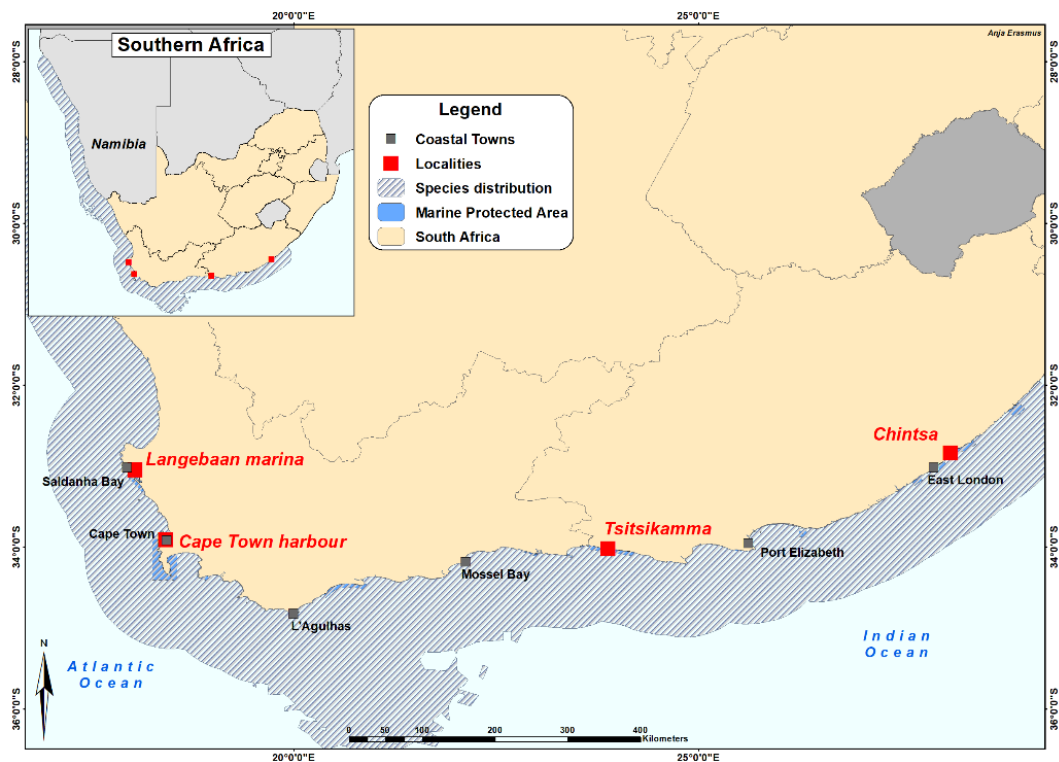


Figure 5. 1: Map indicating sampling localities, from left to right: Langebaan marina, Cape Town harbour, Tsitsikamma and Chintsa. Species distribution data obtained from Holleman et al. (2014) and GBIF.org (2020).

Fish hosts and parasites were collected using the sampling procedure described in Chapter 2. The necessary research permits were obtained prior to sampling from the Department of Agriculture, Forestry and Fisheries (DAFF) and South African National Parks (SANParks) (RES2018/35, MALH-K/2016-005a and RES2019/103), as well as ethical clearance through AnimCare at the North-West University (NWU-00440-16-A5 and NWU-0051-19-A5).

5.2.2 Parasite identification

Preserved specimens were counted and examined under the appropriate microscopes [light- (Nikon Eclipse Ni, Nikon Instruments, Tokyo, Japan) and dissection microscopes (Zeiss Stemi 305, Zeiss, Oberkochen, Germany)] for morphological differences. Each taxon was grouped according to different morphotypes, based on distinct morphological and molecular characteristics, to the lowest taxonomic level possible for each group. Systematic arrangements for each group were done according to the relevant literature, expert identification and taxonomic keys: Acanthocephala (Amin 2013); Cestoda (Caira and Jensen 2017); Copepoda (Kabata 1979; Boxshall and Halsey 2004; Hayes et al. 2021; Smit et al. 2019); Digenea (Vermaak 2020; Vermaak et al. 2021 and references within); Hirudinea (Govedich et al. 2005; Williams and Burrenson 2006); Isopoda (Smit et al. 2002; Hadfield et al. 2008); Monogenea (Whittington 2005); and Nematoda (Moravec et al. 2006; 2016). Detailed parasite descriptions, including new species descriptions and a brief account of their life cycles will be published separately (see also Chapter 3 and 4).

5.2.3 Statistical analyses

Ecological statistics (following Bush et al. 1997; Poulin 2015; Clarke et al. 2014; Benhamou et al. 2017), were used for parasite populations and community composition: Prevalence (P %) calculated as the proportion of the hosts infected with each parasite taxa; mean intensity (MI) as the total number of individuals per parasite taxa divided by the number of infected hosts; and mean abundance (MA) as the total number of individuals of each parasite taxa divided by the number of hosts sampled. Two hierarchical levels were determined for further statistical analysis: infracommunity (all parasites of different taxa within an individual host) and component community (all parasites of all taxa in a host at a given locality at a given point in time).

$$Prevalence (P \%) = \frac{No. of infected fish}{Total no. of fish examined} \times 100$$

$$Mean abundance = \frac{No. of parasites}{No. of fish examined}$$

$$Mean intensity = \frac{No. of collect parasites}{Total no. of infected fish}$$

The parasite diversity was calculated using PRIMER v6, based on the infracommunity abundance data, whereafter the mean $\pm$ 95% confidence interval was plotted using Graphpad Prism v.9.3.1 per locality. Taxa richness (mean number of parasite taxa per fish), abundance (mean number of individual parasites per fish), and Brillouin's diversity index (used for finite parasite assemblages, see Clarke et al. 2014; Poulin 2015) were applied to determine the infracommunity composition. The component community was determined in accordance with Shannon-Wiener Index (H'), Pielou's evenness index (J') and Simpson's Index (Sures et al. 1999). Results were tested for normality (D'Agostino and Pearson's normality test), with data not normally distributed, a non-

parametric test (Kruskal-Wallis ANOVA) followed by Dunn's multiple comparison test was performed to determine significant differences between localities. All parasite taxa were divided into monoxenous and heteroxenous parasites, based on morphological and molecular information from literature, and depending on the type of life cycle they exhibit. The heteroxenous / monoxenous taxa ratio was calculated for taxa richness (S_H/S_M) in each infracommunity (Pérez-del Olmo et al. 2007b; Sures et al. 2017).

Various factors were included in the multivariate statistics. Explanatory factors included condition factor (CF), hepatosomatic index (HSI), fish size (g) (as reported in Erasmus et al. 2021a), parasite life cycle (heteroxenous and monoxenous parasites), as well as water temperature, while overlaying categorical factors included host sex (male or female), seasonality, and habitat type (inter- or infratidal). Community similarity analysis, based on Bray-Curtis similarity coefficients (square root transformed abundance data), was performed for both infracommunity and component community abundances. Additionally, the taxa contributing to similarity (ANOSIM) and dissimilarity (SIMPER) between localities and surveys were also determined. Where the ANOSIM revealed a Global R-value of > 0.75 , the community composition is distinct, and where $\text{Global } R < 0.25$, the community exhibits high similarity based on the factor. Data were also subjected to non-metric multidimensional scaling (nMDS), cluster analysis with Pearson's correlation (> 0.35) analysis to determine the ordination of infracommunities based on relative similarity in composition, and abundance of the taxa at each locality. PERMANOVA with Bray Curtis distances was used to examine the effects of factors on abundance and their interaction on parasite community composition, using Primer v6.

In addition, Canoco v5.12 was used to construct a redundancy analysis (RDA) to summarise the variations in taxon composition explained by environmental variables and interpreted using categorical factors. Interactive-forward-selection with Monte Carlo permutation was performed in order to determine the significant variables (Black et al. 2004). The abundance data per taxa, as well as the environmental variable data for the RDA, were log-transformed ($y = \log(x+1)$), centred and standardised. Finally, a species accumulation curve (UGE), was plotted in Primer v6, to predict the true total number of parasites taxa of *C. superciliosus*. Significant differences were regarded as $p < 0.05$.

5.3 Results

5.3.1 Parasite diversity

During the present study, a total of 30 distinct parasite taxa, representing seven major taxonomic groups (Table 5.1), were identified from the four localities for *C. superciliosus*. These include the digenean *Coitocaecum capense* Bray, 1987, the leech *Zeylanicobdella arugamensis* de Silva, 1963, the gnathiid isopod *Gnathia africana* Barnard, 1914, and the caligid copepod *Caligus mortis* Kensley, 1970, previously reported from this host (Vermaak et al. 2021 and references within). In addition to these, four acanthocephalans, two cestodes, four crustaceans, ten digeneans, one

monogenean and five nematodes, not previously reported, were also found (see Chapter 3). These are representative of 22 heteroxenous taxa (ten larval stages and 12 adult stages) and eight monoxenous taxa (see Table 5.1). A total of 5,757 individual parasites were recovered during all of the surveys.

5.3.2 Infracommunity composition

Langebaan marina yielded a significantly greater (Kruskal-Wallis test = 56.5, $p = 0.0008$) number of parasite individuals (4,909), as well as a high number of taxa (17) compared to the other localities. The high number of individuals was due to *Dollfustrema* sp. Eckmann, 1934 dominating the diversity from this locality (Table 5.1; Figure 5.2). Cape Town harbour had a significantly lower taxa richness (Kruskal-Wallis test = 36.6, $p = 0.0001$) and abundance ($p = 0.001$) compared to the other localities (Figure 5.2a and 5.2b), with only a single metazoan taxon (i.e., *Gyrodactylus* sp. von Nordmann, 1832) present with 50% prevalence. Tsitsikamma on the south coast had an average community composition of 10 taxa. Chintsa, the most eastern locality, had the highest number of taxa (19) during all of the surveys. Brillouin's diversity index also indicated significant differences between Langebaan marina, Tsitsikamma (Kruskal-Wallis test = 17.2, $p = 0.0012$) and Chintsa ($p = 0.0007$) (Figure 5.2d).

Table 5. 1: Overall prevalence (P %) mean intensity (MI) and mean abundance (MA) of metazoan parasites in Clinus superciliosus from four sampling localities in South Africa, Langebaan marina (n=19), Cape Town harbour (n=16), Tsitsikamma (n=10) and Chintsa (n=20). The dominant taxa per locality are indicated in bold.

PARASITE TAXA	PARASITES	LIFE CYCLE	P %	MA	MI
Langebaan marina					
Acanthocephala	Polymorphidae sp.	HL	5.3	0.05	1.0
	Arhythmacanthidae sp. 1	HA	5.3	0.05	1.0
Caligidae	<i>Caligus</i> sp.	M	21.1	0.21	1.5
Lernaeopodidae	<i>Alella</i> n. sp.	M	42.1	0.42	2.9
Gnathiidae	<i>Gnathia africana</i>	M	10.5	0.11	2.0
Digenea	<i>Coitocaecum capense</i>	HA	5.3	0.05	1.0
	<i>Coitocaecum</i> sp. 3	HA	26.3	0.26	6.8
	<i>Helicometra</i> sp. 1	HA	21.1	0.21	6.8
	<i>Cardiocephaloides</i> sp.	HL	42.1	0.42	5.3
	Hemiuridae gen. sp. 2	HA	5.3	0.05	4.0
	<i>Dollfustrema</i> sp.	HL	100.0	1.00	244.4
Annelida	Piscicolidae gen. sp.	M	10.5	0.11	1.5
Monogenea	<i>Gyrodactylus</i> sp.	M	52.6	0.53	6.2
Nematoda	<i>Anisakis</i> Type II	HL	26.3	0.26	6.0
	<i>Cucullanus</i> sp.	HA	36.8	0.37	3.3
	Rhabdochonidae sp.	HA	5.3	0.05	2.0
	Ascarididae sp.	HL	10.5	0.11	1.5
Component community		17			
Cape Town harbour					
Monogenea	<i>Gyrodactylus</i> sp.	M	50	0.5	2.1
Component community		1			

Table 5. 1: (continued)

PARASITE TAXA	PARASITES	LIFE CYCLE	P %	MA	MI
Tsitsikamma					
Acanthocephala	Polymorphidae sp.	HL	55.0	0.55	4.6
Cestoda	<i>Nybelinia</i> sp.	HL	5.0	0.05	1.0
Gnathiidae	<i>Gnathia africana</i>	M	75.0	0.75	4.3
Digenea	<i>Coitocaecum capense</i>	HA	55.0	0.55	5.9
	<i>Helicometra</i> sp. 1	HA	55.0	0.55	3.2
	<i>Proctoeces</i> sp.	HA	15.0	0.15	1.0
	Zoogonidae gen. sp.	HA	10.0	0.10	1.0
	<i>Cardiocephaloides</i> sp.	HL	30.0	0.30	2.8
	Hemiuridae gen. sp. 1	HA	65.0	0.65	3.3
Annelida	<i>Pterobdella arugamensis</i>	M	25.0	0.25	1.0
Component community		10			
Chintsa					
Acanthocephala	Polymorphidae sp.	HL	55.0	0.55	3.3
	Arhythmacanthidae sp. 1	HA	5.0	0.05	1.0
	Arhythmacanthidae sp. 2	HA	5.0	0.05	2.0
	Rhadinorhynchidae sp.	HA	5.0	0.05	1.0
Caligidae	<i>Caligus mortis</i>	M	15.0	0.15	1.7
Cestoda	<i>Nybelinia</i> sp.	HL	40.0	0.40	2.4
	<i>Acanthobothrium</i> sp. ^A	HL	80.0	0.80	15.8
Gnathiidae	<i>Gnathia africana</i>	M	60.0	0.60	3.0
	<i>Gnathia pilosus</i>	M	25.0	0.25	2.0
Digenea	<i>Coitocaecum</i> sp. 2	HA	5.0	0.05	1.0
	<i>Helicometra</i> sp. 1	HA	50.0	0.50	3.0
	<i>Proctoeces</i> sp.	HA	10.0	0.10	3.5
	Zoogonidae gen. sp.	HA	20.0	0.20	1.8
	<i>Cardiocephaloides</i> sp.	HL	25.0	0.25	21.6
	Hemiuridae gen. sp. 1	HA	45.0	0.45	2.0
	<i>Stephanostomum</i> sp.	HL	20.0	0.20	1.3
	<i>Dollfustrema</i> sp.	HL	10.0	0.10	1.0
Annelida	<i>Pterobdella arugamensis</i>	M	15.0	0.15	1.0
Nematoda	Camallanidae sp.	HL	5.0	0.05	1.0
Component community		19			

HA – Heteroxenous adults

HL – Heteroxenous larvae

M – Monoxenous

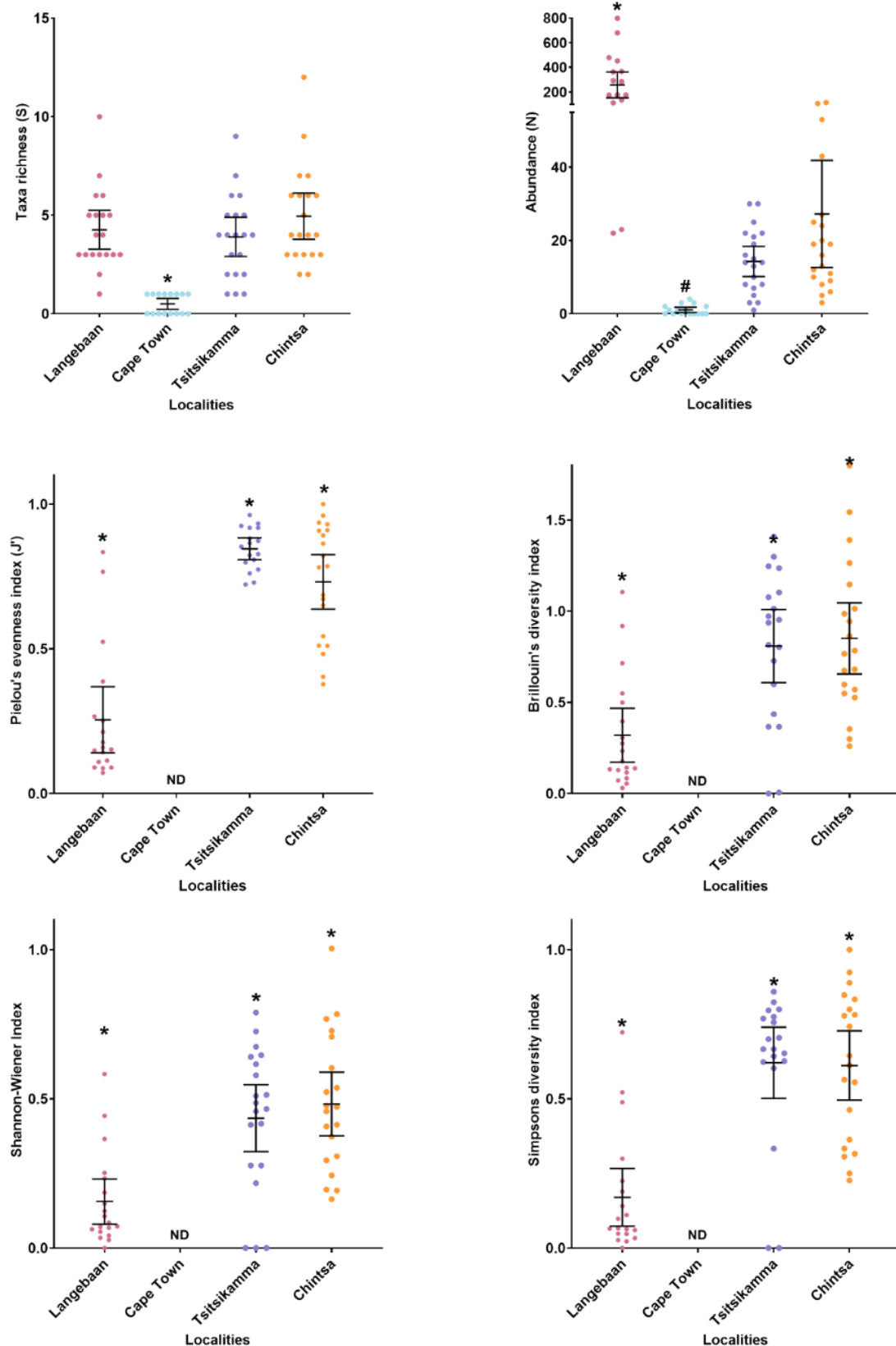


Figure 5. 2: The mean and 95% confidence interval of the calculated parasite infracommunity richness (mean number of taxa), abundance (mean number of individuals), evenness (Pielou's evenness index) and diversity (mean Brillouin's diversity index, Shannon-Wiener index and Simpson's diversity index) of *Clinus superciliosus* sampled at Langebaan marina, Cape Town harbour, Tsitsikamma and Chintsa. Common symbols (*, #) indicate significant differences between localities.

Langebaan marina has a relatively heterogeneous composition dominated by the digenean *Dollfustrema* sp., while Cape Town harbour is only represented by one species (*Gyrodactylus* sp.) in relatively low numbers. The mean number of parasite taxa per host was 4.44, while individual host richness reached up to 13 taxa. The mean parasite abundance per host was calculated at 76.8, however in actuality some hosts reached a total of 798 individual parasites. The highest mean number of parasite taxa for all fish examined was 5 for Chintsa, followed by Langebaan marina (4.3) and Tsitsikamma (3.9) (Figure 5.2). When considering the Brillouin's Diversity Index, Chintsa had a diversity index value of 0.9, compared to the significantly different Langebaan marina ($p = 0.0007$) with an index value of 0.3, which is due to the dominant species *Dollfustrema* sp. The ratio of heteroxenous to monoxenous calculated per locality indicated the highest ratio at Tsitsikamma and the lowest at Cape Town harbour, which can be expected considering the taxa richness from these localities.

5.3.3 Component community composition

Pielou's evenness index calculated for surveys, showed significant differences (Kruskal-Wallis test = 29.2) between Langebaan marina and Tsitsikamma ($p < 0.0001$) and Chintsa ($p < 0.0001$) (Figure 5.2). The degree of diversity with respect to the relative abundance of different parasite taxa in an assemblage is measured by Pielou's evenness index, where values closer to 1 indicate a more even distribution, hence a more stable ecosystem. Tsitsikamma and Chintsa were considered the most evenly distributed due to the fact that no single dominant taxon was present at these localities, where Chintsa was also the most diverse locality in terms of parasite taxa. This is supported by the SIMPER analysis (see Appendix Table C. 1– C. 2), indicating that the most dominant taxon contributed to 31.8% and 48.8% of the diversity, respectively, whereas Cape Town harbour and Langebaan marina had a single dominant taxon each, contributing to more than 50% of the diversity (91.92% for *Dollfustrema* sp. at Langebaan marina, 100% for *Gyrodactylus* sp. at Cape Town harbour). These results were also supported by Simpson's Index that indicates the probability that individuals within a community belong to the same species, in this case, the majority of individuals from Langebaan marina belong to the same taxa (Figure 5.2).

The nMDS plot, based on the Bray-Curtis similarity coefficients, showed a stress level of 0.09 with an overlap between Tsitsikamma and Chintsa but also a generally good separation of infracommunities between the localities (Figure 5.3 and 5.4). The ANOSIM analysis (see Appendix Table C. 3) revealed a Global R-value of 0.82 indicating substantial differences between the data for the chosen factor, locality, with a significance level of sample statistics as 0.1%, which is considered significant. A Pearson's correlation vector overlay, representing the correlation between taxa and nMDS axes, was applied. Correlation vectors are only given for taxa with correlations greater than 0.35, where the vector of each taxa shows the direction of increased abundance across the plot. The ratio of heteroxenous taxa to monoxenous taxa (S_H/S_M) was the highest for Tsitsikamma (4 : 1), followed by Chintsa (3.8 : 1) and Langebaan marina (2.4 : 1).

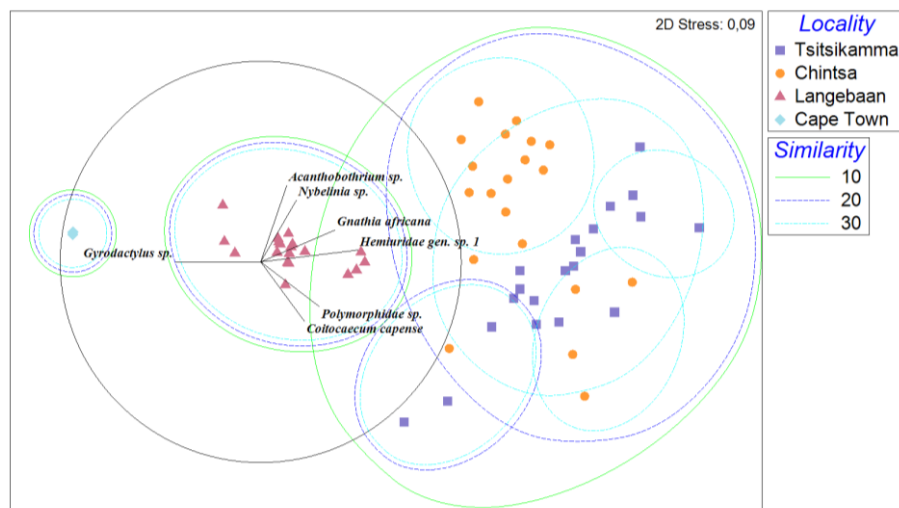


Figure 5. 3: Ordination plot (nMDS) based on parasite infracommunity diversity and abundance in *Clinus superciliosus* sampled at Langebaan marina, Cape Town harbour, Tsitsikamma and Chintsa. The ordination plot illustrates the similarity between samples, with a Pearson's correlation vector overlay showing taxa with a correlation greater than 0.35. Similarity levels (10; 20; 30) were selected based on the hierarchical cluster analyses (Resemblance = 60) of Bray Curtis coefficients.

5.3.4 Factors contributing to community composition

Pearson's correlation coefficients for the predefined explanatory factors were overlaid on the parasite community (Figure 5.4) and only water temperature had a vector longer than 0.5. Higher water temperatures were associated with the Tsitsikamma and Chintsa grouping. From the plot, it can also be seen that fish with a greater size are associated with Langebaan marina and Cape Town harbour. Both these localities are infratidal habitats, compared to the intertidal habitat at Tsitsikamma and Chintsa. Based on the PERMANOVA results, size had a significant ($p = 0.911$) effect on parasite abundance, while size and sex had a significant interaction ($p = 0.964$).

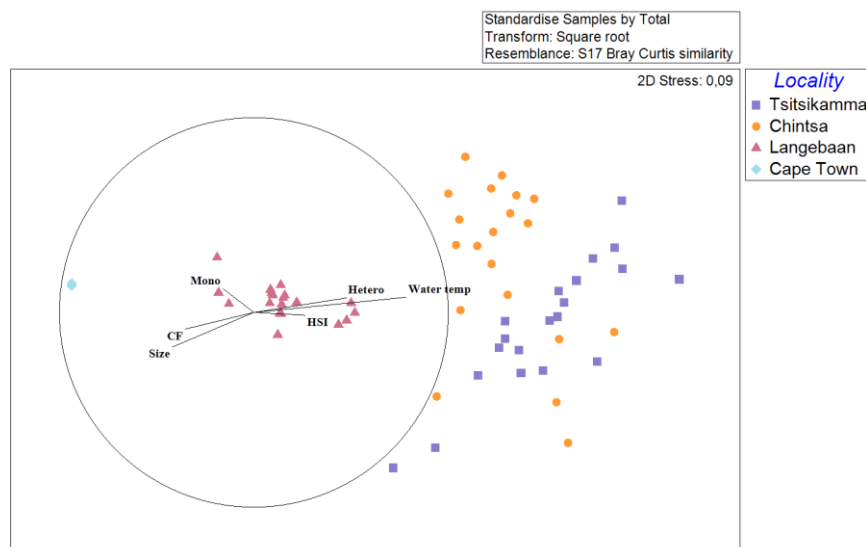


Figure 5. 4: Ordination plot (nMDS) of parasite infracommunity diversity and abundance in *Clinus superciliosus* sampled at Langebaan marina, Cape Town harbour, Tsitsikamma and Chintsa with a Pearson's correlation vector overlay (> 0.2) showing predefined factors. CF – Condition Factor; Hetero – Heteroxenous life cycle; HSI – Hepatosomatic Index; and Mono – Monoxenous life cycle.

The RDA triplot depicts a distinct spatial variation, where Monogenea, Digenea, Nematoda and Lernaepodidae drive the diversity at Langebaan marina, and Isopoda, Cestoda and Acanthocephala drive the diversity at the Tsitsikamma and Chintsa localities (Figure 5.5). Only life cycle type ($p = 0.001$), water temperature ($p = 0.005$), and host size ($p = 0.003$) were considered to significantly influence the community composition at the various localities. Based on the Monte-Carlo interactive forward selection, parasite community composition at the various localities was significantly influenced by the following factors: life cycle (55.5% for monoxenous and 18.1% for heteroxenous) and water temperature (11.8%).

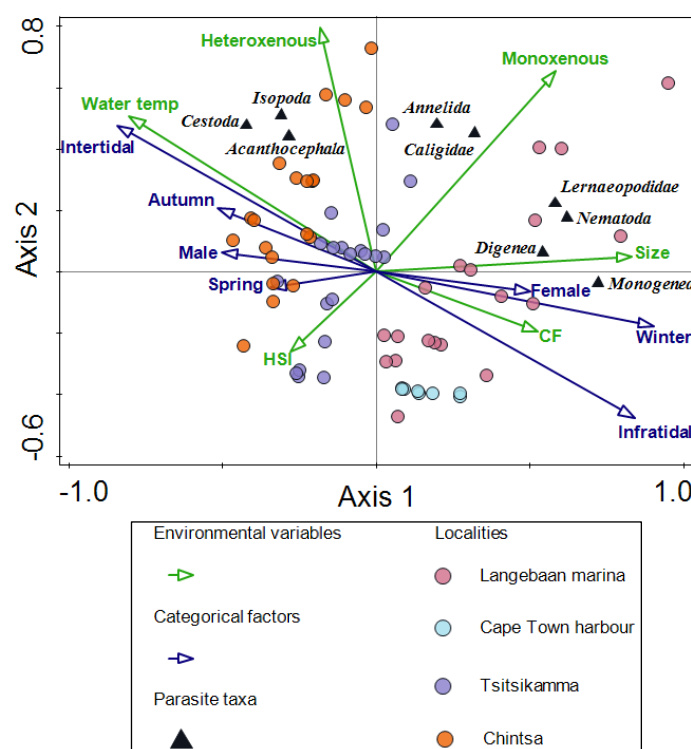


Figure 5. 5: RDA triplot illustrating associations between the parasite infracommunity taxa abundance of *Clinus superciliosus* explained by environmental variables and interpreted using categorical factors, sampled at Langebaan marina, Cape Town harbour, Tsitsikamma and Chintsa. The triplot explains 36% of the variation, with 22.6% on the first axis and 13.4% on the second axis.

5.4 Discussion

5.4.1 General features of the parasite community composition of *Clinus superciliosus*

This is the first comparative parasite community analysis of *C. superciliosus* from the western and southern coast of South Africa, using abundance data of the infracommunity composition. The taxa-rich list of metazoan parasites collected from *C. superciliosus* in the present study is the result of a full examination of all metazoan parasites, compared to previous studies consisting of single taxonomic records, or parasites associated with marine hosts from single sampling localities (Reed 2015; Smit and Hadfield 2015). The parasite fauna of *C. superciliosus* along the South African coast was taxa-rich (30 taxa) and dominated by digeneans (11 taxa). Our results show that even though Langebaan marina had the highest parasite abundance, Chintsa had the

highest diversity index values (Brillouin's index = 0.9 ± 0.4 ; Shannon's index = 0.5 ± 0.2 ; Simpson's index = 0.6 ± 0.3), as well as the highest evenness values (0.7 ± 0.2). This is consistent with general biodiversity trends for South Africa, with the west coast having a high biomass while the east coast is associated with high biodiversity (Griffiths et al. 2010).

Considering their intermediate and definitive hosts, the whole parasite assemblage of a host can give an indication of the local food web, reflecting an ecosystem's free-living species richness (Morris et al. 2019; Schwelm et al. 2021b). The nine trophically transmitted heteroxenous larval stages reflect the fish host's position within these local food webs. Fish with a more general diet are known to have more abundant and diverse parasites than fish with a restricted diet (Rueckert et al. 2008; Dallarés et al. 2016). The diet of *C. superciliosus* consists of various crustaceans, molluscs, and other small fish (Erasmus et al. 2020b), which are known intermediate hosts of many parasites (e.g., Holleman et al. 2012; Pérez-i-García et al. 2015; Dallarés et al. 2016). All of the localities, excluding Cape Town harbour, shared four species of the parasite fauna, including two digenean species, a single acanthocephalan, and a gnathiid. The absence of these taxa at Cape Town harbour can possibly be ascribed to the altered habitat, especially compared to Tsitsikamma and Chintsa. However, even with the high taxa richness, a species accumulation curve estimating taxa richness of this particular host (Figure C.1) indicated that not all the parasite taxa from *C. superciliosus* have been collected and that a greater sampling effort will potentially reveal even more species.

5.4.2 Parasites and environmental factors

To better understand parasite communities, parasites should be considered within host assemblages (Dallarés et al. 2016; Carrassón et al. 2019). Various factors contribute to parasite communities within a specific locality, as well as to the fish and the parasite taxa. Numerous studies have reported correlations between fish size and parasite abundance, seasonal and geographical variation in parasite communities, water temperature, as well as fish condition (Carrassón et al. 2019 and references therein). In the case of the present study, fish were collected from the west coast of South Africa, with temperatures around 15 °C compared to the southeast coast with temperatures around 22 °C. As mentioned above, the west coast of South Africa is known for its high biological productivity and biomass, but low diversity of species (i.e., less intermediate and definitive hosts for parasite transmission), while the southeast coast is characteristic of high diversity but low biomass and productivity (i.e., more intermediate and definitive hosts for parasite transmission (Griffiths et al. 2010). The parasite's life cycle, i.e., heteroxenous (indirect life cycle, mostly endoparasitic) and monoxenous (direct life cycle, mostly ectoparasitic), also has different adaptations to the environmental stressors, where monoxenous parasite abundance often increases in high stressor environments (Pérez-del-Olmo et al. 2007b; Dallarés et al. 2016; Sures et al. 2017).

Parasite abundance and diversity is generally influenced by the habitat, i.e., the fish and the sampling locality. The composition of the host component community, being driven by

ecosystem-level biotic and abiotic factors, is a determining factor of its susceptibility to various parasite taxa (Timi and Poulin 2003; Alarcos and Timi 2012; Nunkoo et al. 2016). Langebaan marina and Cape Town harbour (west coast) are infratidal habitats where fish of larger sizes were sampled, compared to Tsitsikamma and Chintsa (southeast coast). The latter two localities are intertidal habitats known to serve as nursery areas for fish, and thus smaller fish were sampled. Parasites can interact with other ecological drivers, such as metal and organic pollution, nutrient input, climatic events, and oxygen concentrations to name a few, which can have both detrimental and beneficial effects on ecosystems (Hatcher and Dunn 2011). Significant interaction between size and gender can be linked to the fact that some females are larger when they are gravid. However, three times more males were sampled during all of the surveys, possibly revealing information on the foraging behaviour of this species. Generally, the condition factors (CF) of the fish at the infratidal habitats were higher, however, the HSI (hepatosomatic index) was higher at the southeast coast localities compared to the west coast (CF and HSI as reported in Erasmus et al. 2021a). This suggests that the parasite load can be related to the biology of the fish host, as well as their productivity (Vidal-Martínez et al. 2019). While literature reports that the number of individual cestodes and trematodes tend to increase under eutrophic conditions (Marcogliese 2001; Lafferty 2008), both Tsitsikamma and Chintsa have nutrient rich water, linked to frequent upwelling events in the vicinity (Port Alfred upwelling cell) (Erasmus et al. 2020b; Wepener and Degger 2020). Even though these localities are not eutrophic, the high nutrient levels from upwelling events can result in similar effects on the fauna. This was the case in the present study, where cestodes occurred more often than other parasites, and a high diversity of trematodes was also present (up to nine different taxa). Generally, eutrophication has a positive relationship with overall parasite species richness. Nonetheless, high nutrient inputs can also have negative effects on ecosystems and can cause species decline (Overstreet and Howse 1977; Lafferty 2008).

The absence of Trypanorhyncha cestodes at Langebaan marina and Cape Town harbour might be explained by these highly human-impacted localities (Rueckert et al. 2008), where these localities were dominated by monoxenous parasites compared to Tsitsikamma and Chintsa. Reportedly, monoxenous parasites are known to increase in stressed environments, while heteroxenous parasites decrease in stressed environments (Diamant et al. 1999; MacKenzie 1999; Pérez-del-Olmo et al. 2007a; Rueckert et al. 2008; Dallarés et al. 2016; Sures et al. 2017). In the present study, five of the eight taxa of monoxenous parasites sampled were present at Langebaan marina, compared to the southeast coast localities where four of the eight taxa were collected. As for Cape Town harbour, only one taxon was sampled, a monoxenous species, supporting the literature of decreased heteroxenous parasites in localities with increased stress (Rueckert et al. 2008; Sures et al. 2017). Monoxenous parasites reportedly show a dissimilar susceptibility to pollution than heteroxenous parasites and the heteroxenous and monoxenous (S_H/S_M) species richness ratio can be used as a measure of pollution impact. Higher ratios are expected in unpolluted marine habitats compared to samples from polluted habitats (Pérez-del

Olmo et al. 2007b; Sures et al. 2017). This was also the case in the present study, where Cape Town harbour had the lowest ratio, compared to the unpolluted east coast localities. The dominating occurrence of *Dollfustrema* sp. in nearly all organs at Langebaan marina, and very high intensities of infection, can be ascribed to the ease of mode of transmission from this locality, where cercariae can more easily find the fish host with the limited water movement of the area (Vermaak 2020). However, Langebaan marina also had nine unique taxa compared to all of the other localities, seven of which are heteroxenous parasites, indicating the availability of food sources and appropriate habitats for the intermediate and definitive hosts to occur (Rueckert et al. 2008). *Coitocaecum capense* was only collected at Tsitsikamma from the southeast coast localities, with one additional report at Langebaan marina, while a total of seven unique taxa were present at Chintsa compared to all of the other localities.

In addition to habitat differences, host size, and food availability, possible environmental stressors could also cause an increase in some species, compared to the other localities. Monogeneans have been reported to increase with an increase in stress (higher production of eggs), while digeneans species have been reported to increase in high temperatures, which stimulates transmission (Dallarés et al. 2016). These environmental variables will not only affect the parasites but also the intermediate and definitive hosts' occurrence and distribution at various localities (Hogue and Swig 2007; Lafferty 2008; Vidal-Martínez et al. 2019). For future studies, it is thus important to explore parasite ecology, specifically their life cycles, to better understand the increase or decrease in certain parasite species.

5.5 Conclusion

The parasite community of *C. superciliosus* is characterised by high abundance, diversity and richness. The food preference of the fish, including various molluscs, crustaceans and small fishes, contributes greatly to this diversity. The taxa that were observed to be unique to specific localities, can be linked to intermediate and definitive host distribution within different water temperature regions, as well as host size, since larger fish have a different diet than smaller fish. Monoxenous parasites, although not exclusively, associated more with Langebaan marina and Cape Town harbour, while the heteroxenous taxa associated more with the southeast coast. The parasite community composition of the southeast coast fish was less dominated by a single taxon, due to the more complex invertebrate communities found in intertidal areas compared to the infratidal areas, as well as greater human influence at the marina and the harbour. Further studies on parasite specificity and life cycles are needed in order to determine the full ecological impact of these parasites on their host, as well as the ecosystem as a whole. In addition, region- or system-specific relationships should also be considered for future studies, as they may affect the general conclusions.

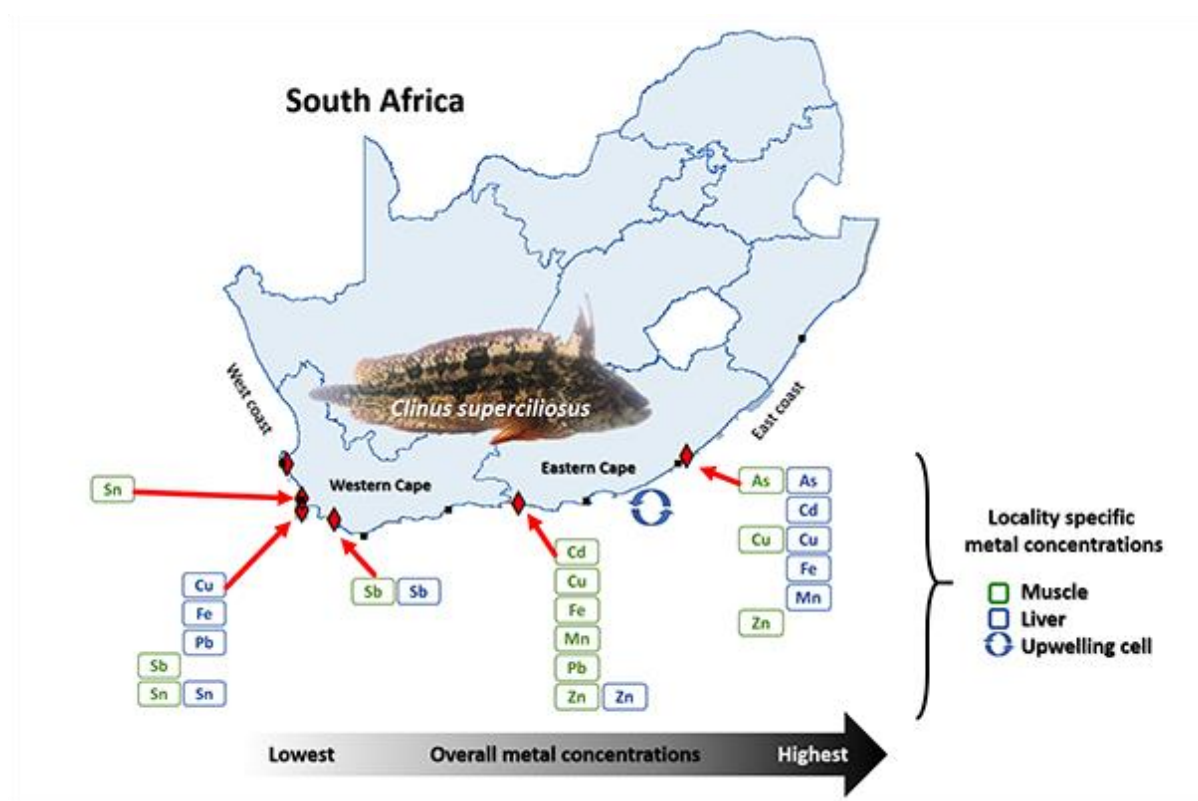
CHAPTER 6

Metal and metalloid concentrations in the southern African endemic inter- and infratidal super klipfish, *Clinus superciliosus*, from the west and south coasts of South Africa

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6.1 Introduction

Metal and metalloid pollutants can be monitored using indicator species such as fish and invertebrates (de Silva et al. 2009). Bioindicator species are those species that accumulate pollutants in their tissue and can in turn be used to monitor pollutant bioavailability within habitats (Rainbow and Phillips 1993). The majority of marine bioindicator studies include barnacles and bivalves and occasionally polychaetes (Rainbow and Phillips 1993; Amoozadeh et al. 2014). Certain marine fish species, e.g., intertidal species, have limited living and foraging ranges, making them ideal bioindicators. They can take up metals and metalloids through ingestion and adsorption, reflecting bioavailable elements specific to the particular organism (Rainbow and Phillips 1993). Since fish are usually at the apex of food chains, they may accumulate higher concentrations than invertebrate indicators and pose a threat to human health, if consumed (Authman et al. 2015). Bioaccumulation and toxicity of metallic elements are dependent on environmental factors that influence metal speciation and bioavailability, e. g., temperature, salinity, pH; and biological factors such as size, sex, age, life cycle and feeding habits (Wang and Rainbow 2008; Linbo et al. 2009; Griboff et al. 2018). Essential metals are, however, required for normal biochemical and physiological processes. These include copper (Cu), iron (Fe), manganese (Mn), tin (Sn), and zinc (Zn), while non-essential metals include cadmium (Cd) and lead (Pb) (Rainbow, 1985). Different species can also have contrasting patterns of accumulation and elimination due to their unique abilities of shell formation, shedding and reproduction, to name a few (Wang and Rainbow 2008). It is thus important to consider different species, from various localities, to better understand the metal metabolism in aquatic organisms.

The highly dynamic South African coastline is encompassed by two major ocean currents with varying local nearshore circulation processes. The Benguela Current transports cold nutrient rich water along the west coast in a northerly direction, while the Agulhas Current transports warm water southwards along the east coast (Van As et al. 2012). Due to these distinct currents, the marine environment has high biodiversity and very productive marine resources (Hutchings et al. 2009; Wepener and Degger 2019). The coastline is also affected by anthropogenic activities, where development within a 100 m coastal strip poses the greatest threat to the marine biodiversity (Driver et al. 2012; Clark et al. 2019). Challenges such as natural resource degradation, destruction of habitats, overexploitation and pollution are some of the major contributors. Metal pollution along South Africa's coastline can be a direct result of the activities that contribute to the ocean economy (WWF-SA 2016) and need to be monitored for human and environmental health. In South Africa, many forms of pollutants originating from the surrounding landscape, as well as transported via ocean currents, impacting the coastal biodiversity and ecosystem health, are poorly understood. These pollutants include metals and metalloids (Harris et al. 2019). Larger scale oceanographic processes such as offshore or inshore upwelling and ocean currents, combined with land derived metal inputs such as freshwater runoff, along with seasonality can result in metal accumulation within nearshore ecosystems (Newman and Taljaard

2019; Erasmus et al. 2020b). Local water circulation speed and direction can vary in summer and winter, where different wind driven water-flows affect bay circulation, causing depositional zones within sheltered areas (Newman and Taljaard, 2019). Along the South African coastline, there is a lack of data on larger geographical scale monitoring of metal pollution. Available data are limited to the Mussel Watch Program (MWP) around the Cape Peninsula using the invasive *Mytilus galloprovincialis* (Mediterranean mussel) (Sparks et al. 2014) and endemic *Perna perna* (brown mussel) from six South African harbours sampled from Cape Town on the south west coast to Richards Bay on the north east coast (Greenfield et al. 2011, 2014; Wepener and Degger 2020).

The present study determined the bioaccumulation of selected metals and metalloids along the South African west and south coast using the southern African endemic super klipfish, *Clinus superciliosus*, (Linnaeus, 1758) as indicator species. This species was selected as a possible bioaccumulation indicator as it meets many of the criteria for ideal bioindicator organisms (Barbaro et al. 1978). It is relatively easy to sample, abundant and restricted to sheltered areas, and reportedly contribute 40% to the total biomass of rocky-pool fishes (Bennett 1984; Branch and Griffiths 1988). It is viviparous and probably breeds throughout the year (von der Heyden et al. 2008), and feeds on various invertebrates and smaller fish (Wilson 2009). Accordingly, the main aim of the present study was to investigate the potential of this locally common species as metal bioindicator. We hypothesized that the proposed bioindicator will reflect metal bioaccumulation based on their proximity to the surrounding land-use activities. Concentrations of the following essential metals, Cu, Fe, Mn, Sn and Zn, and non-essential metals and metalloids, arsenic (As), Cd, Pb and antimony (Sb), in muscle and liver, as well as fry from *C. superciliosus* were measured at six localities from the west to the east coast of South Africa. The influence of intrinsic factors such as size and sex on bioaccumulation were also evaluated.

6.2 Materials and methods

6.2.1 Sampling localities

Sampling was conducted between 2018 and 2019 at six localities from the cool temperate west coast to the warm temperate south coast of South Africa, within the distribution range of *C. superciliosus* (Table 6.1 and Figure 6.1).

Table 6. 1: Environmental conditions and characteristics of sampling localities.

Sampling localities	Coordinates	Biogeographic region*	Water temperature (°C)	Salinity (ppt)	Habitat type	Main activity	Adjacent land use**
Langebaan marina	33°02'44.5"S; 18°02'19.1"E	Cold temperate	15.9	36	Infratidal	Yacht club marina within commercial harbour, with aquaculture of mussels and oysters in the bay	Shrubland Cultivated Land Mines & Quarries Residential Built-up
Cape Town harbour	33°54'29.1"S; 18°25'05.8"E	Cold temperate	16	35.8	Infratidal	Busy commercial port	Shrubland Urban and Industrial Built-up
Simons Town marina	34°11'30.5"S; 18°26'00.3"E	Cold temperate	16.6	35	Infratidal	Yacht club marina with a Blue Flag status, within the greater False Bay	Shrubland Urban and Industrial Built-up
Old harbour Hermanus	34°25'15.9"S; 19°14'37.6"E	Warm temperate	16.7	35.4	Infratidal	Old harbour, used for small scale fishing activities with 12 abalone farms	Urban Built-up Shrubland
Tsitsikamma	34°01'15.2"S; 23°52'43.2"E	Warm temperate	19.9	35	Intertidal	No-take Marine Protected Area	Forested Land Cultivated Land Villages
Chintsa	32°50'11.5"S; 28°07'01.2"E	Warm temperate	22.6	36.4	Intertidal	Recreational fishing area between two Marine Protected Areas	Forested Land Grassland Residential Built-up

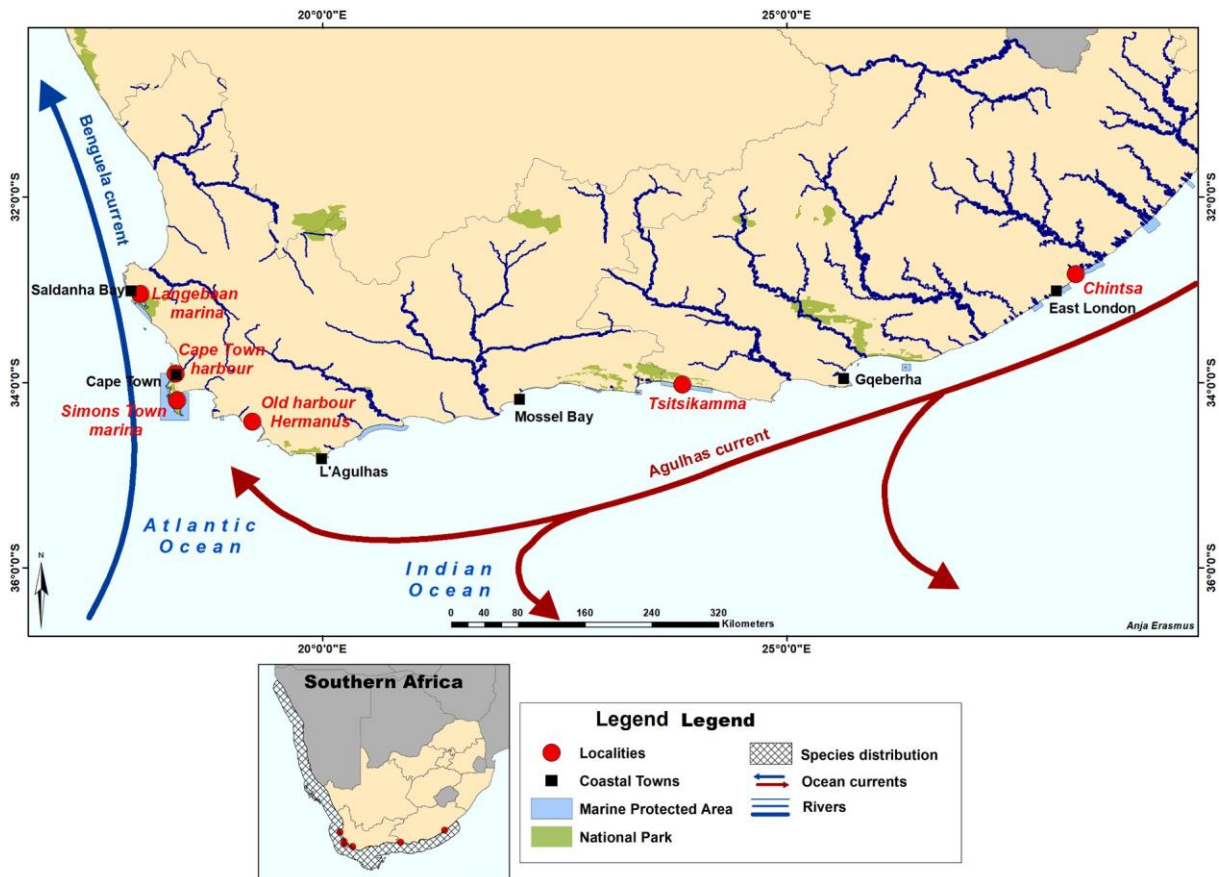


Figure 6. 1: Map of the six different localities (from left to right: Langebaan marina, Cape Town harbour, Simons Town marina, Old harbour Hermanus, Tsitsikamma, and Chintsa) sampled from 2018–2019, within the distribution range of *Clinus superciliosus*. This study map also indicates the major ocean currents along the South African coastline.

The necessary permits were obtained prior to sampling from the Department of Agriculture, Forestry and Fisheries (DAFF) and South African National Parks (SANParks) (RES2018/35, MALH-K/2016-005a and RES2019/103), as well as ethical clearance through AnimCare at the North-West University (NWU-00440-16-A5 and NWU-0051-19-A5). Fish were collected using baited traps and transported to the field station for dissection. Fish were identified, total body weight and length were measured, photographed, and humanly killed by percussive stunning and pithing (SOP NWU-00267-17-A5). The relative condition factor for each fish ($CF = \text{total weight} \times 100 / \text{total length}^3$) was also determined to establish the responses to environmental influences (Dekić et al., 2016). Muscle (boneless) and liver tissue samples were collected and stored frozen at 20 °C in pre-cleaned acid-washed polypropylene tubes, for elemental analysis. Liver tissue was selected as it is the main detoxifying and elimination organ following metal uptake, while muscle tissue represents the longer-term bioaccumulation tissue due to metal sequestration mechanisms.

6.2.2 Sample preparation for elemental analysis

Muscle and liver samples were thawed in the laboratory and weighed to the nearest mg. Following freeze drying (FreeZone® 6, Labconco) for a period of 96 h, samples were reweighed to determine moisture content. Dried homogenized samples (± 100 mg muscle or ± 80 mg liver) were microwave digested in 20 mL TFM® vessels using a Mars 6 microwave digestion system (CEM Corporation, Kamp-Lintfort) with 4 mL HNO₃ (sub-boiled from 65%; p.a. quality, Merck). Digests were transferred to 5 mL volumetric flasks and brought to volume with MilliQ water. Digest solutions were then stored at room temperature until analysis.

6.2.3 Elemental analysis

Multi-element analysis of As, Cd, Cu, Fe, Mn, Pb, Sb, Sn, and Zn, was done on muscle and liver samples, using quadrupole inductively coupled plasma-mass spectrometry (ICP-MS) (PerkinElmer, Elan 6000) equipped with an auto-sampler system (PerkinElmer, AS-90). The operational specifications were 1000 W plasma power, 14 L/min gas flow, 0.95 L/min nebulizer gas flow and 1 mL/min sample flow rate. Yttrium and Thulium (Certipur®, Merck) were used as internal standard for quality measurements at a concentration of 10 µg/L. Quantification was done using the following mass lines: ⁵⁵Mn, ⁵⁷Fe, ⁶⁵Cu, ⁶⁶Zn, ⁷⁵As, ¹¹¹Cd, ¹²⁰Sn, ¹²¹Sb, and ²⁰⁶Pb. These elements were selected due to their availability, persistence and toxicity in the environment, while some of them are vital for fish health at recommended levels.

Calibration of the ICP-MS was performed using a series of 11 dilutions of a multi-element standard solution. With this calibration, the concentrations of the analytes were calculated in the samples using corresponding regression lines with a correlation factor of 0.999. The detection limits were calculated as three times the standard deviation of the blank measurements per digestion batch and related to a mean sample weight of 114 mg for muscle and 68 mg for liver, respectively.

For quality control of the analyses of some metals, standard reference materials (fish homogenate reference material from International Atomic Energy Agency in Monaco: IAEA-407; dogfish liver certified reference material from the National Research Council in Canada: DOLT-3; and Oyster tissue NIST® SRM® 1566b) were used for the validation of the analysis. The elements showed an average recovery within 20% of the certified reference range (Table 6.2).

Table 6. 2: Mean recovery rates (in percentage) of elements obtained for the different certified reference material for muscle (IAEA-407), liver (DOLT-3) and oyster (1566b) tissue.

Elements	IAEA-407 (%)	DOLT-3 (%)	1566b (%)
As	100	92	93
Cd	94	107	101
Cu	105	110	97
Fe	98	71	80
Mn	102	N.P.	99
Pb	109	85	108
Sb	N.P.	N.P.	79
Sn	85	N.P.	105
Zn	86	96	86

N.P. - not present

6.2.4 Data evaluation and statistical analyses

Univariate statistical analyses were performed using GraphPad Prism v7. Data for metal and metalloid concentrations were tested for normality using D'Agostino and Pearson normality test. When data passed the normality test, one-way ANOVA was performed using Bartlett's statistical test followed by Tukey's multiple comparison test, while for the data that were not normally distributed, a non-parametric test were performed, i.e., Kruskal-Wallis ANOVA, followed by Dunn's multiple comparison test. Significance was regarded as $p < 0.05$.

Due to sample limitations, sex differences in metals and metalloids could only be determined in muscle and liver of *C. superciliosus* from Langebaan marina and Old harbour Hermanus since they had the most even distribution of males and females. At Langebaan marina, fry (sex not determined) was also sampled and element concentrations in whole fry were compared with tissue concentrations from males and females. A correlation analyses of the log-transformed concentrations per locality were performed, to examine the influence of body size (mass (g)) on accumulation for muscle and liver tissue, respectively.

Multivariate statistical analysis was performed using Canoco v5.12, where a Principle Component Analysis (PCA) was realized to assess spatial patterns associated with metal and metalloid concentrations, with size and condition factor as supplementary variables. For this, the data were log-transformed, centred, and standardized.

6.3 Results

During the field sampling, a total of 91 *C. superciliosus* were collected, consisting of 69 males and 22 females across all six localities (Table 6.3). Specimens ranged from 90 mm to 317 mm total

length (TL) with the smallest collected at Chintsa and largest at Simons Town marina, respectively. Fish sampled from Simons Town marina were significantly larger than all of the other localities, while fish from Old harbour Hermanus were significantly larger than both Tsitsikamma and Chintsa.

Table 6. 3: Numbers of males and females, as well as the morphometrics of the fish sampled from each of the six localities; CF = condition factor.

	Males (n)	Females (n)	Mean length (mm)	Length range (mm)	Mean mass (g)	Mass range (g)	CF
Langebaan marina	11	8	174.7 ± 8.1	120–240	75.9 ± 10.7	18.7–180.2	1.26
Cape Town harbour	15	1	164.4 ± 6.3	115–215	58.4 ± 7.1	17.9–119.8	1.22
Simons Town marina	10	2	225.6 ± 11.1	166–317	138.5 ± 21.7	52.9–346.7	1.13
Old harbour Hermanus	14	10	190.1 ± 5.5	155–250	99.3 ± 7.9	49.9–187.3	1.42
Tsitsikamma	10	0	152.5 ± 6.0	110–180	37.1 ± 4.2	18.4–64.5	1.05
Chintsa	9	1	145.5 ± 10.8	90–185	37.1 ± 6.6	8.2–69.3	1.09

6.3.1 Influence of sex and body size on bioaccumulation

Element concentrations in muscle and liver of males, females and fry were compared at Langebaan marina and Old harbour Hermanus, separately, to determine the influence of sex on metal and metalloid accumulation (indicated as mean and standard error of the mean in Figs. A1 and A2). Significant differences between muscle tissue from males and females, and in whole fry samples are presented in the table matrix on each graph. Arsenic, Sb, Sn and Zn concentrations in fry were significantly higher than in male muscle tissue, while metal concentrations in both male and female liver were significantly higher for Cd, Cu and Mn than in fry samples. Since there were no significant differences in elemental concentrations detected between male and female muscle and liver tissues, all subsequent statistical analyses were conducted on pooled concentrations of both sexes.

Considering the influence of size on bioaccumulation of elements of muscle and liver tissue, significant positive correlations (with a slope greater than 0.7) were only noted for Mn and Sn in Simons Town marina's liver tissue. Positive correlations (with a slope greater than 0.7) for Cd and Fe liver tissue were also noted at this locality, however, these were not significant (Table D. 1, Figures D. 3 and D. 4). Significant negative correlations (with a slope less than 0.7) were noted for concentrations in liver tissue of Cu at Cape Town harbour, Fe and Mn at Tsitsikamma, as well as As, Cd, Mn and Zn at Chintsa. Of these only Cu, at Cape Town harbour and Mn and Zn at Chintsa, displayed significant ($p < 0.05$) relationships. Based on these results, it was evident that size had limited influence on accumulation of metals and metalloids in muscle and liver tissue. Therefore, all subsequent statistical analyses were conducted on pooled samples of all sizes at each locality.

6.3.2 Spatial patterns of elemental accumulation in *Clinus superciliosus*

The concentrations of As, Cd and Cu (Figure 6.2), Fe and Pb (Figure 6.3), and Sn and Zn (Figure 6.4) were significantly higher ($p < 0.05$) in muscle and liver samples from Chintsa compared to Cape Town harbour, Old harbour Hermanus and Langebaan marina, respectively. Fish from Chintsa and Tsitsikamma had significantly ($p < 0.05$) higher Cu (Figure 6.2), Fe (Figure 6.3) and Zn (Figure 6.4) concentrations in tissues than all of the other localities. Langebaan marina *C. superciliosus* had significantly lower concentrations of Sb and Sn (Figure 6.4) than all of the other localities, while Langebaan marina and Old harbour Hermanus fish had significantly lower concentrations of Sn (Figure 6.4) than all of the other localities. Mean standard error of the mean values is presented in the supplementary material (Table D. 2).

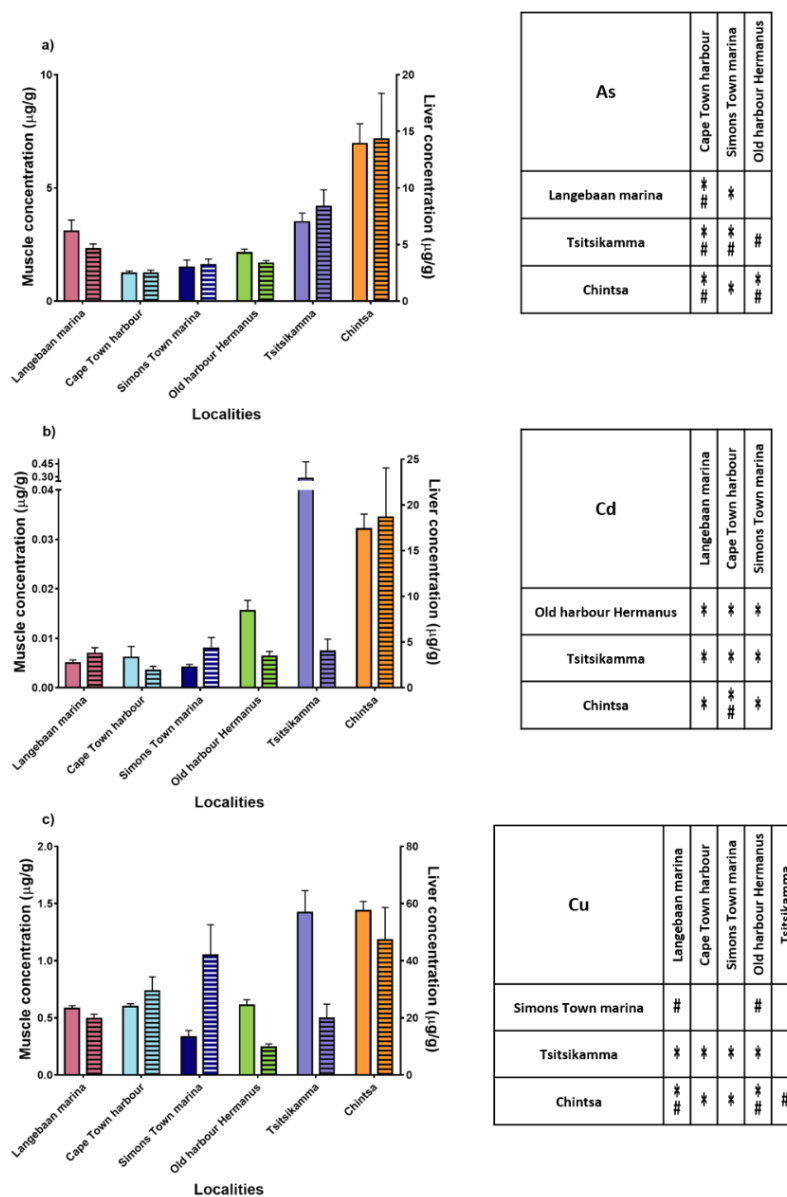


Figure 6. 2: Mean concentrations ($\mu\text{g/g}$ dry weight) [a] As, b) Cd, c) Cu] with standard error of the mean in *Clinus superciliosus* tissue, collected from six localities. Significant differences ($p < 0.05$) between localities are indicated in the table matrix as * for muscle and # for liver. The open bars represent muscle tissue on the left axis and the striped bars represent liver tissue on the right axis.

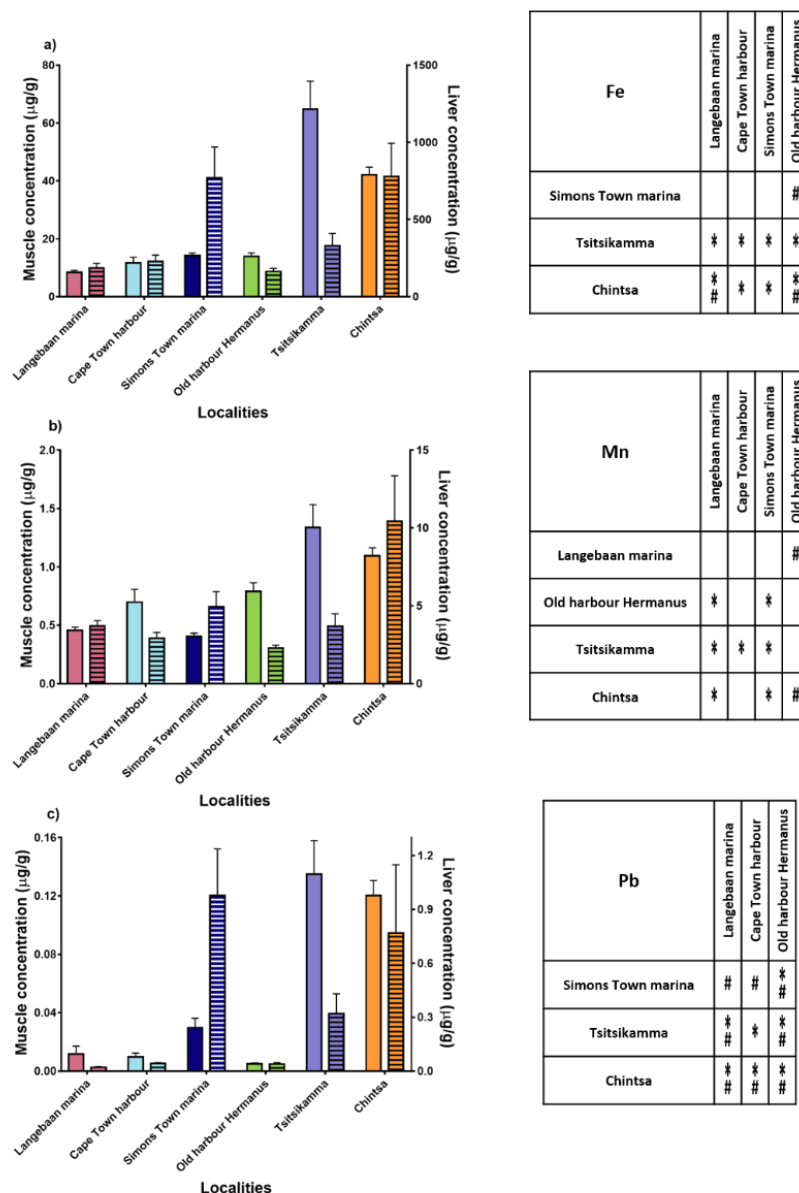


Figure 6. 3: Mean concentrations ($\mu\text{g/g}$ dry weight) [a] Fe, b) Mn, c) Pb] with standard error of the mean in *Clinus superciliosus* tissue, collected from six localities. Significant differences ($p < 0.05$) between localities are indicated in the table matrix as * for muscle and # for liver. The open bars represent muscle tissue on the left axis and the striped bars represent liver tissue on the right axis.

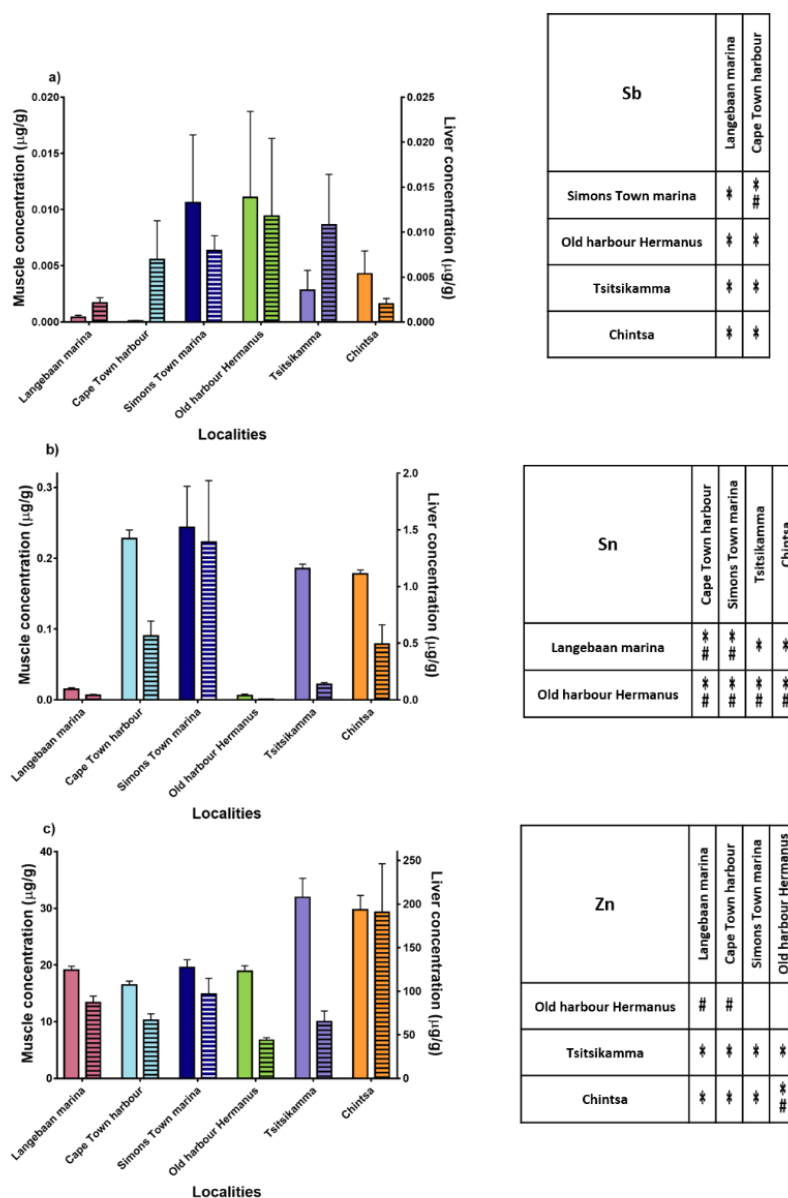


Figure 6. 4: Mean concentrations ($\mu\text{g/g}$ dry weight) [a] Sb, b) Sn, c) Zn] with standard error of the mean in *Clinus superciliosus* tissue, collected from six localities. Significant differences ($p < 0.05$) between localities are indicated in the table matrix as * for muscle and # for liver. The open bars represent muscle tissue on the left axis and the striped bars represent liver tissue on the right axis.

The element bioaccumulation heat maps indicated that Tsitsikamma fish had the highest concentrations for the majority of the elements in muscle tissues, while Chintsa *C. superciliosus* had the highest concentrations for the majority of the elements in liver tissues (Figure 6.5). Simons Town marina fish also exhibited hotspots for some of the elements in both muscle (Sb and Sn) and liver (Cu, Fe, Pb and Sn) tissue.

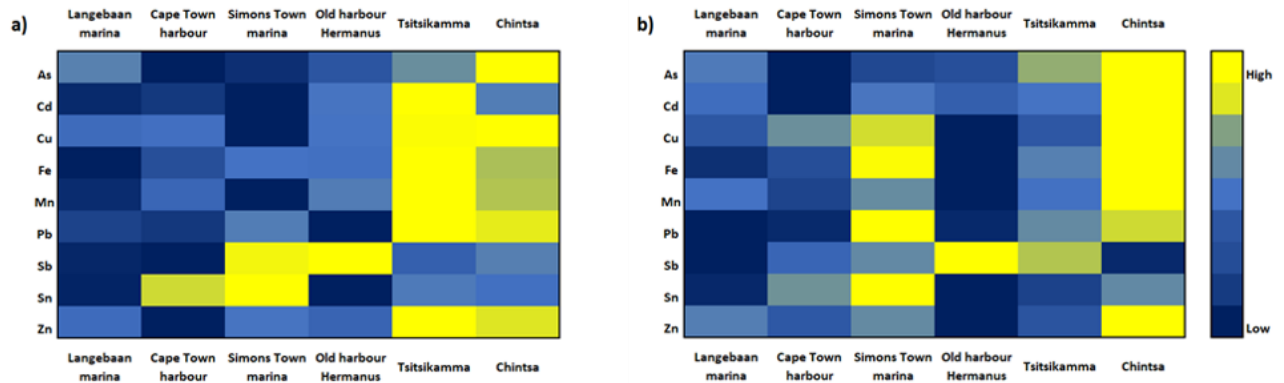


Figure 6. 5: Heat maps indicating mean concentrations ($\mu\text{g/g}$ dry weight) of metals and metalloids in male *Clinus superciliosus* a) muscle and b) liver tissue, indicating hot spots (yellow) and cold spots (blue) between localities for each of the elements analysed.

Distinct spatial differences could be observed for element concentrations in muscle (Figure 6.6A), where Langebaan marina, Cape Town harbour, Simons Town marina and Old harbour Hermanus *C. superciliosus* displayed the most similar concentrations and grouped together. These localities were associated with larger specimens and higher CF, while Tsitsikamma and Chintsa fish grouped together and associated with higher concentrations for the majority of the elements (except Sb and Sn). Considering the liver concentrations (Figure 6.6B), fish from Langebaan marina and Old harbour Hermanus grouped together, while all of the other localities were scattered. Cape Town harbour and Simons Town marina associated with larger, female specimens and higher concentrations of Pb, Sb and Sn.

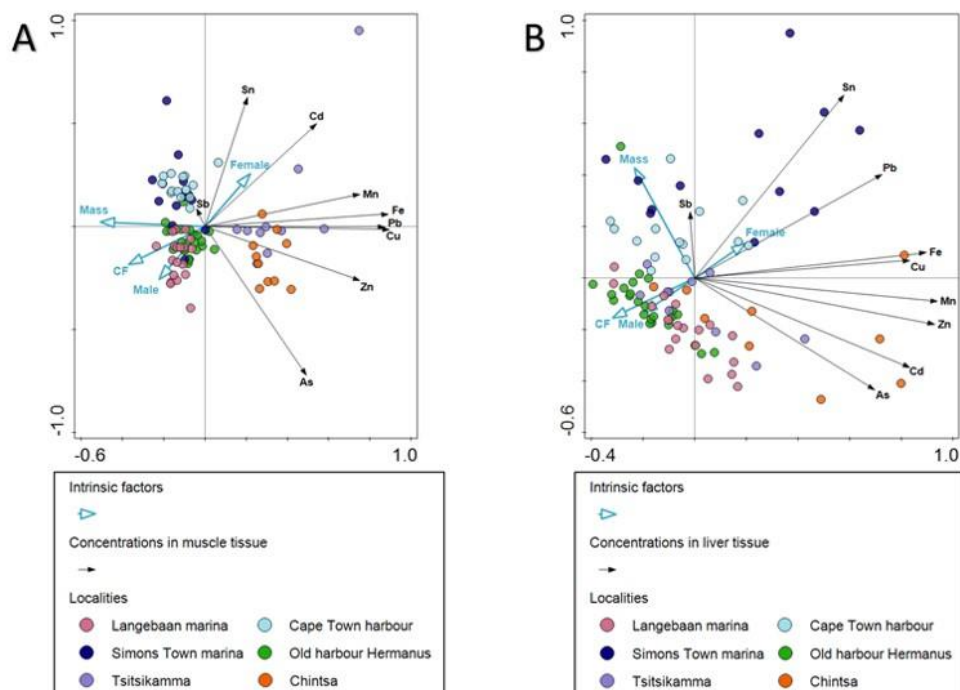


Figure 6. 6: PCA biplot of log-transformed elemental concentrations with sizes of male fish as supplementary variables, from six localities along the South Africa coastline. The muscle biplot (A) describes 59.2% of the variation with 45.1% on the first axis and 14.1% on the second. The liver biplot (B) describes 71.1% of the variation with 58.9% on the first axis and 12.2% on the second.

6.4 Discussion

6.4.1 Effect of sex and body size on element bioaccumulation

The processes of metal uptake and accumulation in organisms are influenced by physiological and biochemical principles, where any change may contribute to variation in individuals and localities. These include changes in body size, sex, reproductive status and nutrition level (Mubiana et al. 2006). Sex-dependent differences in metal accumulation has been ascribed to different energetic requirements, maturation rates and condition and maternal offloading to offspring (Cazan and Klerks 2015; Murillo-Cisneros et al. 2018), specifically in live-bearing fish, such as *C. superciliosus*. The number of hormones and protein composition can account for the sex-specific behaviour of metals (Al-Yousuf et al. 2000). There were no significant sex-related differences of the metals and metalloids found in the present study. While it has been reported that offspring of live-bearing females exposed to Cd and Cu showed elevated muscle tissue Cd levels (Cazan and Klerks 2015), this was not the case in the live-bearing *C. superciliosus*, in the present study. It has, however, been reported that exposure of females to high metal concentrations resulted in a reduced tolerance in the offspring to the exposed metal (Cazan and Klerks 2015) that can in turn lead to reduced populations in polluted localities.

Various studies have demonstrated relationships between metal accumulation and size of fish and attributed these to metabolic activities related to the age of the fish (Al-Yousuf et al. 2000; McKinley et al. 2012). Some metals are essential for fish growth and development in moderate concentrations (Cu, Fe and Zn), while Cd and Pb can lead to reduced growth rates and larval development even in low concentrations (Authman et al. 2015). In the present study, concentrations of metals and metalloids in smaller fish, from Tsitsikamma and Chintsa, were higher compared to medium and large fish from Langebaan marina, Cape Town harbour, Simons Town marina, and Old harbour Hermanus. From these results, Cu in liver at Cape Town harbour and Mn and Zn at Chintsa decrease significantly with increase in size, while Mn and Sn in liver increased significantly with increase in size at Simons Town marina. This is not enough evidence to indicate biodilution or bioconcentration of all elements with an increase in size. Newman (1995) found that decreasing metal concentrations of larger organisms are caused by a dilution effect due to unequal growth rate compared to metal accumulation, as well as size-specific uptake and elimination rates, size-specific metabolism and internal regulation of essential Zn and non-essential Cd (Wang and Fisher 1997; Boyden 1974; Cubadda et al. 2001). Exposure to high concentrations of Cd have been reported to inhibit calcium uptake through the gills and affect body size in different species of marine and freshwater fish species (Authman et al. 2015). Another study conducted by Riget et al. (1996) indicated that size-dependant concentrations of metals varied with location and year. The present study, as well as Mubiana et al. (2006), indicated that the influence of size on metal accumulation can be reduced by sampling fish within a narrow size margin.

6.4.2 Spatial patterns of element accumulation in *Clinus superciliosus*

Larger-scale spatial metal bioaccumulation studies along the South African coast revealed that mussels from the pristine south coast, Tsitsikamma, had unexpected high metal concentrations when compared to localities that were affected by human activities (Wepener and Degger 2020). Data from the present study indicated the same pattern for the intertidal fish, *C. superciliosus*. Tsitsikamma and Chintsa exhibited the highest concentrations for the majority of the elements for both muscle and liver tissues (except for Sb and Sn of muscle tissue, and also Pb for liver tissue). These high concentrations within the muscle tissue can be indicative of through geogenic weathering, as well as frequent upwelling events. Since Cd (and associated Cu, Pb and Zn) has a high affinity for organic matter, the Port Alfred upwelling centre along the south-east coast is likely responsible for some of these concentrations (RSA-DEA 2018; Erasmus et al. 2020b). Concentrations of As, Cd, Fe, and Mn in *C. superciliosus* muscle from this study were higher during the present study compared to a study from 2016 (Erasmus et al. 2020b), while Cu, Pb and Zn were higher during the 2016 study. This fluctuation in element bioaccumulation over a short period of time was also found for mussels (Wepener and Degger 2020) and can be attributed to periodic upwelling events (Erasmus et al. 2020b; Wepener and Degger 2020). The As levels recorded in pelagic *Loligo reynaudii* (chokka squid) and *Sardinops sagax* (sardine) from the South African east coast (Uren et al. 2020) were higher than those measured in the super klipfish from Chintsa and Tsitsikamma localities. These authors also attributed the high metalloid concentrations to the upwelling conditions.

High element concentrations in liver of super klipfish at Chintsa can be indicative of more short-term exposures of these elements. Chintsa is a small coastal town located between the Gonubie and Kei Marine Protected Areas of the Amathole MPA where little to no point sources of pollution are present. A major consideration along the south coast, however, is diffuse sources of pollutants, where the high amount of freshwater runoff in the rainy season on the east coast of South Africa, compared to the south and west coasts, plays a major role at this locality. Chintsa is surrounded by a number of estuaries and located just south of the inflow of the Great Kei River. These cumulatively higher loads of elemental concentrations, compared to other localities in the present study, may be increased due to mobilization caused by illegal sand mining operations in the Eastern Cape (Chevallier 2014). The east coast of South Africa is known for strong water circulation, thereby reducing the risk of pollution. No previous studies have been conducted along the coast of Chintsa, with most studies focusing on the coast of East London to the south and to the north, Richards Bay and Durban (Wepener and Degger 2020; Greenfield et al. 2011). When comparing the bioaccumulation from the present study to concentrations of other intertidal species from similar climatic regions, *C. superciliosus* has much higher concentrations. A study conducted by Rahmanpour et al. (2014) in the Persian Gulf compared concentrations of As, Cd and Pb in liver of *Johnius belangerii* (Belanger's croaker) from different habitats including mangroves, rocky shores, coral reefs and muddy flats. With the exception of Cd at Chintsa the

concentrations of Cd and Pb were enriched within the coral reefs and muddy flats of the aforementioned study when compared to all the localities of the present study. Comparisons of metals with intertidal mudskippers (*Periophthalmodon schlosseri*) from Malaysian waters, which are regarded as international waterways of trade, in the middle of various anthropogenic and domestic sources, revealed higher concentrations of essential metals (Cu and Zn) in *C. superciliosus* at Tsitsikamma (Ikram et al. 2010). The highest non-essential metal concentrations (i.e., Cd and Pb) in *C. superciliosus* from this study were still lower than those measured in the mudskippers from the polluted Malaysian waters (Ikram et al. 2010). These results are surprising since Chintsa and Tsitsikamma are both relatively pristine localities compared to the Malaysian waters, where point sources of pollution are present and large scale industrial and urban activities occur in the vicinity. High concentrations of essential metals (Cu and Zn) can however, be derived from the surrounding environment in naturally elevated concentrations.

Liver concentrations of metals and metalloids from Simons Town marina, which is situated on the western edge of False Bay, were either the highest or second highest compared to the other localities, indicating high level exposure to these elements. Previous studies in the False Bay area focused on long-term metal accumulation and identified “hotspots” of concern for Cd, Pb and Zn (Taljaard et al. 2000; Mdzeke 2004). Concentrations detected during the present study can possibly be attributed to sources from the nearby naval base and seasonal ocean currents within the greater False Bay area (Newman and Taljaard 2019). A monitoring study conducted by Reinecke et al. (2014) reported concentrations in False Bay for Cd (9.7 µg/g; 5.3 µg/g), Cu (5.5 µg/g; 2.1 µg/g), Pb (6.1 µg/g; 10.8 µg/g) and Zn (87.6 µg/g; 156.7 µg/g) in barnacles and mussels, respectively. These concentrations are above the levels in *C. superciliosus* from the present study (except for Cu), and can be attributed to the different feeding habits and thus exposure routes in these invertebrates when compared to *C. superciliosus*. Concentrations of Cu were seven-fold higher in *C. superciliosus* liver tissue, which is possibly due to a recent exposure event. Antifouling paints, containing Cu, are used for ship hulls, underwater surfaces and buoys, as well as timber, which contributes greatly to elevated anthropogenic Cu levels in the marine environment (RSA-DEA 2018). In addition to these anthropogenic exposures, liver is a metabolically active tissue able to accumulate high concentrations of metals, where metallothioneins are able to store and detoxify metals in fish, representing sequestered products (Ikram et al. 2010).

Limited data is available on past concentrations of metals in aquatic organisms within the vicinity of Walker Bay and Old harbour Hermanus, however, Anchor Environmental Consultants (2015) reported an increase in nutrient input from the Klein Estuary as a result of anthropogenic sources of agriculture and effluent from waste water treatment works. Concentrations of most elements, with the exception of Sb were low when compared to levels at the other locations. Antimony was detected in the highest concentrations at Old harbour Hermanus compared to the other localities, however, these are only trace amounts most likely from geogenic weathering with no known anthropogenic Sn sources (e.g., flame retardants, batteries, chemicals, medicine and,

ceramics and glass) in the vicinity (Filella et al. 2002). Based on the extent of surrounding land-use activities at Cape Town harbour, it would be expected that element concentrations would be the highest at this location. However, this was not the case and the low overall element concentrations were congruent with results obtained for elements in the brown mussel, *P. perna* (Wepener and Degger 2020) where levels were lower than locations along the east coast, including Tsitsikamma. When compared to Cu in intertidal mudskippers (*P. schlosseri*) from a contaminated site on the west coast of peninsular Malaysia (Ikram et al. 2010) concentrations in muscle tissue (0.85 µg/g) were comparable to concentrations from Cape Town harbour but, liver concentrations (6 µg/g) were much higher in *C. superciliosus*. This can be indicative of a recent Cu exposure at the Cape Town harbour due to antifouling paint from ship hulls or underwater surfaces within the harbour. The levels of elements in bivalves, i.e., *M. galloprovincialis* (Fatoki et al. 2011) and *P. perna* (Wepener and Degger 2020) from Cape Town harbour, were higher for all elements analysed. These higher concentrations can be attributed to the filter mode of feeding resulting in exposure via water and particulate matter.

Saldanha Bay (wherein Langebaan marina is located) has long been a focus of metal monitoring studies, where State of the Bay reports for 2015–2019 (Clark et al. 2017, 2019) described elevated concentrations of Cd, Cu, Pb and Zn originating from port activities, mining activities like smelters, iron ore and crude oil facilities, fishing and the closely located naval base. However, concentrations of most of these metals are significantly reduced by the strong water circulation within the bay (Lee and Chown 2007), and the filtering of metals from the water column by mussels and oysters from the large-scale mariculture facilities in the bay (Clark et al. 2017, 2019). This is reflected in the higher metal concentrations in mussels from Saldanha Bay (Sparks et al. 2014) when compared to levels in fish muscle from the present study, (e.g., Cd - 5.6 µg/g and 3.8 µg/g, in bivalves and fish muscle respectively). However, for both *M. galloprovincialis* (Sparks et al. 2014) and *C. superciliosus* (this study), the highest metal concentrations were recorded for Zn and Fe. Concentrations of As in pelagic squid and sardines from the South Atlantic Ocean (Uren et al. 2020) were comparable to concentrations in *C. superciliosus* from localities sampled along the west coast during the present study.

Considering the number of anthropogenic activities at the various localities, as well as the high spatial variability in metal concentrations detected in bivalves (Greenfield et al. 2011; Wepener and Degger 2012; Sparks et al. 2014; Farrington et al. 2016; Clark et al. 2017), it is expected that Tsitsikamma and Chintsa would exhibit the lowest element concentrations, however, this was not the case in the present study. The overall spatial bioaccumulation patterns as indicated by the PCA ordination shows distinct spatial separation in muscle tissue concentrations between the two south-east coast locations, and the west coast locations. There is also an association between the west coast samples and larger specimens, which can be attributed to the infratidal habitats compared to intertidal habitats at the south and east coast localities. Therefore, the differences in feeding habits and consequent element exposure routes

probably contribute to the differences in bioaccumulation observed. Furthermore, the large-scale aquaculture activities and filtering of suspended material from the water column in Saldanha Bay on the west coast probably contributes the lower concentrations detected at this location. The major ocean currents on either side of the South African coast, also contributes to the element concentrations detected at each locality. Local physical ocean conditions such as water movement due to strong tidal sweeps, upwelling, seasonal wind driven water movement in bay areas can cause depositional zones (Newman and Taljaard 2019). Seasonal increased storm water runoff during the rainy periods also results in increased concentrations compared to localities with little runoff, or decreased concentrations due to dilution of effluent through current movements (Watling and Watling 1983; Wepener and Degger 2020).

6.4.3 Fish condition and elemental accumulation in *Clinus superciliosus*

Widdows and Donkin (1992) reported that the condition of marine organisms is one of the main factors to influence internal contaminant distribution and retention. Considering the CFs of fish from the present localities, average fish condition for Tsitsikamma and Chintsa (1.05 and 1.09, respectively) were lower than condition factors for Langebaan marina (1.26), Cape Town harbour (1.22), Simons Town marina (1.13), and Old harbour Hermanus (1.42). The CFs correspond to the concentrations of metals detected during the present study. All of the CFs are, however, still greater than 1, indicating no negative influence of stressors. The variation may be due to the effect of tidal exposure within sheltered harbours compare to exposed rocky shores, as well as reproductive and nutritional status (Mubiana et al. 2006). Feeding habits of different fish age classes and fish occupying different habitats (Bennett et al. 1983; Holleman et al. 2012) can also contribute to the variation in CF between localities.

6.5 Conclusion

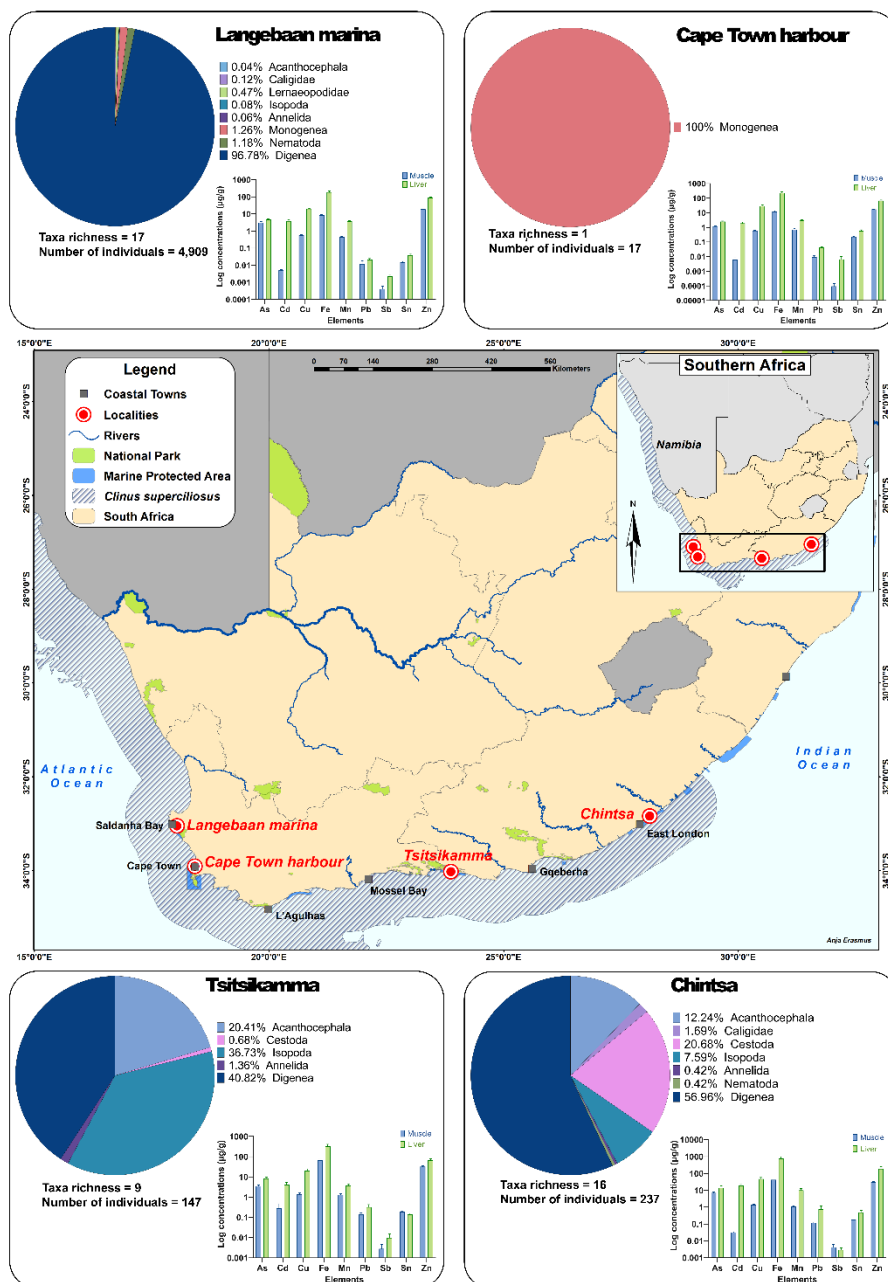
Even though intertidal ecosystems around the world encompasses the smallest area of the ocean environment, these are highly diverse areas. According to Sink et al. (2012) coastal development within 100 m of the shore is prevalent in the Western Cape, particularly at Saldanha Bay, Cape Town, False Bay and Hermanus, putting increased pressure on the natural resources and increasing land derived pollution sources. As a result, a decline has been reported in habitat quality around various regions and countries, in shallow marine environments (Wepener and Degger 2019) and also results in contamination of nursing areas of transient marine fish species. The data obtained from the present study on element accumulation in intertidal klipfish, do not support the hypothesis that metal bioaccumulation in *C. superciliosus* will reflect accumulation depending on the surrounding land-use activities. Sex and size of fish also did not influence element bioaccumulation in muscle and liver tissues of *C. superciliosus*. The factors driving element concentrations in klipfish are rather larger scale oceanographic process and diffuse sources, (e.g., geogenic weathering, ocean currents, upwelling, etc.) and not direct point sources

of pollution. It might therefore be useful to compare these results to pelagic fish species in order to disentangle the real local concentrations compared to the larger scale effects. Hotspots of selected metals and metalloids were noted at specific localities that could be attributed to localised land-use activities or runoff during the rainy season. The limited data on metal accumulation patterns in intertidal fish species highlights the need for long-term monitoring, as well as further studies on various residential and transient inter- and infratidal fish species. The results of such studies combined can contribute to national marine pollution monitoring programmes.

CHAPTER 7

Pollution is not always equivalent to a stressful environment: differential responses of parasite taxa to natural and anthropogenic stressors

Submitted in Marine Pollution Bulletin



7.1 Introduction

Past studies focused on the use of bioindicator species as part of monitoring programs to determine concentrations of various compounds within the marine environment (Rainbow and Wang 2005; Degger et al. 2011a; Degger et al. 2011b; Greenfield et al. 2014). However, recent studies confirmed that bioindicator species, such as fish and mussels, cannot be evaluated in isolation, and should include their associated parasites (Sures et al. 2017b; Selbach et al. 2022). Parasites are characterised as organisms living in close association with one another, where one is dependent (e.g., for feeding and transmission) on the other at some point during its life cycle, without killing it (Morris and Costello 2019). Besides the fact that parasites receive food from their hosts, they are also subjected to predation and are thus involved in the productivity and energy flow of ecosystems, regulating the overall health of systems (Preston et al. 2013; Sures et al. 2017b; Selbach et al. 2022). Parasites have been defined as ecosystem engineers, in some cases changing the behaviour of their hosts to orchestrate transmission to the next host (Morris and Costello 2019). When considering ecosystem health, these parasites can be indicative of various indirect processes previously unknown. Over the past decade, various researchers focused on parasites as bioindicators of anthropogenic impacts (Sures 2003; 2004; 2006; Williams and MacKenzie, 2003; Marcogliese 2005; Vidal-Martínez et al. 2010). Parasites are considered as effect indicators within these ecosystems, where they are positively or negatively impacted by change in their environment, resulting in a change in parasite community composition (Sures et al. 2017b). Parasite diversity can be directly linked to the health of an ecosystem (Nachev and Sures 2009). Previous studies on the effect of pollution on parasite communities, concluded that pollution (such as metals, sewage and eutrophication) has a negative effect on populations of some digeneans and monogeneans (Blonar et al. 2009). To better understand the connection between free-living and parasitic diversity within community change, the degree to which parasites contribute to ecosystem functions should be investigated. During past research studies, this could have resulted in the underestimation of the severity of polluted conditions (Sures et al. 2017a).

Traditional biomonitoring along the South African coast involves the use of bivalves as indicator species, for example the Mussel Watch Program (MWP) (Farrington et al. 2016), due to their ability to effectively accumulate contaminants in the dissolved phase and adsorbed to particulate matter (Wepener and Degger 2020). As part of these monitoring programs, *Mytilus galloprovincialis* and *Perna perna*, were monitored from 1985–2008 by the Department of Environmental Affairs (DEA) (Sparks et al. 2014). A study by Erasmus et al. (2021a) on metals in intertidal fish at selected sites along the South African coastline confirmed the findings by other authors studying element concentrations in mussel bioindicators (Degger et al. 2011a; Moodley et al. 2016; Wepener and Degger 2020) that element bioaccumulation is derived from both natural (upwelling) and geogenic diffuse sources, as well as anthropogenic point sources. However, recent studies have shown that these traditional indicator species do not necessarily reflect the

true exposure in ecosystems, owing to the disregard of the parasite biomass (Wood and Johnson 2015), as well as the fact that some species can adapt to varying environmental conditions. The future of ecotoxicology research should increasingly involve a multidisciplinary approach that includes parasites and other cryptofauna to ecosystem health (Marcogliese et al. 2015) through combining topics that have been under studied in the past.

Environmental parasitology has developed as a discipline that studies the impact of anthropogenic activities related to the occurrence and abundance of parasites and which subsequently relates deviations of normal parasite distribution to environmental health. However, substances such as metals, commonly considered to be pollutants might also occur under natural conditions, whose concentrations might be high due to geogenic release at localities with low anthropogenic activity (e.g., Erasmus et al. 2020b). In the present study, parasite taxa are considered as effect indicators when disentangling general anthropogenic input of elements from naturally occurring high element concentrations. Accordingly, the composition of the metazoan parasite community of the marine fish, *Clinus superciliosus* (Linnaeus, 1758) (Clinidae) (super klipfish), from four localities along the South African coast was studied. The hypothesis tested was that metazoan parasite community composition will respond to element bioaccumulation in the fish host, thereby reflecting the degree of external (environmental) element exposure. Thus, the aim of the present study was to determine whether the elemental exposure in fish causes a change in the parasite community composition at selected localities that represent varying degrees of anthropogenic pressures.

7.2 Materials and methods

7.2.1 Study area

Based on the field survey results presented in Chapter 5 (Erasmus et al. 2022), and Chapter 6 (Erasmus et al. 2021a), four localities were selected to reflect varying degrees of element exposure due to both natural and anthropogenic sources. Thus, the focus will be on two localities on the west coast and two localities on the south to east coast (Figure 7.1).

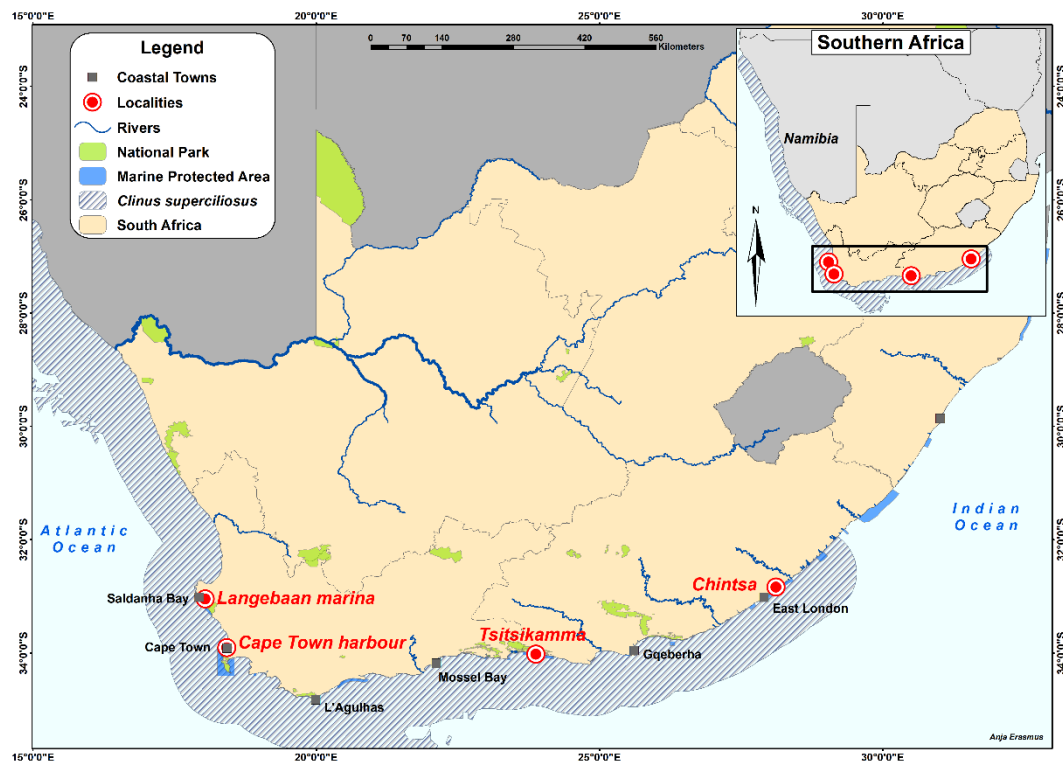


Figure 7. 1: Map of the study area, indicating the number of taxa and concentrations of metals detected from each locality.

7.2.2 Sampling procedure

A minimum of ten klipfish per locality were targeted (with no preference for fish size and gender), dissected and internal and external parasites were identified (diversity) and counted (abundance) per fish. Tissue (muscle and liver) samples were collected, prepared and analysed for As, Cd, Cu, Fe, Mn, Pb, Sb, Sn, Zn elemental concentrations. All methods used were described in preceding chapters (Chapters 2–6). Due to the low abundances and / or biomass of parasites collected from the fish hosts, it was not possible to analyse the elements in the parasites. Therefore, only element concentrations in the fish hosts were determined, and these levels were related to the infection intensity and community composition of the various parasite taxa.

7.2.3 Data analysis

Parasite abundances per group and the total diversity were determined for the four localities, as described in Chapter 3 and Chapter 5. The prevalence, mean intensity, mean abundance and Shannon Wiener diversity index were calculated per fish host for the data set (see Supplementary Table E. 1 for the results of the applicable localities). Element concentrations were determined as described in Chapter 6 (see Supplementary Table E. 2 for the extracted dataset). Based on the results of Chapter 6, the different localities were considered as anthropogenically impacted (Langebaan marina and Cape Town harbour) and natural (Tsitsikamma and Chintsa) rather than polluted and unpolluted conditions.

Conventional linear correlation models were performed in Graphpad Prism v9, to explore the model-fit relationship of the Brillouin's diversity index per fish host, with the concentrations of various elements in muscle and liver tissue, respectively. Metal concentrations were tested for normality and directly compared accordingly using an unpaired t-test followed by a Mann-Whitney test, in fish infected and fish uninfected with monogeneans at Cape Town harbour, since this was the only locality where uninfected fish were sampled.

Multivariate statistical analysis on log-transformed concentrations (centred and standardised) was performed using Canoco v5.12. A constrained redundancy analysis (RDA) was realised for muscle and liver respectively, to determine the effect of element accumulation in fish hosts on the parasite community composition. Generalised linear models (GLMs) were constructed for the taxa with the highest associations with elements based on the results from the RDA. Significance of the statistical differences were determined at $p < 0.05$.

7.3 Results

A total of 55 fish were sampled during the present study, with 5,310 individual parasites representing 29 metazoan parasite taxa (identified to the lowest level possible using morphological and molecular techniques as stated in Chapter 2 and Chapter 3). Due to the low biomass of specimens from each locality, the elemental concentrations could not be measured in the parasites. Thus, the infection intensities were related to elemental concentrations in the fish host using correlation analyses. The mean infection intensities (MI) were relatively low, except for individuals of Digenea (MI = 130) that were unusually high at Langebaan marina (MI = 250) (Table E. 1).

7.3.1 Correlation analysis

All of the elements analysed had a positive Brillouin's diversity index - host element concentration relationship, except for Sn ($p = 0.636$; $r = -0.071$) in muscle samples and Cu ($p = 0.770$; $r = -0.044$) and Sn ($p = 0.505$; $r = -0.101$) in liver samples (Figure 7. 2 and Figure 7. 3). Tin ($p = 0.636$) was the only two elements exhibiting non-significant relationships, while all of the other elements measured in muscle tissue had significant relationships with $p < 0.05$. For elements measured in liver tissue, As ($p < 0.01$), Cd ($p = 0.038$), Fe ($p = 0.044$), Pb ($p < 0.01$) and Sb ($p = 0.043$) had significant correlations (Figure 7.2 and Figure 7.3).

When comparing fish infected and uninfected with monogeneans at Cape Town harbour (results not shown), some of the muscle tissue element concentrations were lower in fish infected (Fe, Cu, Cd, Pb, Sb) than fish uninfected (Mn, Zn, As) with monogeneans. With the exception of Sn and Sb, all elements in liver tissue were lower in uninfected fish when compared to their infected counterparts. None of the differences between infected and uninfected fish were however significant.

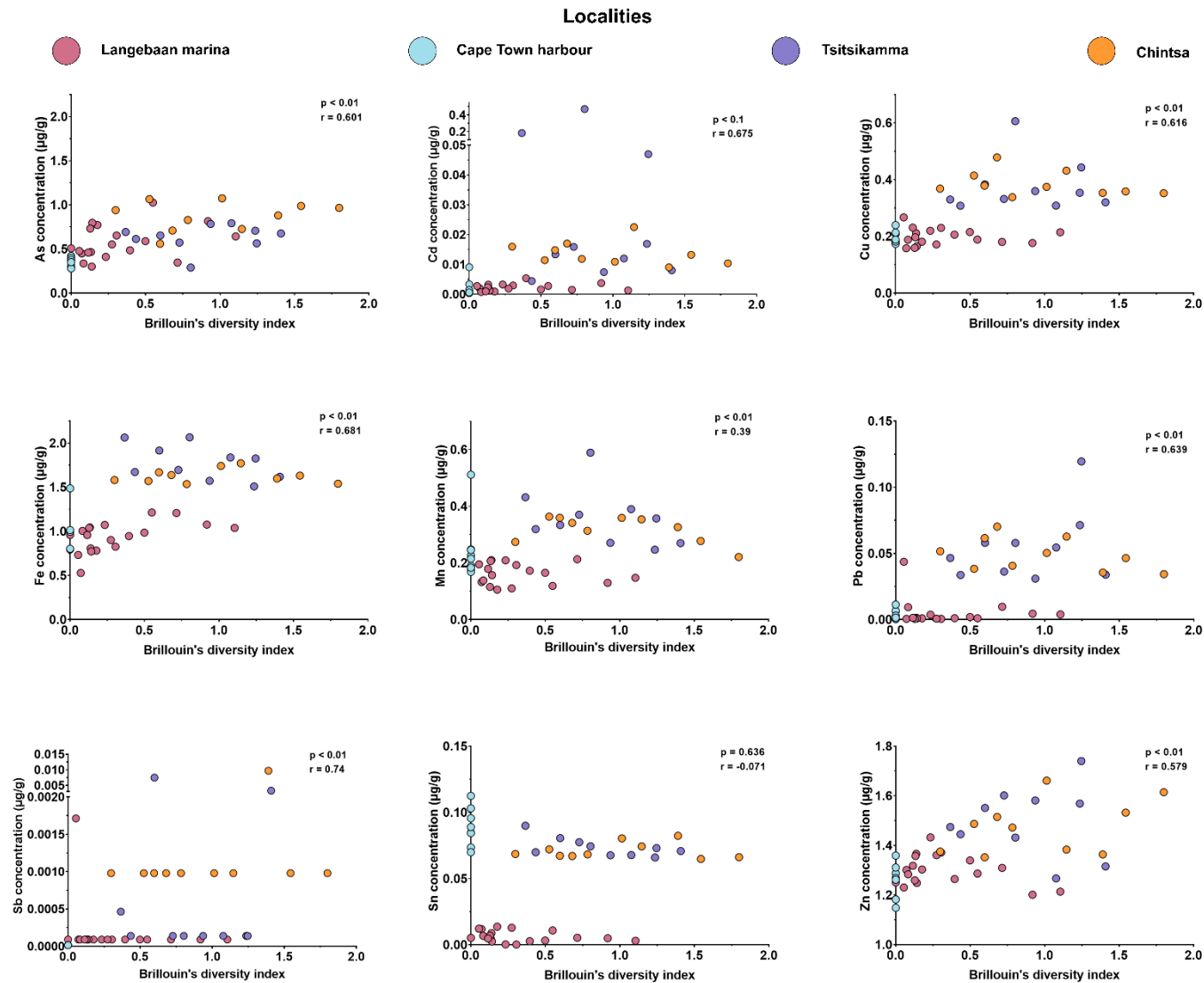


Figure 7. 2: Correlation analysis indicating the relationships between the host element concentrations in muscle tissue and the parasite Brillouin's diversity index from four localities.

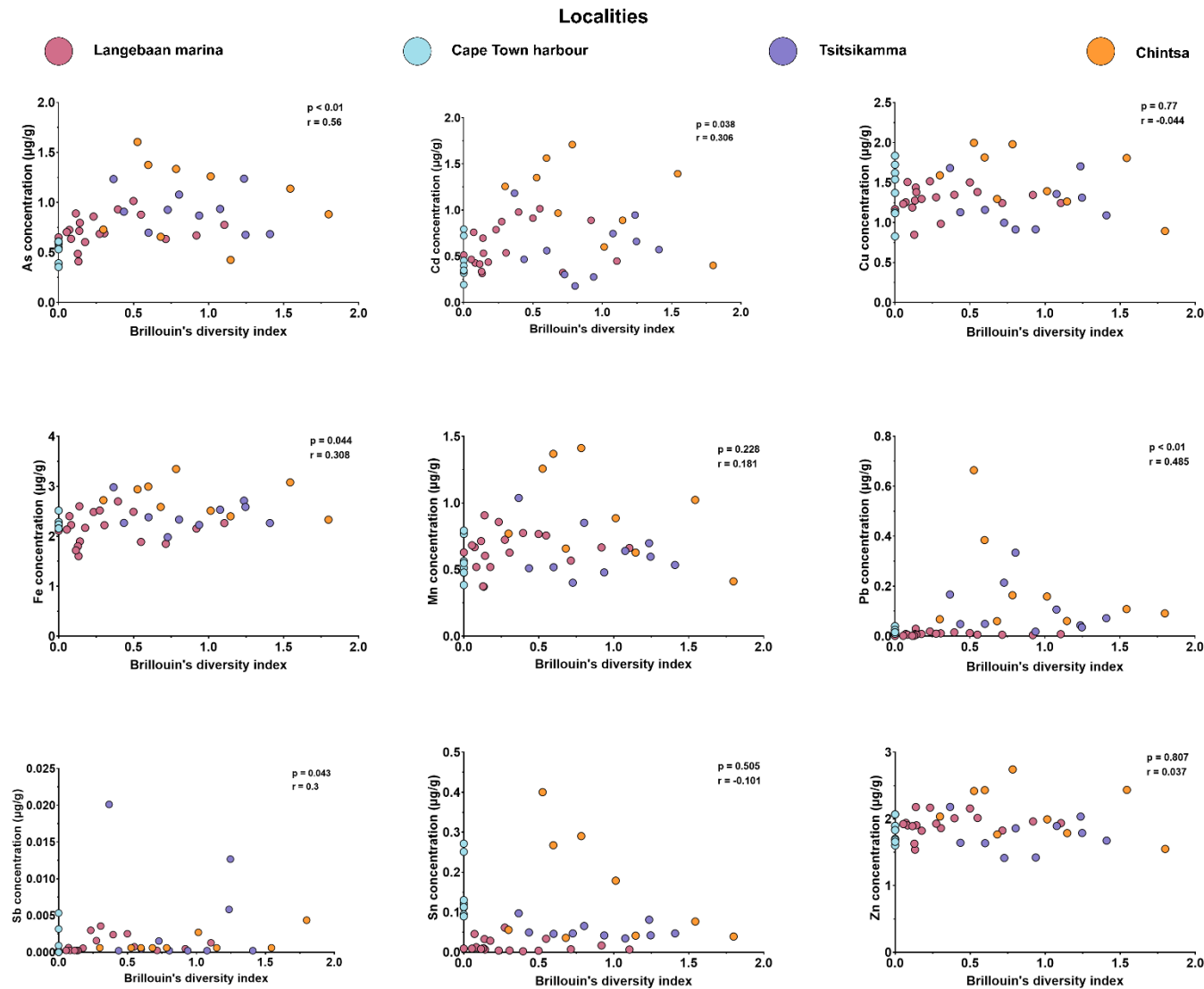


Figure 7. 3: Correlation analysis indicating the relationships between the host element concentrations in liver tissue and the parasite Brillouin's diversity index from four localities.

7.3.2 Host metal accumulation and parasite community composition

When considering the RDA, the response variables (taxa), Acanthocephala, Cestoda and Isopoda had strong associations with element concentrations in muscle and liver tissue of fish hosts from Chintsa and Tsitsikamma (Figure 7.4a and 7.4b). High levels of Cu, Sb and Zn in muscle associated with high abundances of Acanthocephala, while Cestoda associated with As, and Isopoda associated with Cd and Mn in muscle tissue. The same three taxa were associated with high liver concentrations of As, Cd, Mn, Pb, and Zn. These results also indicate that specifically Digenea and Lernaepodidae abundances were higher at the Langebaan marina locality than at any of the other localities sampled, while Monogenea associated with Langebaan marina and Cape Town harbour.

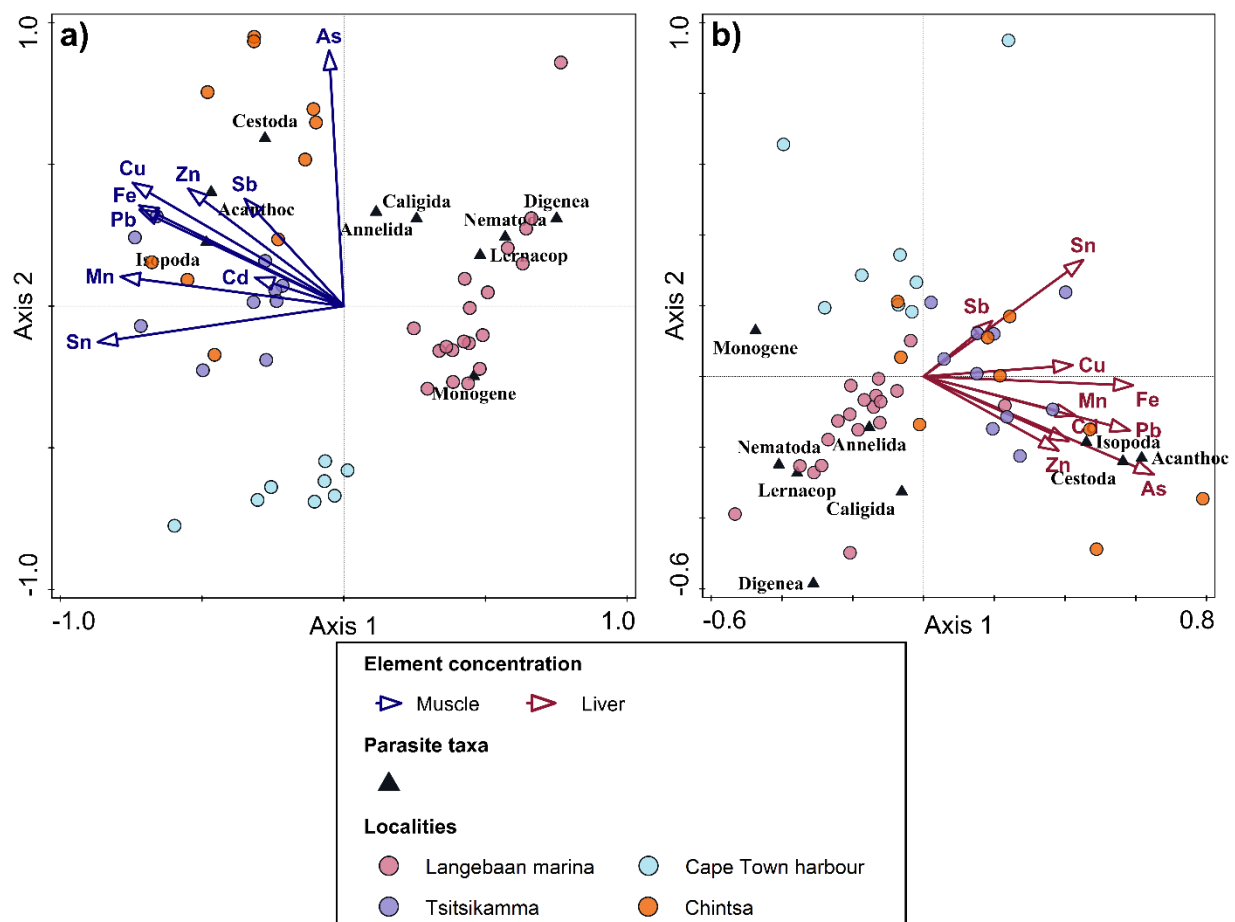


Figure 7. 4: Redundancy analysis depicting the parasite abundance data of each the fish collected at the four localities with log-transformed elemental concentration of the muscle (a) and liver (b) of the host overlaid as environmental variables to elucidate responses to host element exposure. Triplot a) explains 32.9% of the total variation, with 21.6% on the first axis and 11.3% on the second axis, while triplot b) explains 25.8% of the total variation, with 17.3% on axis 1 and 8.5% on axis 2.

Generalised linear models (Table 7.1; Table 7.2) were constructed to determine significant relationships between host element concentrations and specific parasite taxa (Acanthocephala, Cestoda and Isopoda – based on the results from the RDA) abundances. From these results it is possible to link specific taxa to the high element concentrations found at Tsitsikamma and Chintsa. The majority of element concentrations in muscle significantly associated with

Acanthocephala and Isopoda while only As has a significant association with Cestoda. As for liver element concentrations, Acanthocephala and Cestoda had significant associations with the majority of elements, while no significance was detected for Isopoda. This can be linked to the short- and long-term exposure of elements.

Table 7. 1: General Linear Model parameters for taxa with the greatest association with element concentrations in muscle. Significant associations ($p < 0.05$) are indicated in bold.

	Acanthocephala			Cestoda			Isopoda		
	R ² (%)	F	p	R ² (%)	F	p	R ² (%)	F	p
As	10.6	5.3	0.026	23.6	13.9	0.001	0.7	0.3	0.578
Cd	20.4	11.5	0.001	0.2	0.1	0.788	2.7	1.2	0.273
Cu	34.0	23.2	0.00002	4.3	2.0	0.163	9.8	4.9	0.032
Fe	18.3	10.1	0.003	1.9	0.9	0.650	15.9	8.5	0.006
Mn	26.0	15.8	0.0003	0.8	0.4	0.550	9.8	4.9	0.033
Pb	13.0	6.7	0.013	3.8	1.8	0.190	10.3	5.1	0.028
Sb	1.1	0.5	0.508	4.7	2.2	0.142	2.5	1.1	0.289
Sn	5.8	2.8	0.103	2.7	1.2	0.273	6.3	3.0	0.089
Zn	4.7	2.2	0.145	7.6	3.7	0.061	7.9	3.8	0.056

Table 7. 2: General Linear Model parameters for taxa with the greatest association with element concentrations in liver. Significant associations ($p < 0.05$) are indicated in bold.

	Acanthocephala			Cestoda			Isopoda		
	R ² (%)	F	p	R ² (%)	F	p	R ² (%)	F	p
As	44.2	34.8	<0.00001	9.3	4.5	0.039	1.3	0.6	0.548
Cd	2.3	1.1	0.311	18.9	10.2	0.003	0.0	0.0	0.931
Cu	10.8	5.3	0.026	14.5	7.5	0.009	0.5	0.2	0.646
Fe	2.9	1.3	0.262	25.9	15.4	0.0003	0.5	0.2	0.631
Mn	9.0	4.3	0.043	10.4	5.1	0.029	0.1	0.0	0.877
Pb	67.7	92.4	<0.00001	1.9	0.9	0.640	0.2	0.1	0.758
Sb	0.1	0.0	0.836	0.3	0.1	0.737	0.2	0.1	0.777
Sn	21.1	11.7	0.001	2.8	1.2	0.270	0.7	0.3	0.568
Zn	3.1	1.4	0.241	22.2	12.6	0.001	0.0	0.0	0.901

7.4 Discussion

In addition to the factors mentioned influencing parasite abundance and diversity in Chapter 5, and metal uptake in fish hosts in Chapter 6 (condition factor (CF), hepatosomatic index (HSI), fish size (measured in gram), parasite life cycle (monoxenous or heteroxenous), as well as water temperature, host sex, and habitat type), parasites can also play a role in the uptake of metals in their fish hosts, and vice versa. Various researchers have reported on the effects of aquatic contaminants on the parasite community, in terms of richness, abundance and composition (Lafferty 1997; MacKenzie 1999; Marcogliese 2005; Vidal-Martínez 2007; Marcogliese et al. 2010). However, the number of studies conducted on marine host-parasite systems (Sures 2004; Nachev and Sures 2016; Chine et al., 2021) remain scarce and are absent in South Africa. Current host-parasite associations are the result of past adaptations, allowing parasites to exploit

their hosts for transmission, sustain viable populations and survival, where hosts had developed tolerance or resistance to parasites (Timi and Poulin 2020). Tolerance is regarded as limiting the amount of damage caused, while resistance limits the parasite infection altogether (Marcogliese et al. 2010). These defence mechanisms are also applicable to the parasite species, where they tend to be more, or less abundant in areas of high anthropogenic pressures.

7.4.1 Correlation analysis

The correlation analysis revealed positive relationships between the diversity and elemental concentrations for the majority of the metals in both tissues, indicating an increase in parasite diversity with an increase in element concentrations (Nachev and Sures 2016; Sures et al. 2017a). Toxic substances can reduce the diversity of parasite species with complex life cycles (heteroxenous), while the monoxenous parasites are less affected (Sures et al. 2017a). The seemingly contradicting results found in this study may be explained by the fact that the high elemental concentrations at Tsitsikamma and Chintsa are derived from geogenic weathering and larger scale oceanographic processes rather than diffuse and point sources of metal contamination (Degger et al. 2011a; Wepener and Degger 2020; Erasmus et al. 2021a). Depending on the intensity of these naturally derived concentrations, organisms (free-living and parasitic) can build up a tolerance towards these elements, reflecting a generally good condition in the host, even though host species have a high parasite diversity. In general, it is considered that fish with a lower condition factor are more susceptible to infection (Morris and Costella 2019). This may lead to the incorrect assumption that high contaminant levels (such as the elevated elements recorded in the present study) will result in lower condition factors. As discussed in the preceding chapter, the unsheltered and highly kinetic intertidal rocky shore habitat of the klipfish on the southeast coast relative to the sheltered, calm west coast localities that were situated in harbours, would rather contribute to the lower condition factors recorded here (Morato et al. 2001; Hossain et al. 2015; Jisr et al. 2018). The lower parasite diversity recorded at the west coast localities may be explained by the limited habitat diversity in the harbours that resulted in less hosts for parasites with complex life cycles. This is reflected in the dominance of single taxa, i.e., *Dollfustrema* sp. at Langebaan marina, and *Gyrodactylus* sp. at Cape Town harbour, dominating the diversity at these localities. In addition to habitat alteration and contaminants, fishing has also been reported as an additional driver of parasite abundance decline, caused by a reduction in habitat availability and resources for parasites to thrive (Wood et al. 2013).

7.4.2 Host metal accumulation and parasites community composition

There is a clear spatial separation of parasite community composition in klipfish, where Langebaan and Cape Town harbour has distinct groupings, representing the west coast localities where there are more anthropogenic pressures in the form of host habitat alteration. The southeast coast localities displayed a more similar and even diversity as reflected in their ordination on the RDA tri-plot. These groupings also reflect the abundance and diversity results

presented in Chapter 5, with a more even distribution of parasite taxa compared to the west coast localities. The elemental concentrations measured in host fish from these localities indicated that the majority of elements associate with the southeast coast localities.

Nachev and Sures (2016) noted that the choice of suitable parasite bioindicator taxon for element accumulation studies has to be based on their abundance within their hosts, with a high biomass, for sufficient tissue analyses. However, due to the low parasite biomass in the host klipfish, the bioindicator approach that was followed for the present study was to relate parasite abundances, diversity and community composition of the taxa at multiple localities, to the metal levels in the host fish. Three taxa i.e., Acanthocephala, Cestoda and Nematoda were responsible for the similarity in the ordination of parasite community at the southeast coast localities. These taxa have been reported to have a high potential as accumulation indicators and tolerance to contaminants (Marcogliese 2004, 2005; Jirsa et al. 2008; Sures et al. 2017a; Erasmus et al. 2020a; Timi and Poulin 2020), while as effect indicators they can indicate changes on species to community level, due to natural changes and anthropogenic pressures. Circumstances such as ecosystem stress and environmental degradation dictate the kind of indicator used in various studies, which is dependent on the taxon and the functional guild it occupies (Sures et al. 2017a).

Digeneans are known to reflect the degree of disturbance based on the indirect effects on intermediate hosts and ecosystem alteration, while high levels of Zn are known to reduce the presence of monogeneans (Sures et al. 2017a). This appears to be the case in the present study, where the absent trematodes in the Cape Town harbour were associated with increased Zn concentrations in both the liver and muscle tissue of the klipfish host. The dominance of Digenea in structuring the parasite community composition of klipfish in the Langebaan marina can be attributed to the altered host habitat through anthropogenic modifications, resulting in hydro-morphological changes in the water current. Due to reduced current movement cercariae are able to readily infect the intermediate host, *C. superciliosus*, as opposed to the highly kinetic wave action that occurs in the intertidal rocky shores (Vermaak 2020). The absence of digeneans from *C. superciliosus* in Cape Town harbour could be attributed to the high Sn concentrations recorded in the klipfish from this locality (Erasmus et al. 2021a). Antifouling paints containing Sn reportedly cause imposex in marine snail species (Bighiu 2017). As marine snails are intermediate hosts to trematodes, this reduces transmission of these parasitic species within harbours and marinas using antifouling paints on boat hulls.

Similarly, to the acanthocephalans and cestodes, isopods were also associated with high element concentrations. Species from this taxon are known to moult at least three times between different life stages, resulting in the possible accumulation and consequent sequestration of metallic elements, as reported for other macroinvertebrate taxa in freshwater systems (Rauch and Morris 1999; Erasmus et al. 2020c). Whilst it was not possible to analyse element levels in parasites from the klipfish, it is well known that various elements accumulate at higher concentrations in marine fish parasites when compared to their hosts (Nachev and Sures 2016).

Sures and Reimann (2003) reported that acanthocephalans in particular, were excellent indicators of environmental metal exposure. This is also the case for nematodes and cestodes, where the majority of metals (As, Cd, Cu, Fe, Mn, Pb, Zn) analysed in parasite species accumulated to higher levels than in their respective fish hosts (Nachev and Sures 2016; Sures et al. 2017a).

7.5 Conclusion

When considering any species as an ideal bioindicator, their tolerance and resistance to parasites should be taken into account, i.e., through environmental parasitology. Although the results from the present study are conservative in that only metazoan taxa were considered, and no element concentrations were measured in the parasites, these results are still in accordance with current literature. The present study thus demonstrated that localities with high metal exposure (concentrations determined in the fish host) did not reflect the expected lower parasite diversity. This can be due to the fact that these elevated concentrations are of natural origin and that the organisms exposed (hosts and parasites) have probably adapted to tolerate these high levels. It is also true that the taxa known as good bioaccumulation indicators (specifically acanthocephalans and cestodes), were the most abundant and diverse at the localities with these elevated element exposure were evidenced through accumulation in the host. Based on these results, and other studies supporting the concept that the presence and evenness of parasite diversity is evidence of a healthy, functioning and resilient ecosystem (Marcogliese 2005; Hudson et al. 2006; Sures et al. 2017a), despite having high contaminant concentrations.

Even though the present study was not able to obtain sufficient biomass for metal analysis in the parasites concerned, their elevated abundances provide a clear indication of elemental exposure. On the other hand, various studies reported the decrease of contaminant concentrations for aquatic organisms infected with acanthocephalans and cestodes compared to their uninfected counterparts (Sures et al. 2017a; Biswal and Chatterjee 2020; Timi and Poulin 2020). In some cases, parasites can even act synergistically or antagonistically with pollutants to affect host fitness, resulting in biomarker responses falsely attributed to only pollution (Sures et al. 2006). Parasites as accumulation indicators have supported the conclusion that helminths (Acanthocephala, Cestoda, and Nematoda) are useful tools due to their site of infection and measuring metals in orders of magnitude higher than their hosts (Nachev and Sures 2016; Sures et al. 2017b; Morris and Costello 2019; Erasmus et al. 2020a). These parasite taxa are thus useful indicators of elevated element exposure in their hosts, and can even be used for considering free-living ecosystem health.

It can be concluded that high element concentrations are not always a result of anthropogenic impacts. Future studies should better describe the term “polluted conditions”, and disentangle the various anthropogenic alterations (eutrophication, acidification, organic

enrichment, organic pollutants, temperature fluctuations) that contribute to element accumulation in hosts, as well as parasites and their community composition.

CHAPTER 8

General Conclusion



8.1 General conclusion

With multiple species, particularly small-bodied organisms such as parasites, still undescribed from the marine environment (Smit and Hadfield 2015), new studies investigating novel taxa are highly encouraged. Linking these marine biotas to ecosystem functions are of even greater importance. Even though parasite taxa play a vital role in ecosystems, their contribution has been understudied over the past centuries, where the focus remained on taxonomic descriptions, specifically in South Africa. In contrast to the interactive role of parasites in ecosystems along the South African coast, contamination by metallic elements received some attention over the last century. Following on the previous work conducted in South Africa, the next step to an integrated approach to ecosystem health would be to combine the knowledge of parasites and contamination by using environmental parasitology. In addition to environmental parasitology, other approaches are also considered during ecosystem health studies. To properly define the roles that parasites play in ecosystems, the functional diversity approach could be applied. This defines the range of functional traits that can be used to distinguish each organism within their respective communities. Previous studies have supported the use of both diversity and functional approaches to assess the influence of multiple stressors on communities (Ferreira et al. 2012; Erasmus et al. 2021b). Before these techniques can be applied to the majority of ecosystems in South Africa, complete species identifications have to be performed.

The present study forms part of a larger project investigating the hidden diversity in marine habitats, particularly parasite taxa, along the South African coast, within and outside Marine Protected Areas (MPA), investigating multiple fish host species. The primary aim of the present study was thus to ascertain the role of parasites as effect indicators in the marine environment. This was established by conducting host-specific sampling to obtain parasite species infecting *Clinus superciliosus*, as well as calculating the effect of elemental concentrations and other factors on the parasite community composition. In the present study, it is suggested that marine species such as the endemic intertidal klipfish, *C. superciliosus* (Clinidae), with information on their biology, reproduction and diet already known, is an appropriate model organism for ecosystem health studies.

In the subsequent paragraphs the main findings of each chapter will be highlighted to discuss, the taxonomic resolution on various levels, various factors at different localities that drive the community composition at ecosystem level, and to disentangle pollution as an anthropogenic or natural pressure within aquatic ecosystems. This will be followed by ideas for future research.

8.2 Parasite diversity of *Clinus superciliosus*

Precise identification of parasite taxa is crucial for a magnitude of reasons including food web assemblages, pathogenicity, restoration potential, and many more (McManus and Bowles 1996; Poulin 2011). While technological advances in optical microscopes gained much progress for

morphological identification techniques, expertise on the specific taxa is to this day still underrepresented, specifically in South Africa (Smit and Hadfield 2015). The advent of molecular tools to aid in parasite identifications was a significant contribution to biodiversity studies (especially in parasitology) by enhancing our estimation of species diversity and resolving cryptic species (Poulin 2011; Pérez-Ponce de León et al. 2018). Parasites generally have a poor taxonomic resolution compared to free-living species, as a result of their hidden lifestyles, effort of identification, lack of expertise, and particularly small size (Poulin and Morand 2011).

The results from the present study, support the use of both morphological and molecular techniques as an integrated approach to taxonomy, to identify and differentiate parasite taxa. This was evident with the astounding 43 species of parasites reported to infect *Clinus superciliosus* (Chapter 3 and 6). Further support was also given through a specific description of a newly discovered species of *Allela* (Copepoda: Siphonostomatoida: Lernaeopodidae) in Chapter 4. Through these results it is evident that taxonomy is ever evolving, with new techniques and equipment constantly updating and reporting novel discoveries. With these ever changing and newly developed advances in parasitology, taxa are repeatedly allocated to different genera, synonymies are created, and redescrptions are common (Tkach et al. 2016). In addition to the advances in laboratory techniques to identify species, there has also been an emerging trend in parasite discoveries. Instead of taxonomic experts sampling fish for one specific taxa, the whole parasite community of a specific host is targeted (for example studies like Vidal-Martínez et al. 2014; Vidal-Martínez et al. 2019). Standard operating procedures for the collection, fixation and preservation of all the various parasite groups are available to researchers (Scholz et al. 2018; Klimpel et al. 2019), and once the investigator has collected the specimens, taxonomic experts can be approached for identification or collaboration to that regard. This also has the benefit of utilising the whole host, instead of sacrificing organisms to investigate one organ, especially when combined with ecotoxicological studies.

Morphological identification has sometimes proven unfeasible due to the occurrence of species complexes and a lack of proper identification guides available for the more ubiquitous taxa. Furthermore, a limited number of sequences are available on GenBank for proper comparison, resulting in identification only up to the genus or family level. Throughout the progress of the present study, it became even more evident that the more data available, on databases such as FishBase, WoRMS, and GenBank, the better the taxonomic resolution would be. In addition to the taxa found during the present study, various suggestions of possible intermediate and definitive hosts have been considered, from where future opportunities to complete life cycle studies could follow. *Octopus vulgaris* are considered as a possible host to *Proctoeces* (Trematoda: Fellodistomidae), and possibly *Acanthobothrium* (Cestoda: Onchobothriidae) species. It is thus recommended that the molecular data from especially larval species are linked to GenBank, where adults from the definitive hosts can be connected to intermediate or paratenic host species when elucidating their life cycles (Criscione et al. 2005;

Georgieva et al. 2013). Due to the ambiguity of sequence data, the aim should be to characterise the specimen morphologically and then relate that to the subsequent molecular data (as in Schwelm et al. 2021a). This integrated approach to taxonomy will thus limit the misidentifications, re-designations and duplications of species data.

Only two of the previously reported taxa infecting *C. superciliosus* were not collected during surveys from the present study, while one of these species, *Elthusia xena*, has only been reported from outside the current sampling area. Taxa from all of the various parasitic groups (Euglenozoa, Myxozoa, Ciliophora, Myxozoa, Digenea, Monogenea, Cestoda, Acanthocephala, Annelida, Nematoda, Copepoda, Isopoda) have now been reported to infect *C. superciliosus*, with the majority of the taxa collected during the present study recording new host records, and even several novel parasite species. Additionally, an economically important species of myxozoa, *Kudoa thyrsites*, was also reported during the present study. Thus, the hypothesis set that host-specific parasite sampling will yield more parasite species than parasite-specific sampling from previous studies was supported by the results obtained. The role of MPAs in the conservation of not only free-living species but also parasites, has proven significant through the results of this study, where the high diversity and evenness was also related to ecosystem health, indicating functionality, stability and resilience.

8.3 Factors influencing biodiversity

In addition to the diversity findings, host level influences were also investigated during the present study, since parasites play a vital role in food web connectivity. To do so, prevalence (P %), mean intensity (MI) and mean abundance (MA) per host were calculated, as well as various indices on infracommunity and component community level, and compared between the various localities. Other factors influencing distribution patterns and species occurrence were investigated to affect parasite community composition through redundancy analysis. Condition factor (CF), hepatosomatic index (HSI), fish size (measured in gram), parasite life cycle, as well as water temperature, host sex, and habitat type were considered. These factors and the conclusions deduced from the present study can also give further information on the free-living species from specific habitats (Lafferty et al. 2008).

From the results presented in Chapter 5, the two main factors influencing parasite community composition are life cycle (monoxenous or heteroxenous) and water temperatures. From literature it is well known that more complex life cycles are present in healthy ecosystems compared to altered ecosystems (Hudson et al. 2006). This was also reflected in the present study where a greater parasite diversity was sampled at the southeast coast localities, concurring that these localities are in a healthier state. Higher water temperatures at the southeast coast localities, known to increase the transmission of parasite species (Selbach et al. 2022), were associated with generally high abundance and diversity. The temperature difference between sampled localities is due to the ocean currents on the opposing coastal areas, where the north

flowing Benguela Current transports cold nutrient-rich water from the polar zones and the south flowing Agulhas Current transports warm water from the equators (Lutjeharms et al. 2000). Habitat availability refers to a habitat for intermediate hosts within the intertidal rocky shore, but also to the habitat within the various hosts for parasite species to thrive. This can in turn be linked to host condition where, if the host has a lower condition factor, they might be easier to infect due to a weakened immune system, and easier for parasites to access due to the altered hydro-morphology at the anthropogenically altered Langebaan marina. We hypothesised that parasite community composition will be mostly influenced by environmental factors, such as water temperature and habitat availability. The results from this chapter supported the hypothesis. Additional influences on the parasite community composition were also investigated in Chapter 7 on Environmental Parasitology, and will be discussed in details below.

8.4 Metal and metalloid accumulation in *Clinus superciliosus*

Metallic element concentrations (As, Cd, Cu, Fe, Mn, Pb, Sb, Sn, Zn) detected in the selected host species, *C. superciliosus*, were regarded as naturally high, due to upwelling, geogenic weathering and larger scale oceanographic processes. From these results, sources such as antifouling paints in harbours and marinas, the nearby naval base, and transport by season water currents within the False Bay area (Newman and Taljaard 2019), were identified as hotspots. When compared to other monitoring species on sentinel organisms such as mussels, the concentrations from the present study were lower, attributed to different exposure routes (for example Reinecke et al. 2014). The short- and long-term exposure of these organisms were also measured by considering the different tissues (muscle and liver) analysed. Evidently Cu and Sn were shown to be a short- to long-term hotspot at Simons Town marina and Cape Town harbour, respectively, which can also be attributed to the antifouling paints used within the harbour, marina and the naval base. During the present study, it was apparent that very little ecotoxicological studies are conducted, specifically on intertidal fish species along the South Africa coast, and with similar studies, these results can aid in national marine pollution monitoring programmes and provide critical baseline data for informing governance policies.

The results from this chapter did not support the hypothesis that the proposed bioindicator, *Clinus superciliosus*, will reflect metal bioaccumulation based on their proximity to the surrounding land-use activities. Instead, localities with more natural conditions on the southeast coast had higher concentrations than the west coast localities, located within a marina and a harbour, with multiple anthropogenic activities in the vicinity. The influence of these concentrations on the ecosystem level was addressed in Chapter 7, combining the results and conclusions from Chapter 5 and 6.

8.5 Environmental parasitology

For this chapter the hypothesis stated that the metazoan parasite community composition will respond to element bioaccumulation in the fish host, thereby reflecting the degree of external (environmental) element exposure. Metazoan parasites are herein regarded as effect indicators of element exposure. According to literature, several parasitic groups are considered as useful monitoring tools in ecosystem health studies (Marcogliese 2005; Sures et al. 2017a). In the present study, it was noted that acanthocephalans and cestodes associated with high concentrations of elements at the two east coast localities, which is in accordance with previous studies (Nachev and Sures 2016; Sures et al. 2017a). Parasite responses to anthropogenic pressures are dependent on species and host physiology (Blanar et al. 2009; Vidal-Martínez et al. 2010; Timi and Poulin 2020). Host condition at the various localities reflected these high concentrations, however, the concentrations were not considered as detrimental to these species, with only a slightly lower condition factor. This may lead to the incorrect assumption that higher contaminant concentrations will result in the lower condition factors noted at Tsitsikamma and Chintsa, which was attributed rather to the unstable and highly kinetic nature of these habitats compared to the anthropogenically altered habitat on the west coast. In addition to host condition, it was also evident that the altered habitat conditions caused changes to the ecosystem structure, with reduced habitat availability for intermediate host species. Other anthropogenic factors, such as fishing, have been reported in literature as an additional driver of species abundance decline (free-living and parasitic) by reducing host availability for parasites to complete their life cycle (Wood et al. 2013). Naturally high elements do not influence the metazoan parasite community structure since species are able to adapt to tolerate these concentrations. Rather, anthropogenic alteration of habitats and the loss of intermediate hosts influence community composition.

Due to the direct and indirect reflection of anthropogenic pressures on parasite communities, some studies have also reported the use of parasite taxa as rehabilitation indicators, for the restoration success of altered ecosystems (Huspeni and Lafferty 2004; Marcogliese 2005; Morley et al. 2006; Pérez-del Olmo et al. 2007a). In line with the results from the present study, future parasite community studies at the anthropogenically altered localities can be conducted to determine if the ecosystem has recovered from habitat alteration, fishing, and overexploitation. On the other hand, frequent studies at the Marine Protected and conservation areas can be conducted to establish whether these conservation areas are able to continuously conserve the whole ecosystem and not just free-living species.

The deduction from these results were that pollution is not always high metallic element concentrations, but can also be eutrophication, habitat degradation and overexploitation at human impacted localities, compared to Marine Protected Areas and areas of conservation where these concentrations are naturally high and abovementioned activities are banned.

8.6 Recommendations for future studies

The present study highlights the need to sample multiple localities within a distribution range, as well as do further explorative parasitological surveys within the biodiversity rich marine and intertidal environments. The present research also supports the statement alluded throughout the thesis, that the majority of parasite species have not been described from the South African marine biota. Linking environmental data to parasite community composition can aid in bridging the gap between parasites, anthropogenic pressures, and ecosystem health. Recommendations to consider for future studies include:

- More focus should be placed on specific host-parasite assemblages, for example recreational and commercial fish species, as information on their community composition can add value to various decision-making processes. It is also important to consider fish cryptofauna, as this information can be useful for establishing red list data. As mentioned in the thesis, parasite species can aid in the determination of ecosystem health, recovery rate of the ecosystem following disastrous events such as an oil spill. This approach can also help to resolve the cointroduction of parasites from invasive host species.
- When considering these host-parasite assemblages, it is also imperative to investigate the organisms that contribute to the food web of each ecosystem. By conducting molecular and morphological characterisation of all available life stages of a specific taxon, future life cycle studies can be elucidated.
- Throughout the process of this thesis, a major gap in reliable and concise databases and keys to taxonomic groups was observed. Although various databases exist on species occurrence and classification, the establishment of comprehensive reference libraries are still longed for in these biodiversity monitoring methods.
- Future studies could consider investigating all tissues of *Clinus superciliosus* for the presence of *Kudoa thyrsites* to determine if the infection is widespread through more than one tissue, as the urinary bladder is not the normal site of infection. This can be done with histological techniques, looking at muscle fibres.
- Laboratory-based studies on marine taxa to explore the effect of combined exposure to parasites and pollutants should be considered in future studies, where the experimental designs should also include the biomarkers affecting host species. This will clarify the specific effects on parasites and their hosts with regard to ecosystem health.
- It is recommended that future novel parasite discoveries include multiple analyses for proper descriptions (light- and scanning electron microscopy, molecular analysis of more than one gene region where possible, and descriptions accompanying illustrations), to support findings and reduce the number of misidentifications. The

taxonomy of parasites is very much divided based on expertise, but that does not mean researchers cannot work together with other departments to create a proper full description of new species.

- Another tool to aid in the comparison of previously described species and newly collected specimens is DELTA (DEscriptive Language for TAXonomy). This method is highly recommended in future descriptions and will discourage the use of multiple terms for the same morphological characters and encourage direct comparisons of morphologically similar species.
- Differentiation of various contamination sources, anthropogenic or naturally derived, should be considered for environmental parasitological studies. When considering metallic elements, speciation of metals within the marine environment should be defined, as differing toxicity occurs at different stages.
- Where at all possible in field studies, it is advised that element concentrations in parasites are also included, in order to directly link parasites as effect indicators of pollution. From these results, it can be concluded if parasites have a synergistic, antagonistic or additive effect on their hosts when exposed to various elemental concentrations under natural conditions.

8.7 Concluding remarks

To conclude, the selected host species is vital within intertidal rocky shore ecosystems, where the influence of anthropogenic pressures and natural conditions are reflected in the host and its parasite community composition. Some taxa are associated with higher concentrations of elements, while others were absent under altered habitat conditions. These results are not always linked to pollution events but can also be derived from natural conditions at different localities. For organisms identified as bioindicator species, bioavailable elements in the environment and within the host should be considered in combination. Based on the occurrence data, it can be concluded that parasites and their hosts are able to adapt to these high concentrations and even thrive. Moreover, this study serves as proof that Marine Protected Areas not only conserve free-living biodiversity, but even parasite species, where a healthy ecosystem is one that is rich in parasite diversity. Additionally, the high number of parasites reported from this host species, numerous adult and larval taxa have been identified to the lowest level possible. Nine species have been identified to species level, as well as one full description of a newly discovered species of *Allela* (Lernaeopodidae), and an economically important species, *Kudoa thyrsites*. Further outputs from this thesis will include full descriptions of the remaining novel taxa using the integrated approach to taxonomy, will follow. If these species are not conserved, parasite taxa and their integral contribution to ecosystems can be lost. Findings from these integrated taxonomic approaches and multidisciplinary studies will hopefully provide valuable insight for future researchers of ecological parasitology.

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Appendix A – Chapter 3

Table A. 1: Sequence data from GenBank, verified on BLAST and with published data, including host, locality, accession numbers (18S SSU) and references for sequences used in the phylogenetic analyses for *Myxidium*. TBP – to be provided

Parasite species	Host	Locality	Accession no.	Reference
Novel sequence				
EPD077_ <i>Myxidium</i> sp. (gallbladder)	<i>Clinus superciliosus</i>	Langebaan marina, South Africa	TBP	Present study
Published GenBank sequences				
<i>Myxidium gadi</i>	<i>Pollachius virens</i>	North Sea	DQ377711	Fiala 2006
<i>Myxidium coryphaenoideum</i>	<i>Coryphaenoides rupestris</i>	North Atlantic, Atlantic Ocean	DQ377697	Fiala 2006
<i>Myxidium gadi</i>	<i>Melanogrammus aeglefinus</i>	Scotland, North Sea, UK	DQ377707	Fiala 2006
<i>Myxidium bergense</i>	<i>Helicolenus dactylopterus</i>	North Atlantic, Atlantic Ocean	DQ377702	Fiala 2006
<i>Myxidium incurvatum</i>	<i>Callionymus lyra</i>	North Sea	DQ377708	Fiala 2006
<i>Myxidium laticurvum</i>	<i>Trachinus draco</i>	Denmark	JN033230	Karlsbakk and Koie 2012
<i>Myxidium truttae</i>	<i>Salmo trutta</i>	Northern Scotland, UK	AJ582061	Holzer et al. 2004
<i>Myxidium giardi</i>	<i>Anguilla anguilla</i>	Northern Scotland, UK	AJ582213	Holzer et al. 2004
<i>Myxidium lieberkuehni</i>	<i>Esox lucius</i>	North America	X76639	Jirků et al. 2006
<i>Myxidium finnmarkicum</i>	<i>Merlangius merlangus</i>	North Norway	GQ890672	Mackenzie et al. 2010
Outgroup				
<i>Sphaeromyxa zaharoni</i>	<i>Pterois miles</i>	Red Sea	AY538662	Diamant et al. 2004

Table A. 2: Sequence data from GenBank, verified on BLAST and with published data, including host, locality, accession numbers (18S SSU) and references for sequences used in the phylogenetic analyses for *Henneguya*. TBP – to be provided

Parasite species	Host (Family)	Locality	Accession no.	Reference
Novel sequences				
EPD063_ <i>Henneguya</i> sp. (eye)	<i>Clinus superciliosus</i>	Langebaan marina, South Africa	TBP	Present study
Published GenBank sequences				
<i>Henneguya sinova</i>	<i>Parablennius tentacularis</i>	Black Sea, Turkey	KU726089	Özer et al. 2016
<i>Henneguya salminicola</i>	<i>Oncorhynchus nerka</i>	British Columbia, Canada	AF031411	Hervio et al. 1997
<i>Henneguya lesteri</i>	<i>Sillago analis</i>	Queensland, Australia	AF306794	Hallett and Diamant 2001
<i>Henneguya gurlei</i>	<i>Ameiurus nebulosus</i>	North Carolina	DQ673465	Iwanowicz et al. 2008
<i>Henneguya exilis</i>	<i>Ictalurus punctatus</i>	Mississippi	AF021881	Lin et al. 1999
<i>Henneguya quelen</i>	<i>Rhamdia quelen</i>	Paracauari River, Brazil	MH230064	Abrunhosa et al. 2018
<i>Henneguya bicaudi</i>	<i>Cirrhinus mrigala</i>	Harike Wetland, Punjab	KP099967	Kaur and Attari 2015
<i>Henneguya multiradiatus</i>	<i>Brochis multiradiatus</i>	Peruvian Amazon	MT415832	Mathews et al. 2020
<i>Henneguya aegae</i>	<i>Pagrus major</i>	Leros, Greece	MK007473	Katharios et al. 2020
<i>Henneguya peruviana</i>	<i>Hyphessobrycon loretoensis</i>	Peru	MH744155	Mathews et al. 2018
Outgroup				
<i>Ceratomyxa shasta</i>	<i>Oncorhynchus mykiss</i>	Pacific Northwest of North America	AF001579	Bartholomew et al. 1997

Table A. 3: Sequence data from GenBank, verified on BLAST and with published data, including host, locality, accession numbers (18S SSU) and references for sequences used in the phylogenetic analyses for *Myxobolus*. TBP – to be provided

Parasite species	Host (Family)	Locality	Accession no.	Reference
Novel sequence				
EPD031_ <i>Myxobolus</i> sp. (gallbladder)	<i>Clinus superciliosus</i>	Tsitsikamma, South Africa	TBP	Present study
Published GenBank sequences				
<i>Myxobolus portucalensis</i>	<i>Anguilla anguilla</i>	Lake Balaton, Hungary	AF085182	Andree et al. 1999
<i>Myxobolus squamalis</i>	<i>Oncorhynchus tshawytscha</i>	Rancho Cordova, California	U96495	Andree et al. 1997
<i>Myxobolus cerebalis</i>	<i>Oncorhynchus mykiss</i>	West Virginia	U96492	Andree et al. 1997
<i>Myxobolus hearti</i>	<i>Carassius auratus gibelio</i>	Honghu, China	GU574808	Ye and Wang 2010
<i>Myxobolus pyramidis</i>	<i>Carassius auratus gibelio</i>	China	HQ613411	Xi et al. 2013
<i>Myxobolus wulii</i>	<i>Carassius auratus gibelio</i>	China	HQ613412	Xi et al. 2013
<i>Myxobolus cultus</i>	<i>Carassius auratus gibelio</i>	Jiangsu Province, China	HQ613409	Xi et al. 2013
<i>Myxobolus koi</i>	<i>Carassius auratus auratus</i>	China	FJ710800	Zhang et al. 2010
<i>Myxobolus mucosus</i>	<i>Leuciscus leuciscus</i>	Russia	KP751908	Liu et al. 2016
<i>Myxobolus bizerti</i>	<i>Mugil cephalus</i>	Tunisia	AY129318	Bahri et al. 2003
<i>Myxobolus ichkeulensis</i>	<i>Mugil cephalus</i>	Tunisia	AF378337	Bahri et al. 2003
<i>Myxobolus spinacurvatura</i>	<i>Mugil cephalus</i>	Tunisia	AF378341	Kent et al. 2001
<i>Myxobolus episquamalis</i>	<i>Liza ramada</i>	Egypt	MK329248	Eissa et al. 2020
<i>Myxobolus platessae</i>	<i>Platichthys flesus</i>	SW Iceland	KX886734	Karlsbakk et al. 2016
<i>Myxobolus aeglefini</i>	<i>Gadus morhua</i>	Iceland	KX886731	Karlsbakk et al. 2016
<i>Myxobolus albi</i>	<i>Pomatoschistus microps</i>	Forth Estuary, Scotland	EU420055	Picon-Camacho et al. 2009
<i>Myxobolus groenlandicus</i>	<i>Reinhardtius hippoglossoides</i>	Greenland	F694785	Buchmann et al. 2012
Unpublished GenBank sequences used in alignment				
<i>Myxobolus</i> sp. LP-2017	<i>Cyprinus carpio</i>	Yueyang City, Hunan Province, China	KY203795	Xi and Li 2017
<i>Myxobolus groenlandicus</i>	<i>Reinhardtius hippoglossoides</i>	Greenland	JF694785	Buchmann et al. 2012
<i>Myxobolus wulii</i>	<i>Bothrioneurum vej dovskyanum</i>	Honghu, China	KY784580	Unpublished
<i>Myxobolus cultus</i>	<i>Branchiura sowerbyi</i>	Tokyo, Japan	AB121146	Unpublished
Outgroup				
<i>Ceratomyxa shasta</i>	<i>Oncorhynchus mykiss</i>	Pacific Northwest of North America	AF001579	Bartholomew et al. 1997

Table A. 4: Code used for *Ceratomyxa* sequences in genetic distance, Table 3.5

Code	Sequence labels
1	EPD054_ <i>Ceratomyxa</i> sp. A (gallbladder)
2	EP067_ <i>Ceratomyxa</i> sp. B (gallbladder)
3	EP090_ <i>Ceratomyxa</i> sp. B (gallbladder)
4	EP074_ <i>Ceratomyxa</i> sp. B (gallbladder)
5	EP065_ <i>Ceratomyxa</i> sp. B (gallbladder)
6	EP086_ <i>Ceratomyxa</i> sp. B (gallbladder)
7	EP078_ <i>Ceratomyxa</i> sp. B (gallbladder)
8	EP092_ <i>Ceratomyxa</i> sp. B (gallbladder)
9	EP107_ <i>Ceratomyxa</i> sp. B (gallbladder)
10	EPD065_ <i>Ceratomyxa</i> sp. B (eye)
11	EP106_ <i>Ceratomyxa</i> sp. B (gallbladder)
12	EP109_ <i>Ceratomyxa</i> sp. B (gallbladder)
13	EP087_ <i>Ceratomyxa</i> sp. B (gallbladder)
14	EP060_ <i>Ceratomyxa</i> sp. A (gallbladder)
15	EP061_ <i>Ceratomyxa</i> sp. A (gallbladder)
16	EP065_ <i>Ceratomyxa</i> sp. A (gallbladder)
17	EP065_ <i>Ceratomyxa</i> sp. A (gallbladder)
18	EP065_ <i>Ceratomyxa</i> sp. A (gallbladder)
19	EP104_ <i>Ceratomyxa</i> sp. B (gallbladder)
20	EP083_ <i>Ceratomyxa</i> sp. B (gallbladder)
21	EP088_ <i>Ceratomyxa</i> sp. C (gallbladder)
22	EPD066_ <i>Ceratomyxa</i> sp. C (cyst)
23	EP116_ <i>Ceratomyxa</i> sp. C (gallbladder)
24	EP111_ <i>Ceratomyxa</i> sp. C (gallbladder)
25	EP063_ <i>Ceratomyxa</i> sp. C (gallbladder)
26	EP112_ <i>Ceratomyxa</i> sp. C (gallbladder)
27	EP117_ <i>Ceratomyxa</i> sp. C (gallbladder)
28	EP118_ <i>Ceratomyxa</i> sp. C (gallbladder)

Code	Sequence labels
29	EP119_ <i>Ceratomyxa</i> sp. C (gallbladder)
30	EP089_ <i>Ceratomyxa</i> sp. C (gallbladder)
31	EP066_ <i>Ceratomyxa</i> sp. C (gallbladder)
32	EP114_ <i>Ceratomyxa</i> sp. C (gallbladder)
33	EP085_ <i>Ceratomyxa</i> sp. C (gallbladder)
34	EP062_ <i>Ceratomyxa</i> sp. C (gallbladder)
35	EPD064_ <i>Ceratomyxa</i> sp. A (eye)
36	EP076_ <i>Ceratomyxa</i> sp. B (gallbladder)
37	EP066_ <i>Ceratomyxa</i> sp. B (gallbladder)
38	MG238434_ <i>Ceratomyxa cottoidii</i>
39	JF911815_ <i>Ceratomyxa carcharinid</i>
40	JF911817_ <i>Ceratomyxa melanopteri</i>
41	JF911818_ <i>Ceratomyxa negaprioni</i>
42	KM273026_ <i>Ceratomyxa synaphobranchi</i>
43	KM273022_ <i>Ceratomyxa informis</i>
44	KM273025_ <i>Ceratomyxa appendiculata</i>
45	KM273028_ <i>Ceratomyxa leatherjacketi</i>
46	KM273029_ <i>Ceratomyxa ayami</i>
47	KM273021_ <i>Ceratomyxa longipes</i>
48	KM273027_ <i>Ceratomyxa verudaensis</i>
49	MG266049_ <i>Ceratomyxa sultani</i>
50	MT935658_ <i>Ceratomyxa truncata</i>
51	KM273024_ <i>Ceratomyxa arcuata</i>
52	KJ200360_ <i>Ceratomyxa hamour</i>
53	MG238480_ <i>Ceratomyxa dehoopi</i>
54	MG238496_ <i>Ceratomyxa</i> sp. 4 PBS-2017
55	MG238498_ <i>Ceratomyxa</i> sp. 5 PBS-2017
56	MG238486_ <i>Ceratomyxa</i> sp. 2 PBS-2017
57	AF201375_ <i>Parvicapsula minibicornis</i>

Table A. 5: Sequence data from GenBank, verified on BLAST and with published data, including host, locality, accession numbers (18S SSU) and references for sequences used in the phylogenetic analyses for *Ceratomyxa*. TBP – to be provided

Parasite species	Host (Family)	Locality	Accession no.	Reference
Novel sequences				
<i>Ceratomyxa</i> sp. A	<i>Clinus superciliosus</i>	see Table 4	TBP	Present study
<i>Ceratomyxa</i> sp. B	<i>Clinus superciliosus</i>	see Table 4	TBP	Present study
<i>Ceratomyxa</i> sp. C	<i>Clinus superciliosus</i>	see Table 4	TBP	Present study
Published GenBank sequences				
<i>Ceratomyxa appendiculata</i>	<i>Lophius piscatorius</i>	North Sea, off Scotland	KM273025	Fiala et al. 2015
<i>Ceratomyxa arcuata</i>	<i>Callionymus lyra</i>	North Sea, off Scotland	KM273024	Fiala et al. 2015
<i>Ceratomyxa ayami</i>	<i>Aluterus monoceros</i>	Andaman Sea, off Malaysia	KM273029	Fiala et al. 2015
<i>Ceratomyxa carcharhini</i>	<i>Carcharhinus melanopterus</i>	Australia	JF911815	Gleeson and Adlard 2011
<i>Ceratomyxa cottoidii</i>	<i>Clinus cottoides</i>	Sea View, South Africa	MG238434	Bartošová-Sojková et al. 2018
<i>Ceratomyxa dehoopi</i>	<i>Clinus superciliosus</i>	Kommetjie, South Africa	MG238480	Bartošová-Sojková et al. 2018
<i>Ceratomyxa informis</i>	<i>Merlangius merlangus</i>	North Sea, off Scotland	KM273022	Fiala et al. 2015
<i>Ceratomyxa leatherjacketi</i>	<i>Aluterus monoceros</i>	Andaman Sea, off Malaysia	KM273028	Fiala et al. 2015
<i>Ceratomyxa longipes</i>	<i>Merlangius merlangus</i>	North Sea, off Scotland	KM273021	Fiala et al. 2015
<i>Ceratomyxa melanopteri</i>	<i>Carcharhinus melanopterus</i>	Australia	JF911817	Gleeson and Adlard 2011
<i>Ceratomyxa negaprioni</i>	<i>Negaprion acutidens</i>	Australia	JF911818	Gleeson and Adlard 2011
<i>Ceratomyxa</i> sp. 2 PBS-2017	<i>Clinus superciliosus</i>	Mouille Point, South Africa	MG238486	Bartošová-Sojková et al. 2018
<i>Ceratomyxa</i> sp. 4 PBS-2017	<i>Clinus superciliosus</i>	Kommetjie, South Africa	MG238496	Bartošová-Sojková et al. 2018
<i>Ceratomyxa</i> sp. 5 PBS-2017	<i>Clinus superciliosus</i>	Cape Columbine, South Africa	MG238498	Bartošová-Sojková et al. 2018
<i>Ceratomyxa sultani</i>	<i>Upeneus margarethae</i>	Arabian Gulf	MG266049	Abdel-Baki et al. 2018
<i>Ceratomyxa hamour</i>	<i>Epinephelus coioides</i>	Saudi Arabia	KJ200360	Mansour et al. 2014
<i>Ceratomyxa synaphobranchi</i>	<i>Synaphobranchus kaupii</i>	North Sea	KM273026	Fiala et al. 2015
<i>Ceratomyxa truncata</i>	<i>Sardina pilchardus</i>	Mediterranean Sea in France	MT935658	Mansour et al. 2021
<i>Ceratomyxa verudaensis</i>	<i>Scorpaena porca</i>	Mediterranean Sea, off Croatia	KM273027	Fiala et al. 2015
Outgroup				
<i>Parvicapsula minibicornis</i>	<i>Gasterosteus aculeatus</i>	Oregon, USA	AF201375	Kent et al. 2000

Table A. 6: Sequence data from GenBank, verified on BLAST and with published data, including host, locality, accession numbers (18S SSU) and references for sequences used in the phylogenetic analyses for *Sphaeromyxa*. TBP – to be provided

Parasite species	Host (Family)	Locality	Accession no.	Reference
Novel sequences				
EPD069_ <i>Sphaeromyxa clini</i> (gallbladder)	<i>Clinus superciliosus</i>	Langebaan marina, South Africa	TBP	Present study
EPD087_ <i>Sphaeromyxa clini</i> (gallbladder)	<i>Clinus superciliosus</i>	Cape Town harbour, South Africa	TBP	Present study
EPD032_ <i>Sphaeromyxa clini</i> (gallbladder)	<i>Clinus superciliosus</i>	Chintsa, South Africa	TBP	Present study
Published GenBank sequences				
<i>Sphaeromyxa limocapitis</i>	<i>Gephyroberyx darwinii</i>	Indonesia: Java, Pelabuhan Ratu	KM201343	Bartosova-Sojkova et al. 2015
<i>Sphaeromyxa clini</i>	<i>Clinus superciliosus</i>	Cape Columbine, South Africa	KM201338	Bartosova-Sojkova et al. 2015
<i>Sphaeromyxa clini</i>	<i>Clinus cottoides</i>	Kalk Bay, South Africa	KM201336	Bartosova-Sojkova et al. 2015
<i>Sphaeromyxa clini</i>	<i>Clinus acuminatus</i>	Mouille Point, South Africa	KM201337	Bartosova-Sojkova et al. 2015
<i>Sphaeromyxa clini</i>	<i>Muraenoclinus dorsalis</i>	Mouille Point, South Africa	KM201339	Bartosova-Sojkova et al. 2015
<i>Sphaeromyxa clini</i>	<i>Muraenoclinus dorsalis</i>	Jacobs Bay, South Africa	KM201340	Bartosova-Sojkova et al. 2015
<i>Sphaeromyxa clini</i>	<i>Muraenoclinus dorsalis</i>	Kommetjie, South Africa	KM201341	Bartosova-Sojkova et al. 2015
<i>Sphaeromyxa clini</i>	<i>Muraenoclinus dorsalis</i>	Granger Bay, South Africa	KM201342	Bartosova-Sojkova et al. 2015
<i>Sphaeromyxa longa</i>	<i>Trisopterus minutus</i>	North Sea	KM201344	Bartosova-Sojkova et al. 2015
<i>Sphaeromyxa hellandi</i>	<i>Helicolenus dactylopterus</i>	North Atlantic, Atlantic Ocean	DQ377701	Fiala 2006
<i>Sphaeromyxa hellandi</i>	<i>Melanogrammus aeglefinus</i>	North Sea	DQ377693	Fiala 2006
<i>Sphaeromyxa hellandi</i>	<i>Helicolenus dactylopterus</i>	North Sea	DQ377692	Fiala 2006
<i>Sphaeromyxa zaharoni</i>	<i>Pterois miles</i>	Red Sea	AY538662	Diamant et al. 2004
Outgroup				
<i>Myxidium coryphaenoideum</i>	<i>Coryphaenoides rupestris</i>	Atlantic Ocean: North Atlantic	DQ377697	Fiala 2006

Table A. 7: Sequence data from GenBank, verified on BLAST and with published data, including host, locality, accession numbers (18S SSU) and references for sequences used in the phylogenetic analyses for *Kudoa*. TBP – to be provided

Parasite species	Host (Family)	Locality	Accession no.	Reference
Novel sequences				
EPD071_ <i>Kudoa</i> sp. (urinary bladder wall)	<i>Clinus superciliosus</i>	Langebaan marina, South Africa	TBP	Present study
EPD092_ <i>Kudoa</i> sp. (urinary bladder wall)	<i>Clinus superciliosus</i>	Cape Town harbour, South Africa	TBP	Present study
Published GenBank sequences				
<i>Kudoa thyrsites</i>	<i>Paralichthys olivaceus</i>	Japan	AY382607	Yokoyama et al. 2005
<i>Kudoa thyrsites</i>	<i>Merluccius capensis</i>	South Africa	AY078430	Whipps et al. 2003a
<i>Kudoa thyrsites</i>	<i>Thyrsites atun</i>	South Africa	AY078430	Whipps et al. 2003a
<i>Kudoa thyrsites</i>	<i>Sardinops ocellatus</i>	South Africa	AY542481	Whipps and Kent 2006
<i>Kudoa thyrsites</i>	<i>Coryphaena hippurus</i>	Queensland, Australia	AY152747	Whipps et al. 2003b
<i>Kudoa thyrsites</i>	<i>Scomber scombrus</i>	Engalnd	AY542482	Whipps and Kent 2006
<i>Kudoa thyrsites</i>	<i>Lepidopus caudatus</i>	Azores	AY542482	Whipps and Kent 2006
<i>Kudoa thyrsites</i>	<i>Salmo salar</i>	Chile	AF031412	Hervio et al. 1997
<i>Kudoa thyrsites</i>	<i>Scomber scombrus</i>	Norwegian sea	MH899081	Giulietti et al. 2019
<i>Kudoa guangdongensis</i>	<i>Konosirus punctatus</i>	South China Sea, off Guangdong, Pacific Ocean	LC493825	Li et al. 2020
<i>Kudoa empressemichikoe</i>	<i>Acanthogobius hasta</i>	Fukuoka, Ariake Sea, Japan	LC190926	Kasai et al. 2017
<i>Kudoa whippsi</i>	<i>Ostorhinchus aureus</i>	Ningaloo Reef, Australia	JX090292	Heiniger et al. 2013
<i>Kudoa gunterae</i>	<i>Neoglyphidodon melas</i>	Australia	FJ792712	Burget and Adlard 2010
<i>Kudoa megacapsula</i>	<i>Seriola quinqueradiata</i>	Japan	AB263074	Yokoyama et al. 2006
<i>Kudoa megacapsula</i>	<i>Sphyaena pinguis</i>	Japan	AB188529	Yokoyama et al. 2005
<i>Kudoa minithyrsites</i>	<i>Pempheris ypsilychnus</i>	Great Barrier Reef, Australia	AY152749	Whipps et al. 2003b
<i>Kudoa parathyrsites</i>	<i>Thamnaconus modestus</i>	Inland Sea of Japan, off Shimonoseki, Japan	LC128647	Kasai et al. 2016
Outgroup				
<i>Kudoa lateolabracis</i>	<i>Lateolabrax</i> sp.	Japan	AY382606	Yokoyama et al. 2005

Table A. 8: Morphometric data of *Gyrodactylus* spp. from Clinidae in the present study (South Africa) and Bleniidae from the Mediterranean and Black seas (Dmitrieva et al. 2022)

Morphometric measurements	West coast, South Africa	Black Sea	Mediterranean and Black seas
	<i>Clinus superciliosus</i>	<i>Aidablennius sphynx</i>	<i>Salaria pavo</i> , <i>Salaria basilica</i>
	<i>Gyrodactylus</i> sp.	<i>G. sphinx</i>	<i>G. gerasevi</i>
	n = 2 range (mean) μm	n = 43 range (mean) μm	n = 104 range (mean) μm
Hamulus aperture distance (HA)	10.7–11.1 (10.9)	9.5–14 (11.3 $\pm$ 0.9)	9.5–14 (11.0 $\pm$ 0.8)
Hamulus proximal shaft width (HW)	5.4–7.6 (6.5)	4.5–6.1 (5.2 $\pm$ 0.3)	4.4–6.0 (5.1 $\pm$ 0.3)
Hamulus point length (HPL)	17.4–18.6 (18)	14–18 (15.6 $\pm$ 0.7)	14–17 (15.3 $\pm$ 0.6)
Hamulus shaft length (HShL)	20.4–25.2 (22.8)	23–25 (23.5 $\pm$ 0.6)	20–26 (22.7 $\pm$ 0.8)
Hamulus root length (HRL)	8.5–9.3 (8.9)	6.4–9.6 (8.5 $\pm$ 0.7)	6.8–9.8 (8.8 $\pm$ 0.6)
Hamulus total length (HTL)	31–31.3 (31.2)	28–33 (30.1 $\pm$ 0.9)	27–33 (29.9 $\pm$ 0.9)
Ventral bar total width (VBTW)	16.8–17.2 (17.0)	n = 22 11.0–14.1 (11.8 $\pm$ 0.7)	n = 30 9.15–13.7 (11.5 $\pm$ 1.1)
Ventral bar median length (VBML)	1.3–1.4 (1.3)	n = 22 4.6–6.6 (5.4 $\pm$ 0.5)	n = 33 4.1–6.8 (5 $\pm$ 0.6)

Table A. 9: Sequence data from GenBank, verified on BLAST and with published data, including host, locality, accession numbers (28S) and references for sequences used in the phylogenetic analyses for Tentaculariidae. TBP – to be provided

Parasite species	Stage	Host (Family)	Locality	Accession no.	Reference
Novel sequences					
AE29_Nybelinia sp.	Postlarvae	<i>Clinus superciliosus</i>	Tsitsikamma, South Africa	TBP	Present study
AE30_Nybelinia sp.	Postlarvae	<i>Clinus superciliosus</i>	Tsitsikamma, South Africa	TBP	Present study
Published GenBank sequences					
<i>Heteronybelinia cf estigmena</i>	Larval	<i>Coryphaena hippurus</i>	Indonesia	FJ572931	Palm et al. 2009
<i>Nybelinia sphyrnae</i>	Adult	<i>Sphyrna lewini</i>	Malaysia	DQ642791	Olson et al 2010
<i>Heteronybelinia yamagutii</i>	Larval	<i>Promethichthys prometheus</i>	Indonesia	FJ572932	Palm et al. 2009
<i>Kotorella pronosoma</i>	Adult	<i>Dasyatis say</i>	USA	DQ642788	Olson et al. 2010
<i>Kotorella pronosoma</i>	Larval	<i>Syacium papillosum</i>	Peninsula of Yucatan, Mexico	MK558802	Vidal-Martínez et al. 2019
<i>Nybelinia</i> sp. 4	Larval	<i>Syacium papillosum</i>	Peninsula of Yucatan, Mexico	MK558805	Vidal-Martínez et al. 2019
<i>Nybelinia africana</i>	Adult	<i>Lamiopsis temminckii</i>	Malaysia	DQ642786	Olson et al 2010
<i>Tentaculariidae</i> sp.	Larval	<i>Peltorhamphus novaezeelandiae</i>	Catlins Region, New Zealand	KY909274	Anglade and Randhawa 2017
<i>Nybelinia</i> sp. 1	Larval	<i>Syacium papillosum</i>	Peninsula of Yucatan, Mexico	MK558803	Vidal-Martínez et al. 2019
<i>Nybelinia</i> sp. 3	Larval	<i>Syacium papillosum</i>	Peninsula of Yucatan, Mexico	MK558804	Vidal-Martínez et al 2019
<i>Nybelinia aequidentata</i>	Adult	<i>Rhinoptera neglecta</i>	Malaysia	DQ642790	Olson et al 2010
<i>Nybelinia queenslandensis</i>	Adult	<i>Carcharinus melanopterus</i>	Australia	AF286975	Olson et al 2001
<i>Nybelinia indica</i>	Larval	<i>Heteropriacanthus cruentatus</i>	Hawaii, USA	FJ572930	Palm et al. 2009
<i>Mixonybelinia lepturi</i>	Larval	<i>Gempylus serpens</i>	Indonesia	FJ572933	Palm et al. 2009
<i>Nybelinia africana</i>	Larval	<i>Gempylus serpens</i>	Indonesia	FJ572928	Palm et al. 2009
<i>Nybelinia surmenicola</i>	Larval	<i>Merluccius productus</i>	USA	JN662466	Bryan et al 2012
<i>Nybelinia surmenicola</i>	Larval	<i>Pleurogrammus azonus</i>	Japan	FJ572929	Palm et al. 2009
Outgroup					
<i>Tentacularia coryphaenae</i>	Larval	<i>Lepidocybium flavobrunneum</i>	Indonesia	EF095268	Palm et al. 1997

Table A. 10: Sequence data from GenBank, verified on BLAST and with published data, including host, locality, accession numbers (28S) and references for sequences used in the phylogenetic analyses for Onchoproteocephalidea. TBP – to be provided

Parasite species	Stage	Host (Family)	Locality	Accession no.	Reference
Novel sequences					
AE31_ <i>Acanthobothrium</i> sp.	Plerocercoid	<i>Clinus superciliosus</i>	Chintsa, South Africa	TBP	Present study
AE32_ <i>Acanthobothrium</i> sp.	Plerocercoid	<i>Clinus superciliosus</i>	Chintsa, South Africa	TBP	Present study
AE33_ <i>Acanthobothrium</i> sp.	Plerocercoid	<i>Clinus superciliosus</i>	Chintsa, South Africa	TBP	Present study
AE36_ <i>Acanthobothrium</i> sp.	Plerocercoid	<i>Clinus superciliosus</i>	Chintsa, South Africa	TBP	Present study
Published GenBank sequences					
<i>Acanthobothrium</i> sp. 6A	Adult	<i>Dasyatis say</i>	Northern Gulf of Mexico, USA	GQ470108	Jensen and Bullard 2010
<i>Acanthobothrium</i> sp. 6B	Adult	<i>Dasyatis say</i>	Northern Gulf of Mexico, USA	GQ470110	Jensen and Bullard 2010
<i>Acanthobothrium brevissime</i>	Adult	Stingray	Tampa Bay, Florida, USA	EU170363	Holland et al. 2009
<i>Acanthobothrium brevissime</i>	Larval	<i>Branchiostoma floridae</i>	Tampa Bay, Florida, USA	EU170364	Holland et al. 2009
<i>Acanthobothrium brevissime</i>	Larval	<i>Ensis minor</i>	Tampa Bay, Florida, USA	FJ357445	Holland and Wilson 2009
<i>Acanthobothrium romanowi</i>	Adult	<i>Himantura</i> sp.	Australia	FJ843598	Flyer et al. 2009
<i>Acanthobothrium oceanharvestae</i>	Adult	<i>Himantura</i> sp.	Australia	FJ843594	Flyer et al. 2009
<i>Acanthobothrium</i> sp.	Adult	<i>Himantura</i> sp.	Australia	FJ843592	Flyer et al. 2009
<i>Acanthobothrium masniae</i>	Adult	<i>Himantura</i> sp.	Australia	FJ843604	Flyer et al. 2009
<i>Acanthobothrium zimneri</i>	Adult	<i>Himantura</i> sp.	Australia	FJ843602	Flyer et al 2009
<i>Acanthobothrium</i> sp. 4	Adult	<i>Bathyraxia magellanica</i>	Falkland Islands, Atlantic Ocean	MH688726	Beer et al. 2018
<i>Acanthobothrium</i> sp. 4	Adult	<i>Bathyraxia cousseauae</i>	Falkland Islands, Atlantic Ocean	MH688727	Beer et al. 2018
<i>Acanthobothrium filicollis</i>	Adult	<i>Zearaja nasuta</i>	South Island, New Zealand	MH913266	Bennette et al. 2019
<i>Onchobothrium antarcticum</i>	Larval	<i>Notothenia rossii</i>	King George Island, Antarctica	KF882020	Laskowski and Rocka 2014
<i>Onchobothrium antarcticum</i>	Larval	<i>Notothenia rossii</i>	King George Island, Antarctica	KF882021	Laskowski and Rocka 2014
<i>Acanthobothrium margieae</i>	Adult	<i>Orectolobus japonicus</i>	Taiwan	HQ437683	Flyer 2011
<i>Acanthobothrium margieae</i>	Adult	<i>Orectolobus japonicus</i>	Taiwan	HQ437682	Flyer 2011
<i>Acanthobothrium parviuncinatum</i>	Adult	<i>Urolophus maculatus</i>	Bahia de Los Angeles, Mexico	EF095264	Waeschenbach et al. 2007
<i>Acanthobothrium jeanneae</i>	Adult	<i>Rhynchobatus laevis</i>	Australia	HQ917928	Flyer and Caira 2011
<i>Acanthobothrium wedli</i>	Adult	<i>Zearaja nasuta</i>	South Island, New Zealand	MH913270	Bennett et al 2019
<i>Acanthobothrium santarosaliense</i>	Adult	<i>Heterodontus mexicanus</i>	Santa Rosalia, Mexico	KF685751	Caira et al. 2014
<i>Acanthobothrium</i> sp. 16/64	Plerocercoid	<i>Octopus vulgaris</i>	Central Mediterranean	MN660289	Tedesco et al. 2020
<i>Acanthobothrium</i> sp. 16/04	Plerocercoid	<i>Octopus vulgaris</i>	Central Mediterranean	MN660287	Tedesco et al. 2020
Outgroup					
<i>Litobothrium janovyi</i>	Adult	<i>Alopias superciliosus</i>	Mexico	AF286968	Waeschenbach et al. 2007

Table A. 11: Sequence data from GenBank, verified on BLAST and with published data, including host, locality, accession numbers (COI mtDNA) and references for sequences used in the phylogenetic analyses for Piscicolidae. TBP – to be provided

Parasite taxa	Host (Family)	Locality	Accession no.	Reference
Novel sequences				
AE37_ <i>Pterobdella arugamensis</i> (anterior)	<i>Clinus superciliosus</i>	Chintsa, South Africa	TBP	Present study
AE38_ <i>Pterobdella arugamensis</i> (posterior)	<i>Clinus superciliosus</i>	Chintsa, South Africa	TBP	Present study
AE39_ Piscicolidae gen. sp. (anterior)	<i>Clinus superciliosus</i>	Langebaan marina, South Africa	TBP	Present study
AE40_ Piscicolidae gen. sp. (posterior)	<i>Clinus superciliosus</i>	Langebaan marina, South Africa	TBP	Present study
Published sequences				
<i>Calliobdella vivida</i>	-	Gloucester Point, Virginia	AF003260	Siddall and Bureson (1998)
<i>Branchellion torpedinis</i>	<i>Raja eglanteria</i>	Kiptopeke, Virginia	AF003265	Siddall and Bureson (1998)
<i>Johanssonia arctica</i>	<i>Paralithodes camtschaticus</i>	Varangerfjord, Norway	AY336012	Utevsky et al. (2007)
<i>Piscicola geometra</i>	-	Siverskyi Donets River, Ukraine	AY336015	Utevsky et al. (2007)
<i>Limnotrachelobdella okae</i>	<i>Huso dauricus</i>	Nevelskoy Strait, Russia	AY336022	Utevsky et al. (2007)
<i>Notostomum cyclostomum</i>	<i>Pandalus borealis</i>	Tatar Strait, Russia	AY336028	Utevsky et al. (2007)
<i>Pontobdella muricata</i>	-	Gulf of Piran, Slovenia	AY336029	Utevsky et al. (2007)
<i>Pterobdella (Aestabdella) abditovesiculata</i>	-	Hawaii, USA	DQ414300	(Williams and Bureson 2006) Utevsky et al. 2021
<i>Austrobdella bilobata</i>	-	Tasmania, Australia	DQ414301	Williams and Bureson 2006
<i>Austrobdella californiana</i>	<i>Pleuronichthys verticalis</i>	California, USA	DQ414303	Williams and Bureson 2006
<i>Pterobdella leisotomi</i>	-	Virginia, USA	DQ414305	Williams and Bureson 2006
<i>Austrobdella translucens</i>	-	South Australia, Australia	DQ414306	Williams and Bureson 2006
<i>Branchellion parkeri</i>	-	Tasmania, Australia	DQ414308	Williams and Bureson 2006
<i>Branchellion ravenelii</i>	-	Gulf of Mexico, USA	DQ414309	Williams and Bureson 2006
<i>Calliobdella lophii</i>	-	Hordaland, Norway	DQ414314	Utevsky et al. (2007)
<i>Oceanobdella sexoculata</i>	-	New Brunswick, Canada	DQ414332	Williams and Bureson 2006
<i>Oxytonostoma typica</i>	-	New Brunswick, Canada	DQ414333	Williams and Bureson 2006
<i>Pterobdella amara</i>	-	Queensland, Australia	DQ414334	Williams and Bureson 2006
<i>Piscicola milneri</i>	-	Quebec, Canada	DQ414337	Williams and Bureson 2006
<i>Pontobdella (Stibarobdella) macrothela</i>	-	Virginia, USA	DQ414342	(Williams and Bureson 2006) Utevsky et al. 2021
<i>Pterobdella (Zeylanicobdella) arugamensis</i>	-	Borneo, Indian Ocean	DQ414344	Williams and Bureson 2006 Utevsky et al. 2021
<i>Pterobdella (Zeylanicobdella) arugamensis</i>	<i>Epinephelus</i> sp.	Buleleng Bali, Indonesia	MH299847	Murwantoko et al. 2018
<i>Johanssonia extrema</i>	<i>Coryphaenoides acrolepis</i>	Kuril-Kamchatka Trench, Pacific Ocean	MK967944	Utevsky et al. (2019)
<i>Pterobdellina vernadskyi</i>	<i>Dissostichus mawsoni</i>	Antarctica	MT871866	Utevsky et al. 2021
Outgroup				
<i>Ozobranchus margo</i>	Marine turtles	Virginia	AF003268	Apakupakul et al. (1999); Light and Siddall (1999)

Table A. 12: Sequence data from GenBank, verified on BLAST and with published data, including host, locality, accession numbers (COI mtDNA) and references for sequences used in the phylogenetic analyses for Caligidae. TBP – to be provided

Parasite species	Host (Family)	Locality	Accession no.	Reference
Novel sequences				
Cm01_ <i>Caligus</i> sp.	<i>Clinus superciliosus</i>	Chintsa, South Africa	TBP	Present study
Cm02_ <i>Caligus mortis</i>	<i>Clinus superciliosus</i>	Langebaan marina, South Africa	TBP	Present study
Published GenBank sequences				
<i>Caligus dakari</i>	<i>Argyrosomus japonicus</i>	Witsand, South Africa	MW911362	Hayes et al. 2021
<i>Caligus furcisetifer</i>	<i>Carcharias taurus</i>	Jeffreys Bay, South Africa	MW911361	Hayes et al. 2021
<i>Caligus lalandei</i>	<i>Seriola lalandi</i>	Struisbaai, South Africa	MW911365	Hayes et al. 2021
<i>Caligus tetrodontis</i>	<i>Amblyrhynchotes honckenii</i>	Tsitsikamma, South Africa	MW911366	Hayes et al. 2021
<i>Caligus gurnardi</i>	Salmonids	Arendal, Norway	AY861369	Oines and Heuch 2005
<i>Caligus elongatus</i>	Salmonids	Arendal, Norway	AY386273	Oines and Heuch 2005
<i>Caligus elongatus</i>	Salmonids	Arendal, Norway	AY386272	Oines and Heuch 2005
<i>Caligus belones</i>	Salmonids	Arendal, Norway	AY861368	Oines and Heuch 2005
<i>Caligus uniartus</i>	<i>Siganus guttatus</i>	Barru Regency, South Sulawesi	KC569367	Freeman et al. 2013
<i>Caligus rogercresseyi</i>	Salmonids	Chile	HQ157565	Yasuike et al. 2012
<i>Caligus curtus</i>	Salmonids	Arendal, Norway	AY861366	Oines and Heuch 2005
<i>Caligus pelamydis</i>	Salmonids	Arendal, Norway	AY861367	Oines and Heuch 2005
<i>Caligus centrodoni</i>	<i>Labrus bergylta</i>	Arendal, Norway	AY861370	Oines and Heuch 2005
<i>Caligus quadratus</i>	<i>Neothynnus macropterus</i>	Chile	EF065619	Oines and Schram 2008
<i>Caligus clemensi</i>	Salmonids	British Columbia, Canada	HQ157566	Yasuike et al. 2012
Outgroup				
<i>Cyclops insignis</i>	-	Barskiy Pond, Russia	MK329341	Hołyńska and Wyngaard 2019

Appendix B – Chapter 4

Table B. 1: Terms used for morphological features in the present study, as well as synonyms of the terms used in other descriptions, with their respective definitions

Term used in DELTA	Synonyms (descriptions of other species)	Definition
aliform expansions	lateral swellings; aliform projections; medial protuberance	Located at the base of the cephalothorax
antenna	second antenna	The second cephalic appendage. [Boxshall and Halsey, 2004]
antennule	first antenna	The first cephalic appendage. [Boxshall and Halsey, 2004]
anteriorly		Front end of organism "head end." [Hobbs and Jass, 1988]
anterolateral		Front side of a structure. [Butler, T. H.]
apical		Apex; tip. [Hobbs and Jass, 1988]
appendage	attachment	Paired extensions of a somite along a proximodistal axis and usually composed of serially repeated elements. [Ferrari and Dahms, in press]
armature	elements	The setae and spines carried on the paired limbs and caudal rami. [Boxshall and Halsey, 2004]
article		One in a series of up to 26 segments of flagellum of antennule (few in antenna). Number of articles is of systematic importance. May also be used to refer to segments of other appendages. (syn. segment, joint) [Stachowitsch, 1992]
auxiliary		Supplementary or additional support
barb		A sharp projection near the end of appendage, which is angled away from the main point so as to make extraction difficult.
basal		Segment closest to the body of the organism
basis		The distal segment of the protopod of postantennular appendages, bearing the rami. [Boxshall and Halsey, 2004]
bifid		Split in two, as in spines. [Butler, T. H.]
biramous		Two-branched, used for limbs possessing both endopod and exopod. [Boxshall and Halsey, 2004]
buccal apparatus	anterior end of cephalothorax; terminal head	Head region containing mouth parts and appendages
buccal cavity	mouth cone; mouth tube siphon; oral cone	In parasitic copepod, term applied to space formed around mouth by prolonged upper lip (labrum) and lower lip (labium). (See also mouth cone). [Stachowitsch, 1992]
bulbous		Fat, round, or bulging. [Oxford Languages]
bulla	attachment organ; anchor	A small, chitinous anchor-like structure fused to the tips of the maxillary arms in lernaepodid copepods, that is embedded in the surface of the host fish to serve as an anchor. [Boxshall and Halsey, 2004]
caudal rami		Pair of appendage-like or spine-like processes borne on anal somite in primitive crustaceans. [Brusca and Brusca, 2002]
cephalic		Pertaining to the head [Hobbs and Jass, 1988]
cephalothorax		Anterior region of body including head (cephalon) and thoracic somites (thoracomeres) fused to it. Cephalosome and metasome together form prosome. (See also cephalothorax). [Stachowitsch, 1992]
chela	claw	A claw, or the claw-like terminus of a cheliped. [Bliss, 1982]
clavate		Club shaped; having one end thickened. [Kensley and Schotte, 1989]
corpus		The massive proximal part of the subchelate maxilliped in some parasitic copepods, corresponding either to the basis or fused basis plus syncoxa. [Boxshall and Halsey, 2004]

Table B. 1 (continued)

Term used in DELTA	Synonyms (descriptions of other species)	Definition
coxa		The middle segment of the 3-segmented protopod of postmandibular limbs, the proximal segment of the 2-segmented protopod of the antenna and mandible. [Boxshall and Halsey, 2004]
denticles	small teeth	A solid extension of the epicuticle of a segment. [Ferrari and Dahms, in press]
digitiform		Fingerlike. [Kensley and Schotte, 1989]
distal		Away from body mid-line; toward apex (tips) of appendages. [Hobbs and Jass, 1988]
distinct		Prominent
dorsal		Top, back or upper surface
dorsal shield	cephalic shield; carapace; head shield	Dorsal cuticular shield covering head and thoracic somites (thoracomeres) fused to it. May project anteriorly to form rostrum, posteriorly to form pair of alae. (convex, flat, regionated). (syn. head shield, dorsal shield) [Stachowitsch, 1992]
egg-sacs		A batch of one or more eggs contained within a sac-like membrane that remains attached to the genital opening(s); typically paired. [Boxshall and Halsey, 2004]
elements	setae; spines; tubercles	Located at the end of an attachment
endite	inner lobe; labium	Inwardly (medially) directed lobe of proximal part (protopod) of maxillule. Used to clean filtering apparatus in suspension-feeding copepod. (See also exite). [Stachowitsch, 1992]
endopod		The inner ramus of a biramous appendage. [Boxshall and Halsey, 2004]
excretory duct		In antennal gland, short duct leading from bladder to excretory pore on basal segment (coxa) of antenna. See: Excretory tubule [Stachowitsch, 1992]
exopod		The outer ramus of a biramous appendage. [Boxshall and Halsey, 2004]
genital process	genital tubercle; oviduct orifice	
globular		globe like
habitus		Appearance of the whole animal. [Wilson, 1989]
holotype		The single specimen used as the basis of the original published description of a species and later designated as the type specimen. [AHD]
indistinct		Not prominently visible
labium		The lower lip; a median lobe derived from the fusion of the paragnaths, forming the posterior wall of the oral cone in siphonostomatoids. [Boxshall and Halsey, 2004]
labrum	mouth cone	The upper lip; a posteroventrally-directed, muscular, median lobe forming the anterior wall of the oral cone. [Boxshall and Halsey, 2004]
lamina(e)		Blade or expanded part of scale or appendage segment
lateral	distal	Away from the middle, on the side (opposite medial)
mandible		The paired limb of the somite of the cephalon posterior to antenna 2. The third cephalic appendage. [Boxshall and Halsey, 2004]
manubrium		Stem connecting the bulla and the collar
maxilla	second maxilla	The fifth and last pair of cephalic appendages. [Boxshall and Halsey, 2004]
maxilliped		The first pair of thoracic appendages located on the last somite of cephalosome. [Boxshall and Halsey, 2004]
maxillula	first maxilla	The fourth cephalic appendage. [Boxshall and Halsey, 2004]
medial		Middle (opposite lateral)
mesial		Referring to inner margins or surfaces, closer to midline than sides. [Butler, T. H.]
multiserated		Used of egg sacs containing two or more rows of eggs. [Boxshall and Halsey, 2004]

Table B. 1 (continued)

Term used in DELTA	Synonyms (descriptions of other species)	Definition
myxa	myxal	The medial surface of the corpus of the maxilliped of siphonostomatoids, sometimes produced into a short (myxal) process. [Boxshall and Halsey, 2004]
non-ovigerous		Not bearing developing embryos; Pertaining to the condition of, or a female, not carrying external fertilized eggs. [Butler, T. H.]
orifice	opening; aperture	(posteroventral) opening into cirriped mantle cavity
oviduct		In female: passageway from ovary to uterus and leading to genital aperture. [Moore and McCormick, 1969]
ovigerous		Bearing developing embryos. [Wilson, 1989] Pertaining to the condition of, or a female, carrying external fertilized eggs. [Butler, T. H.]
palp	outer lobe; labium	The basis plus rami of a limb. The palp may be reduced to a poorly-sclerotized extension of the exoskeleton bearing a crown of small setae corresponding to the terminal setae of one or both rami. [Ferrari and Dahms, in press]
papillae		Tube- or finger-shaped projection from inner surface of each ocular peduncle. (syn. ocular papilla) [Stachowitsch, 1992]
peduncle		A stalk-like structure. [AHD]
posterior		of, or pertaining to the rear end. [Butler, T. H.]
posterior process		Posterior part of an elongate median protuberance projecting caudally onto the last (XIV) thoracic sternite. [Perez Farfante and Kensley, 1997]
posterior protuberance		Conspicuous elevation arising from the posteromedian part of the last (XIV) thoracic sternite (Fig. 4A). [Perez Farfante and Kensley, 1997]
posterolaterally		relatively small region along each posterolateral margin of carapace. See: Anterolateral region [Stachowitsch, 1992]
posteroventral		On rear lower side of structure. [Butler, T. H.]
protuberance		outgrowth
proximally		Toward or near a point of attachment. [Butler, T. H.]
proximodistal		An imaginary line through the insertion of a limb on its somite, and the tip of the limb; on biramous limbs, the proximodistal axis is duplicated through each ramus. [Ferrari and Dahms, in press]
ramus		A branch of an appendage; the exopod or endopod. [Boxshall and Halsey, 2004]
reflexed dorsally	reflexed along dorsal surface of the trunk; deflect dorsally forming acute angle with long axis of trunk	Reflexing along the dorsal surface
sclerotized	calcified	With thick and sometimes calcified cuticle. [Wilson, 1989]
serrated		Having a row of short, tooth-like denticles. [Wilson, 1989]
seta		Slender, often plumose, armature element on paired appendages or caudal rami, with internal tissue connection through integument. [Boxshall and Halsey, 2004]
sigmoid		S-shaped
spine		Stout, often thickened and rigid, armature element on paired appendages or caudal rami, with internal tissue connection through integument. [Boxshall and Halsey, 2004]
spiniiform		Shaft very stout; very broadly basally; surface smooth and unarmed; pore may be present. [Ingle, 1992]
subchela		The terminal, movable part of an appendage, which adducts to oppose the surface of the subterminal segment. [Boxshall and Halsey, 2004]
sympod		A compound segment formed by fusion or failure to separate of coxa and basis. [Boxshall and Halsey, 2004]
tapered		wider at base than at tip, decreasing in diameter towards tip

Table B. 1 (continued)

Term used in DELTA	Synonyms (descriptions of other species)	Definition
terminal		At the end. [Butler, T. H.]
trunk		The postcephalic region of the body. [Boxshall and Halsey, 2004]
tubercle		Knoblike process; tip usually blunt. [Butler, T. H.]
uniramous		One-branched; used of limbs possessing only one ramus (either endopod or exopod). [Boxshall and Halsey, 2004]
uniserated		Used for egg sacs containing only a single row of eggs, often disc-like in shape. [Boxshall and Halsey, 2004]
ventral		bottom, front or lower surface

Table B. 2: Sequence data from GenBank, verified on BLAST and with published data, including host, various accession numbers (number of base pairs) and references for sequences used in the phylogenetic analyses for each gene.

Species	Host family	Locality	18S	28S	COI	Reference
Novel sequences						
<i>Allela</i> sp. A	<i>Clinus superciliosus</i>	Langebaan marina, Saldanha Bay	TBP	TBP	TBP	Present study
<i>Allela</i> sp. A	<i>Clinus superciliosus</i>	Langebaan marina, Saldanha Bay	TBP	TBP	TBP	Present study
<i>Allela</i> sp. A	<i>Clinus superciliosus</i>	Langebaan marina, Saldanha Bay	TBP	TBP	TBP	Present study
Published GenBank sequences						
<i>Clavella addunca</i> [sic]	<i>Melanogrammus aeglefinus</i>	Fair Isle, Scotland	AY627028 (1800 bp)	-	-	Huys et al. 2006
<i>Clavella perfida</i>	<i>Gadus chalcogrammus</i>	Hokkaido, Japan	-	-	LC179743 (683 bp)	Yanagimoto and Ichimura 2018
<i>Clavellisa emarginata</i>	<i>Alosa fallax</i>	River Minho, Iberia	KR349122 (790 bp)	-	-	Bao et al. 2015
<i>Parabrachiella exilis</i>	<i>Mugil cephalus</i>	Buenos Aires province, Argentina	-	-	KY026073 (681 bp) KY026074 (699 bp)	Montes et al. 2017
<i>Parabrachiella kabatai</i>	<i>Isacia conceptionis</i>	Buenos Aires province, Argentina	-	-	KY026076 (666 bp) KY026078 (627 bp)	Montes et al. 2017
<i>Parabrachiella platensis</i>	<i>Mugil liza</i>	Buenos Aires province, Argentina	-	-	KY026080 (666 bp) KY026085 (666 bp)	Montes et al. 2017
<i>Parabrachiella</i> sp	Haemulidae	Buenos Aires province, Argentina	-	-	KY026079 (666 bp)	Montes et al. 2017
<i>Parabrachiella baponosa</i>	<i>Chelidonichthys gurnardus</i>	Beatrice, Scotland	AY627027 (1818 bp)	-	-	Huys et al. 2006

Table B. 2 (continued)

Species	Host family	Locality	18S	28S	COI	Reference
Published GenBank sequences						
<i>Salmincola californiensis</i>	<i>Oncorhynchus mykiss</i>	Watauga River Watershed, North Carolina	-	KY113083 (914 bp)	-	Ruiz et al. 2017
<i>Salmincola edwardsii</i>	<i>Salvelinus fontinalis</i>	Watauga River Watershed, North Carolina	-	KY113081 (928 bp)	-	Ruiz et al. 2017
Sequences not included in alignments						
<i>Clavella adunca</i>	-	-	MF077734	MF077820	-	Khodami et al. 2017 ^a
<i>Parabrachiella merlucii</i>	-	-	MF077733	-	-	Khodami et al. 2017 ^a
<i>Clavellopsi</i> sp.	-	-	HM545893	-	-	Wang et al. 2010 ^b
<i>Clavellotis dilatata</i>	-	-	-	-	KX815899 KX815900	Castro-Romero et al. 2018 ^b
<i>Parabrachiella anisotremi</i>	-	-	-	-	KX815889 KX815890	Castro-Romero et al. 2018 ^b
<i>Parabrachiella auriculata</i>	-	-	-	-	KX815906 KX815907	Castro-Romero et al. 2018 ^b
Lernaeopodidae gen. 1 sp. 1	-	-	-	-	KX815903	Castro-Romero et al. 2018 ^b
Lernaeopodidae gen. 2 sp. 1	-	-	-	-	KX815891	Castro-Romero et al. 2018 ^b
Lernaeopodidae gen. 2 sp. 3	-	-	-	-	KX815905	Castro-Romero et al. 2018 ^b
<i>Parabrachiella hugu</i>	-	-	-	-	KT030285	Baek and Hwang 2015 ^b
Outgroups						
<i>Ergasilus yaluzangbus</i>	<i>Gymnocypris stewartii</i>	Lasa River, Tibet	DQ107578 (1458 bp)	DQ107540 (696 bp)	-	Song et al. 2008
<i>Ergasilus</i> sp	<i>Mugil liza</i>	Samborombon Bay, Argentina	-	-	KU557411 (678 bp)	Castro-Romero et al. 2016

[sic] seen in context

^a Retracted publication^b Unpublished sequences

Appendix C – Chapter 5

Table C. 1: Results from the SIMPER analysis, indicating the average dissimilarity between localities

	Langebaan marina	Cape Town harbour	Tsitsikamma
Langebaan marina			
Cape Town harbour	91.1%		
Tsitsikamma	97.3%	100%	
Chintsa	96.9%	100%	77%

Table C. 2: Results from the SIMPER analysis showing the mean similarity for each locality, as well as the mean abundance (MA), mean similarity $\pm$ SD, contribution (%) and cumulative contribution (%) for the most abundant taxa per locality

Taxa per locality	MA	Mean similarity	Contribution %	Cumulative %
Langebaan marina		Mean similarity = 71.8%		
<i>Dollfustrema</i> sp.	9.42	66.02 $\pm$ 4.49	91.92	91.92
Cape Town harbour		Mean similarity = 100%		
<i>Gyrodactylus</i> sp.	10	100 $\pm$ 0	100	100
Tsitsikamma		Mean similarity = 38.5%		
<i>Gnathia africana</i>	4.03	12.3 $\pm$ 0.87	31.84	31.84
Hemiuridae gen sp. 1	3.32	8.41 $\pm$ 0.69	21.84	53.68
<i>Coitocaecum capense</i>	2.82	5.66 $\pm$ 0.55	14.7	68.38
Polymorphidae sp.	2.58	5.6 $\pm$ 0.59	14.54	82.91
<i>Helicometra</i> sp. 1	2.17	4.43 $\pm$ 0.6	11.26	94.18
Chintsa		Mean similarity = 37.4%		
<i>Acanthobothrium</i> sp.	5.42	18.2 $\pm$ 0.98	48.76	48.76
<i>Gnathia africana</i>	2.04	4.72 $\pm$ 0.68	12.63	61.39
<i>Helicometra</i> sp. 1	2.5	4.36 $\pm$ 0.47	11.68	73.07
Polymorphidae sp.	1.81	3.25 $\pm$ 0.56	8.7	81.76
Hemiuridae gen. sp. 1	1.53	2.79 $\pm$ 0.47	7.48	89.24
<i>Nybelinia</i> sp.	1.52	2.05 $\pm$ 0.38	5.5	94.74

Table C. 3: Results from the ANOSIM analysis indicating the R Statistic matrix for each locality. $R > 0.75$ (bold values) indicates a distinct community assemblage while $R < 0.25$ indicates high similarity between localities

	Langebaan marina		Cape Town harbour		Tsitsikamma	
	R statistic	Sign. Level (%)	R statistic	Sign. Level (%)	R statistic	Sign. Level (%)
Langebaan marina						
Cape Town harbour	0.999	0.1				
Tsitsikamma	0.889	0.1	0.862	0.1		
Chintsa	0.926	0.1	0.927	0.1	0.411	0.1

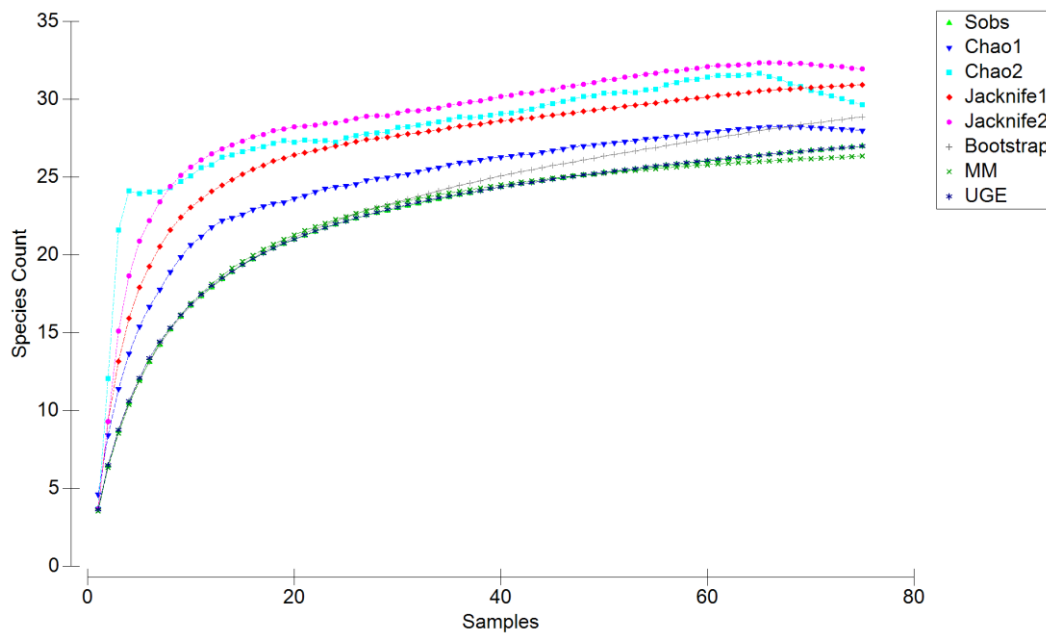


Figure C. 1: Species accumulation curve used to estimate the expected diversity from 75 host individuals

Sobs - Curve of observed species counts.

Chao 1 - Chao's estimator based on number of rare species.

Chao 2 - Chao's estimator using just presence-absence data.

Jackknife 1 - Jackknife estimator based on species that only occur in one sample.

Jackknife 2 - Second order jackknife estimator

Bootstrap - Bootstrap estimator based on proportion of quadrats containing each species.

Michaelis-Menton - Curve fitted to observed S curve. Curve parameters are sent to results.

S(n) = $S_{max} - BS(n)/n$

UGE - Calculated species accumulation curve (Ugland, Gray and Ellingsen (2003). The species-accumulation curve and estimation of species richness. Journal of Animal Ecology, 72, 888-897).

Appendix D – Chapter 6

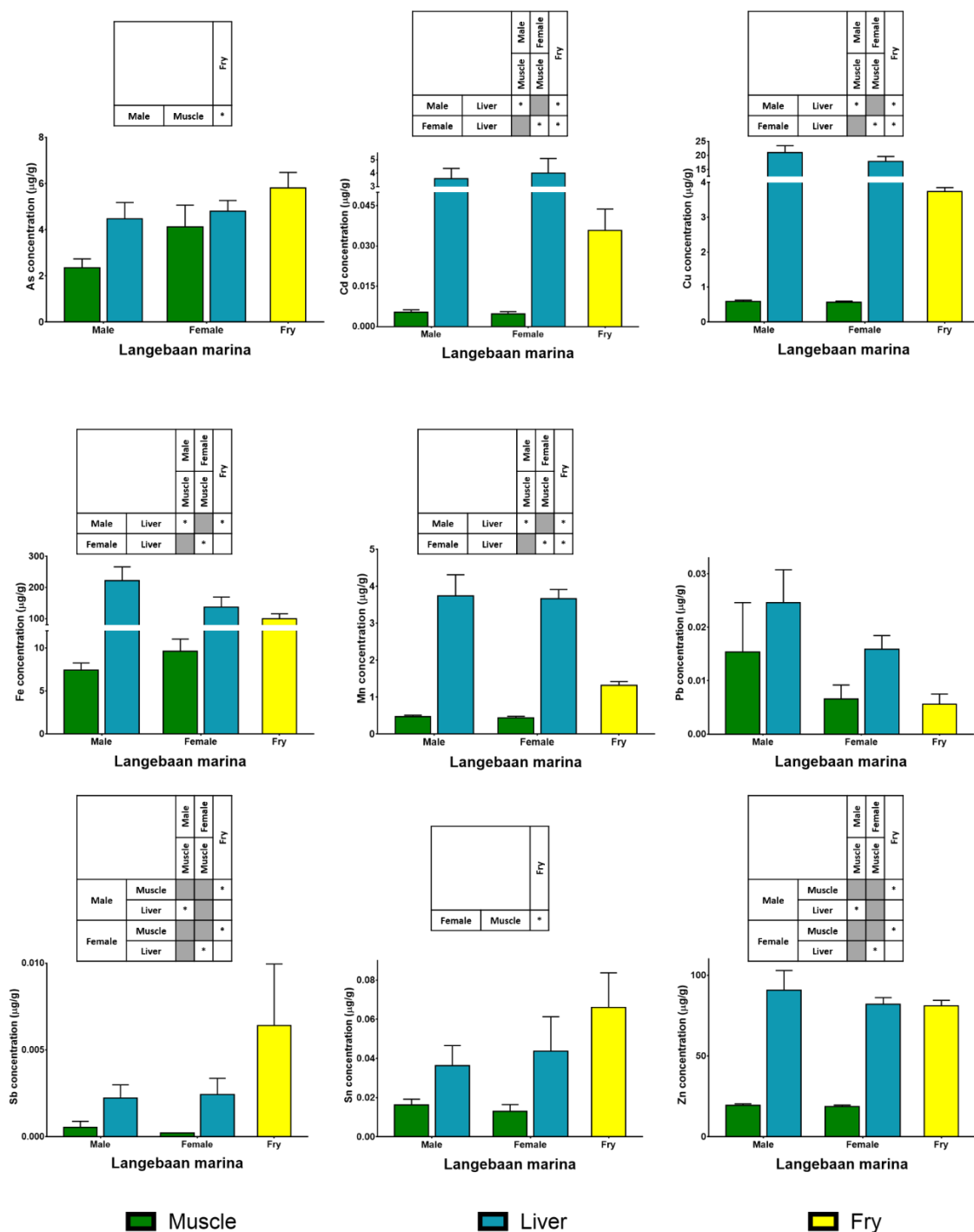


Figure D. 1: Mean $\pm$ standard error of the mean concentrations (µg/g dry weight) of muscle and liver of male, female and fry of *Clinus superciliosus*, collected from Langebaan marina. Significant differences ($p < 0.05$) are indicated in the matrix table as *.

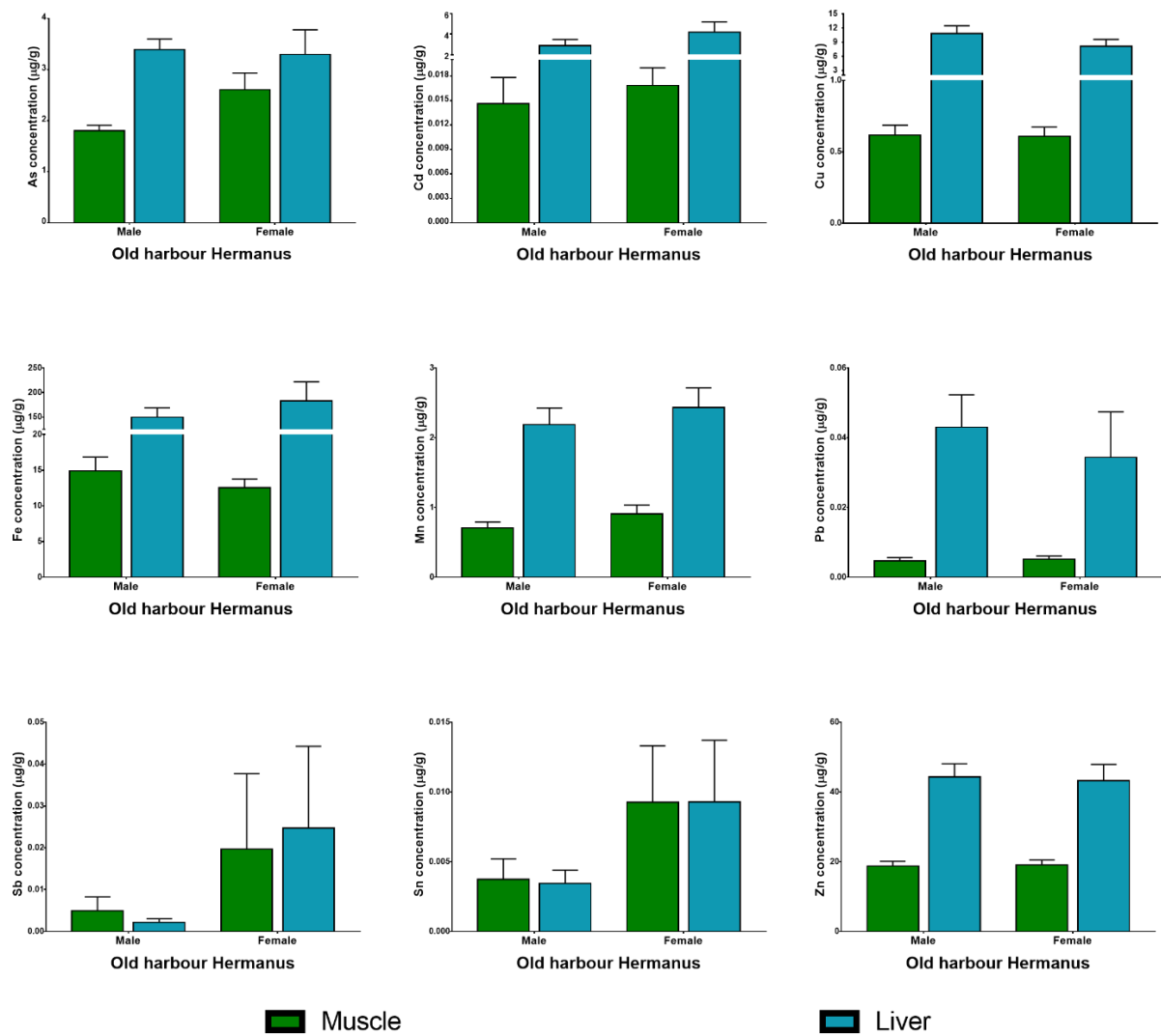


Figure D. 2: Mean $\pm$ standard error of the mean concentrations (µg/g dry weight) of muscle and liver of male and female *Clinus supercilius*, collected from Old harbour Hermanus. No significant differences were detected between sexes.

Table D. 1: Correlation analysis for metal and metalloid concentrations related to fish size calculated for each element at each locality, as well as the overall correlation of all the localities. Data were plotted on a log-transformed plot where significance ($p < 0.05$) is indicated as *. Slope values > 0.7 , and < -0.7 are highlighted, indicating increased and decreased concentrations, respectively, with increase in size.

			As	Cd	Cu	Fe	Mn	Pb	Sb	Sn	Zn
Langebaan marina	Muscle	r ²	0.272	0.031	0.258	0.00003	0.696	0.022	0.005	0.259	0.145
		F	6.37	0.539	5.9	0.0006	38.9	0.379	0.081	5.96	2.87
		Slope	0.376 ± 0.149	0.0008 ± 0.001	-0.054 ± 0.022	-0.004 ± 0.155	-0.116 ± 0.019	-0.006 ± 0.009	0.097 ± 0.0003	0.008 ± 0.003	-0.087 ± 0.051
		p	0.022*	0.473	0.027*	0.981	< 0.0001*	0.546	0.779	0.026*	0.109*
	Liver	r ²	0.004	0.169	0.005	0.004	0.016	0.135	0.007	0.252	0.017
		F	0.064	3.44	0.091	0.065	0.283	2.65	0.121	5.73	0.285
		Slope	0.034 ± 0.136	0.367 ± 0.198	-0.046 ± 0.154	0.073 ± 0.287	-0.068 ± 0.128	-0.009 ± 0.006	-0.0003 ± 0.0009	0.031 ± 0.013	-0.078 ± 0.145
		p	0.803	0.081	0.767	0.802	0.602	0.122	0.732	0.029	0.600
Cape Town harbour	Muscle	r ²	0.578	0.231	0.116	0.024	0.00007	0.012	0.167	0.016	0.027
		F	19.2	4.21	1.83	0.347	0.0009	0.167	2.81	0.228	0.392
		Slope	0.268 ± 0.061	-0.008 ± 0.004	-0.046 ± 0.034	0.164 ± 0.279	0.003 ± 0.11	-0.002 ± 0.006	-0.0002 ± 0.094	0.010 ± 0.022	-0.050 ± 0.080
		p	0.0006*	0.060	0.197	0.566	0.976	0.689	0.116	0.641	0.542
	Liver	r ²	0.002	0.206	0.358	0.047	0.121	0.005	0.347	0.213	0.176
		F	0.025	3.11	6.68	0.590	1.65	0.064	6.37	3.24	2.56
		Slope	0.033 ± 0.208	-0.629 ± 0.357	-1.23 ± 0.476	-0.296 ± 0.385	-0.299 ± 0.233	0.004 ± 0.015	0.023 ± 0.009	-0.332 ± 0.184	-0.451 ± 0.282
		p	0.877	0.103	0.024*	0.458	0.224	0.804	0.027*	0.097	0.135
Simons Town marina	Muscle	r ²	0.464	0.091	0.052	0.0006	0.157	0.003	0.0096	0.047	0.100
		F	8.64	1.001	0.553	0.006	1.87	0.026	0.097	0.488	1.11
		Slope	-0.53 ± 0.18	-0.0012 ± 0.0012	-0.067 ± 0.091	0.012 ± 0.151	-0.057 ± 0.041	0.002 ± 0.015	-0.004 ± 0.013	0.067 ± 0.096	-0.163 ± 0.154
		p	0.015*	0.341	0.474	0.94	0.202	0.875	0.762	0.501	0.316
	Liver	r ²	0.180	0.239	0.00001	0.257	0.484	0.143	0.217	0.544	0.156
		F	2.19	3.14	0.0001	3.45	9.37	1.67	2.76	11.9	1.84
		Slope	0.423 ± 0.286	0.816 ± 0.461	-0.007 ± 0.608	1.18 ± 0.633	0.879 ± 0.287	0.35 ± 0.271	0.005 ± 0.003	0.782 ± 0.226	0.62 ± 0.457
		p	0.170	0.107	0.991	0.093	0.012*	0.225	0.128	0.006*	0.205

Table D. 1: (continued)

		As	Cd	Cu	Fe	Mn	Pb	Sb	Sn	Zn	
Old harbour Hermanus	Muscle	r ²	0.157	0.066	0.0001	0.043	0.238	0.031	0.0000001	0.031	0.051
		F	4.11	1.56	0.003	0.989	6.88	0.694	0.0000002	0.697	1.18
		Slope	-0.268 ± 0.132	0.007 ± 0.006	-0.005 ± 0.086	-0.214 ± 0.215	-0.262 ± 0.1	-0.002 ± 0.002	0.031 ± 0.021	-0.005 ± 0.006	0.146 ± 0.134
		p	0.055	0.225	0.958	0.331	0.016*	0.414	0.999	0.413	0.290
	Liver	r ²	0.004	0.132	0.161	0.112	0.0004	0.004	0.005	0.015	0.009
		F	0.078	3.33	4.24	2.77	0.008	0.094	0.118	0.331	0.202
		Slope	0.038 ± 0.134	0.555 ± 0.304	0.558 ± 0.271	0.493 ± 0.296	0.013 ± 0.150	0.006 ± 0.021	0.008 ± 0.022	0.003 ± 0.006	0.08 ± 0.178
		p	0.782	0.082	0.052	0.110	0.929	0.762	0.735	0.571	0.657
Tsitsikamma	Muscle	r ²	0.019	0.107	0.008	0.267	0.049	0.046	0.124	0.255	0.048
		F	0.156	0.955	0.065	2.92	0.411	0.383	1.13	2.74	0.402
		Slope	-0.13 ± 0.329	-0.316 ± 0.324	-0.053 ± 0.209	-0.663 ± 0.388	-0.144 ± 0.225	0.037 ± 0.06	-0.006 ± 0.005	-0.024 ± 0.015	0.201 ± 0.317
		p	0.703	0.357	0.805	0.126	0.54	0.553	0.319	0.136	0.544
	Liver	r ²	0.068	0.106	0.044	0.260	0.355	0.0003	0.089	0.211	0.161
		F	0.587	0.953	0.366	2.81	4.39	0.002	0.779	2.14	1.53
		Slope	-0.356 ± 0.465	-0.664 ± 0.681	-0.391 ± 0.646	-0.945 ± 0.563	-0.746 ± 0.356	-0.011 ± 0.233	-0.013 ± 0.015	-0.06 ± 0.041	-0.647 ± 0.523
		p	0.466	0.358	0.562	0.132	0.069	0.964	0.403	0.182	0.251
Chintsa	Muscle	r ²	0.113	0.041	0.121	0.001	0.216	0.0001	0.001	0.010	0.037
		F	1.02	0.344	1.1	0.011	2.2	0.0007	0.010	0.077	0.308
		Slope	0.178 ± 0.176	0.003 ± 0.004	0.048 ± 0.046	-0.009 ± 0.089	-0.07 ± 0.047	-0.0004 ± 0.014	0.0003 ± 0.003	-0.002 ± 0.007	0.066 ± 0.118
		p	0.341	0.574	0.325	0.919	0.176	0.979	0.923	0.788	0.594
	Liver	r ²	0.394	0.335	0.357	0.404	0.680	0.125	0.061	0.476	0.515
		F	4.55	3.53	3.88	4.74	14.9	0.999	0.457	6.35	7.42
		Slope	-0.728 ± 0.342	-0.757 ± 0.403	-0.664 ± 0.337	-0.646 ± 0.297	-0.867 ± 0.225	-0.213 ± 0.213	0.001 ± 0.002	-0.279 ± 0.111	-0.844 ± 0.31
		p	0.071	0.103	0.090	0.066	0.006*	0.351	0.521	0.040*	0.030*

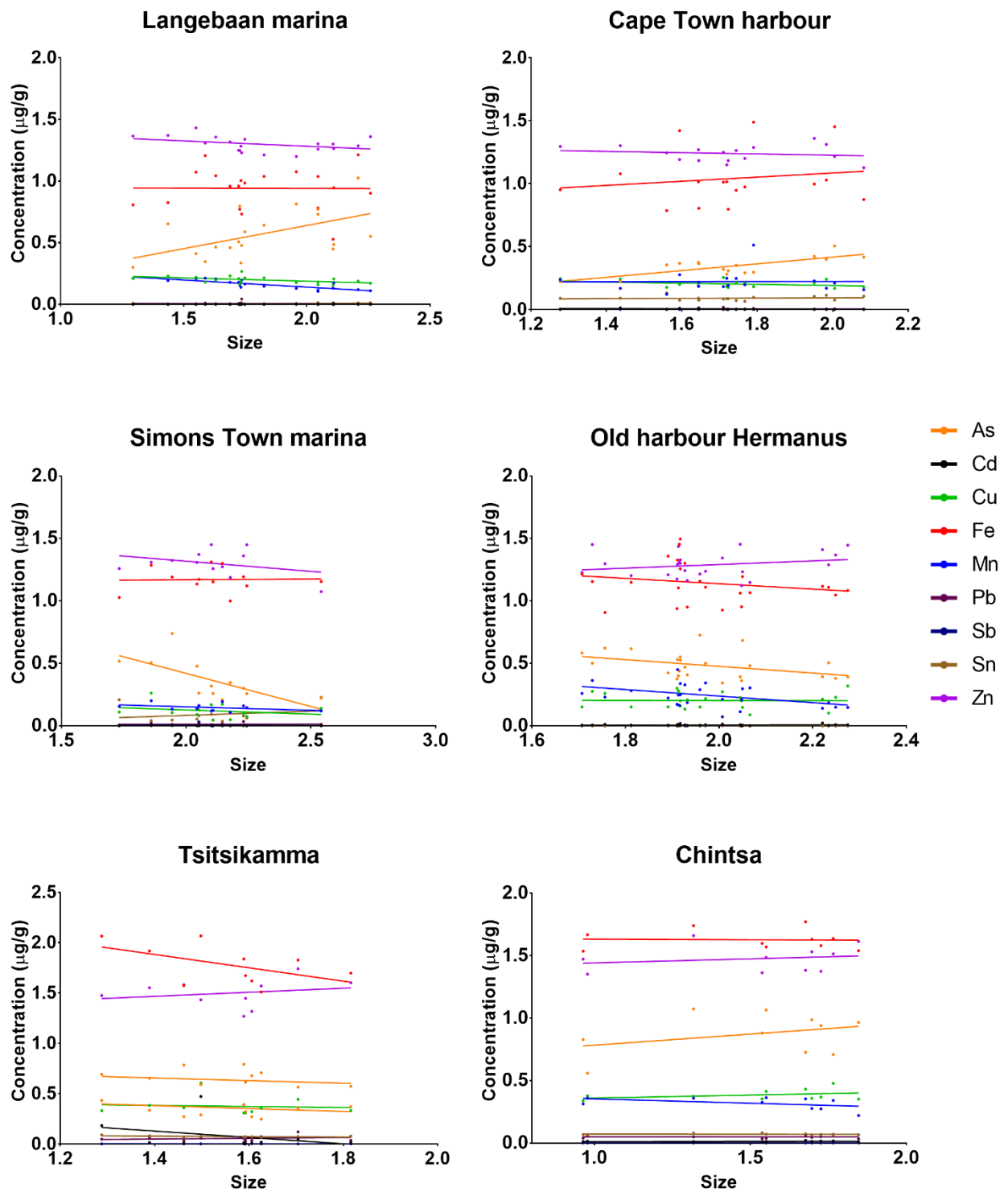


Figure D. 3: Correlation of the log-transformed muscle concentrations related to the log-transformed fish size (mass) per locality.

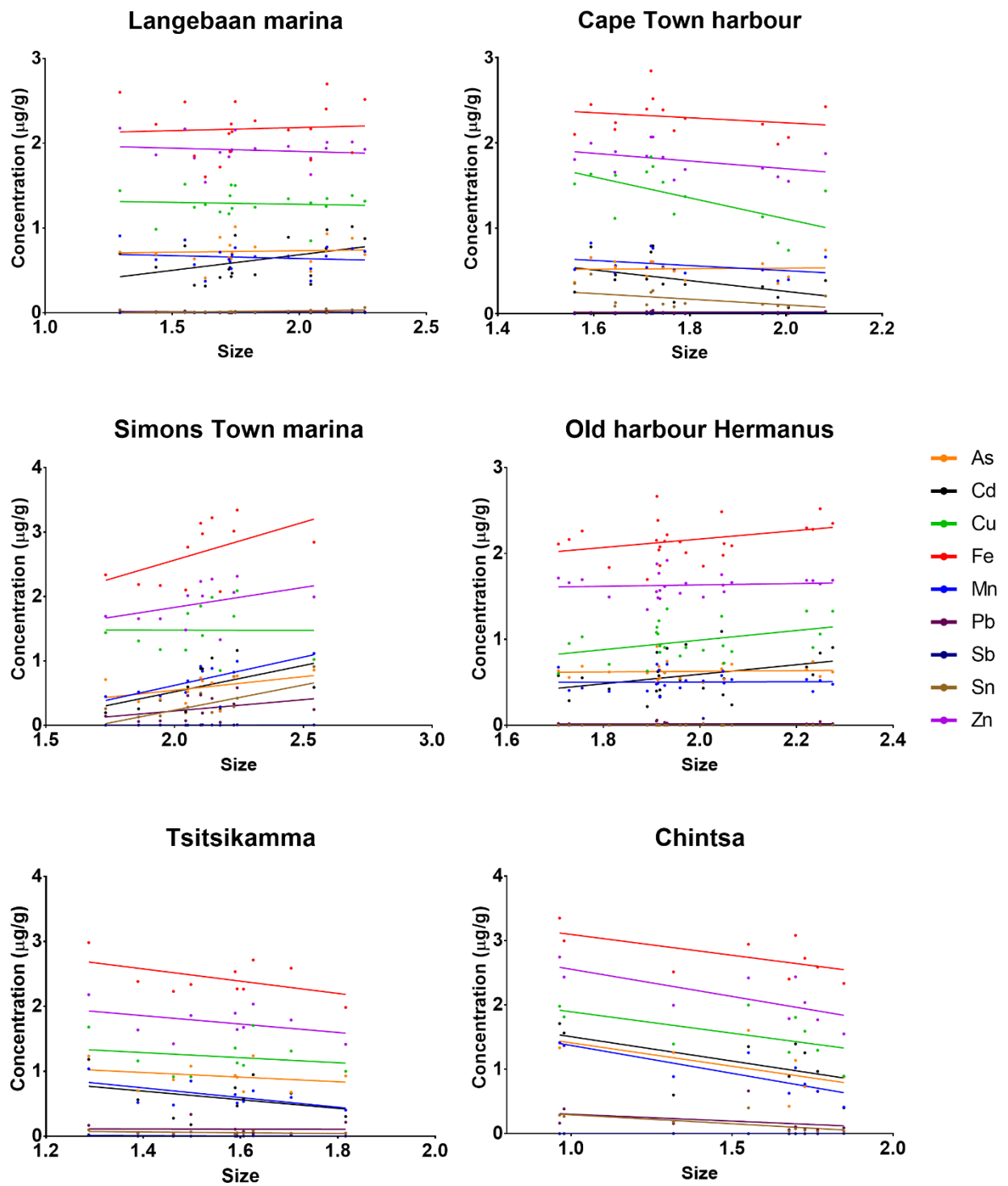


Figure D. 4: Correlation of the log-transformed liver concentrations related to the log-transformed fish size (mass) per locality.

Table D. 2: Mean $\pm$ standard error of the mean for elemental concentrations in *Clinus superciliosus* sampled from six localities, for muscle and liver tissue, respectively.

	LANGEBAAN MARINA		CAPE TOWN HARBOUR		SIMONS TOWN MARINA		OLD HARBOUR HERMANUS		TSITSIKAMMA		CHINTSA	
	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver
As	3.1 $\pm$ 0.48	4.6 $\pm$ 0.44	1.2 $\pm$ 0.098	2.5 $\pm$ 0.25	1.5 $\pm$ 0.32	3.2 $\pm$ 0.51	2.1 $\pm$ 0.17	3.4 $\pm$ 0.23	3.5 $\pm$ 0.39	8.4 $\pm$ 1.5	7 $\pm$ 0.88	14 $\pm$ 4
Cd	0.005 $\pm$ 0.00062	3.8 $\pm$ 0.62	0.0062 $\pm$ 0.0022	1.9 $\pm$ 0.45	0.0042 $\pm$ 0.00056	4.3 $\pm$ 1.2	0.016 $\pm$ 0.0021	3.4 $\pm$ 0.55	0.28 $\pm$ 0.19	4 $\pm$ 1.3	0.032 $\pm$ 0.003	19 $\pm$ 5.4
Cu	0.58 $\pm$ 0.024	20 $\pm$ 1.6	0.6 $\pm$ 0.027	29 $\pm$ 5	0.33 $\pm$ 0.057	42 $\pm$ 11	0.61 $\pm$ 0.048	9.7 $\pm$ 1.1	1.4 $\pm$ 0.19	20 $\pm$ 5	1.4 $\pm$ 0.081	47 $\pm$ 11
Fe	8.4 $\pm$ 0.78	187 $\pm$ 30	12 $\pm$ 2	229 $\pm$ 40	14 $\pm$ 0.97	770 $\pm$ 199	14 $\pm$ 1.2	164 $\pm$ 20	65 $\pm$ 9.7	329 $\pm$ 80	42 $\pm$ 2.6	778 $\pm$ 216
Mn	0.46 $\pm$ 0.028	3.7 $\pm$ 0.34	0.7 $\pm$ 0.11	2.9 $\pm$ 0.37	0.4 $\pm$ 0.028	4.9 $\pm$ 0.98	0.79 $\pm$ 0.074	2.3 $\pm$ 0.18	1.3 $\pm$ 0.19	3.7 $\pm$ 0.81	1.1 $\pm$ 0.069	10 $\pm$ 2.9
Pb	0.012 $\pm$ 0.0055	0.021 $\pm$ 0.0038	0.0096 $\pm$ 0.0026	0.041 $\pm$ 0.0057	0.03 $\pm$ 0.0065	0.98 $\pm$ 0.26	0.0049 $\pm$ 0.00067	0.039 $\pm$ 0.0075	0.14 $\pm$ 0.023	0.32 $\pm$ 0.11	0.12 $\pm$ 0.01	0.77 $\pm$ 0.38
Sb	0.00041 $\pm$ 0.0002	0.0023 $\pm$ 0.00057	0.000095 $\pm$ 0.000047	0.0065 $\pm$ 0.004	0.011 $\pm$ 0.006	0.0079 $\pm$ 0.0015	0.011 $\pm$ 0.0077	0.012 $\pm$ 0.0083	0.0028 $\pm$ 0.0018	0.0098 $\pm$ 0.0051	0.0043 $\pm$ 0.002	0.0029 $\pm$ 0.001
Sn	0.015 $\pm$ 0.0022	0.039 $\pm$ 0.0092	0.23 $\pm$ 0.012	0.56 $\pm$ 0.13	0.24 $\pm$ 0.058	1.4 $\pm$ 0.54	0.0061 $\pm$ 0.0019	0.0059 $\pm$ 0.002	0.19 $\pm$ 0.0064	0.14 $\pm$ 0.017	0.18 $\pm$ 0.0053	0.49 $\pm$ 0.17
Zn	19 $\pm$ 0.66	87 $\pm$ 7.2	17 $\pm$ 0.65	67 $\pm$ 7.4	20 $\pm$ 1.4	97 $\pm$ 18	19 $\pm$ 0.97	44 $\pm$ 2.9	32 $\pm$ 3.4	65 $\pm$ 12	30 $\pm$ 2.5	190 $\pm$ 56

* < LOQ

Appendix E – Chapter 7

Table E. 1: Statistical calculations for parasite taxa from each locality sampled, N – number of infected hosts; P – prevalence; A – abundance; MA – mean abundance; MI – mean intensity.

		Acanthocephala	Caligidae	Cestoda	Lernaeopodidae	Isopoda	Annelida	Monogenea	Nematoda	Digenea
Langebaan marina	N	2	4	0	8	2	2	10	9	19
	P	10.5	21.1	0.0	42.1	10.5	10.5	52.6	47.4	100.0
	A	2	6	0	23	4	3	62	58	4751
	MA	0.1	0.2	0.0	0.4	0.1	0.1	0.5	0.5	1.0
	MI	1.0	1.5	0.0	2.9	2.0	1.5	6.2	6.4	250.1
Cape Town harbour	N	0	0	0	0	0	0	8	0	0
	P	0.0	0.0	0.0	0.0	0.0	0.0	50.0	0.0	0.0
	A	0	0	0	0	0	0	17	0	0
	MA	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
	MI	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.0	0.0
Tsitsikamma	N	7	0	1	0	10	2	0	0	9
	P	70.0	0.0	10.0	0.0	100.0	20.0	0.0	0.0	90.0
	A	30	0	1	0	54	2	0	0	60
	MA	0.7	0.0	0.1	0.0	1.0	0.2	0.0	0.0	0.9
	MI	4.3	0.0	1.0	0.0	5.4	1.0	0.0	0.0	6.7
Chintsa	N	7	2	8	0	6	1	0	1	10
	P	70.0	20.0	80.0	0.0	60.0	10.0	0.0	10.0	100.0
	A	29	4	49	0	18	1	0	1	135
	MA	0.7	0.2	0.8	0.0	0.6	0.1	0.0	0.1	1.0
	MI	4.1	2.0	6.1	0.0	3.0	1.0	0.0	1.0	13.5

Table E. 2: Mean element concentrations of muscle and liver tissue, respectively, for each localities sampled.

		As	Cd	Cu	Fe	Mn	Pb	Sb	Sn	Zn
Langebaan marina	Muscle	3.1	0.005	0.58	8.4	0.46	0.012	0.00041	0.015	19
	Liver	4.6	3.8	20	187	3.7	0.021	0.0023	0.039	87
Cape Town harbour	Muscle	1.2	0.0062	0.6	12	0.7	0.0096	0.000095	0.23	17
	Liver	2.5	1.9	29	229	2.9	0.041	0.0065	0.56	67
Tsitsikamma	Muscle	3.5	0.28	1.4	65	1.3	0.14	0.0028	0.19	32
	Liver	8.4	4	20	329	3.7	0.32	0.0098	0.14	65
Chintsa	Muscle	7	0.032	1.4	42	1.1	0.12	0.0043	0.18	30
	Liver	14	19	47	778	10	0.77	0.0029	0.49	190