Observations on myxozoans (Myxozoa: Myxosporea) and the spatial and temporal variation in parasite assemblages of the nosestripe klipfish, *Muraenoclinus dorsalis* Bleeker, 1860 (Perciformes: Clinidae)

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DECLARATION

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ABSTRACT

The coast of South Africa is one of the most biologically diverse marine systems in the world but little is known about the parasites occurring in this environment. A survey of the parasites of an intertidal clinid, *Muraenoclinus dorsalis* Bleeker, 1860, captured from Granger Bay, Kommetjie and Jacobsbaai, South Africa during Summer 2008/2009 and Winter 2009 revealed twenty-three parasitic species. Five myxozoans, all likely new to science, were found to infect *M dorsalis. Ceratomyxa* sp. and *Sphaeromyxa* sp. 1 were found in the gall bladder and bile ducts, *Kudoa* sp. in skeletal muscle, and a *Myxobolus* sp. on the eyes. Spores of *Ortholinea* sp. were also detected in gall bladder squashes, but the actual location of infection is unknown. The endoparasite community composition and structure, and their persistence over space and time were also probed. Non-metric multidimensional scaling, cluster analysis and Analysis of Similarity showed that community composition during both summer and winter differed most between Kommetjie and Jacobsbaai, the sites geographically furthest apart. This observation implies a decay in similarity over geographic distance. The endoparasite component communities from Granger Bay showed no significant dissimilarity in composition between summer and winter while the component communities from Kommetjie showed little dissimilarity (Global R: 0.105; p-value = 0.002). These results suggest that season or season-associated factors play weak roles at both localities. Contrastingly, the summer and winter component communities from Jacobsbaai showed significant dissimilarity (Global R: 0.201; p-value = 0.003) because of the higher parasite load in winter. This is attributed to the sheltered nature of Jacobsbaai, where violent winter waves do not disturb the fish but rather push up the tide thereby extending the submergence period of *M dorsalis.* A longer period of submergence may provide parasites with increased opportunities to infect a host. Nestedness analyses

confirmed a nested subset structure in all component communities, likely a result of differentiated colonization, passive sampling, or a combination of both. This result lends evidence to the theory that parasite community structure is persistent over space and time and that there are laws in parasite ecology.

CHAPTER 1: Introduction to Myxozoa Grassé, 1970 and Parasite Community Structure

Parasites employ the most popular lifestyle on Earth (Price, 1980) and can be utilized as effective tools to explore the origins, distribution, and maintenance of biodiversity (Marcogliese and Cone, 1997). However, information on parasite communities continues to lag behind that of free-living organisms (Kennedy and Bush, 1994; Rohde, 2002) despite the escalating concern of the rise of disease due to factors such as climate change, pollution, introduced species, and harvesting (Lafferty et al., 2004). This thesis aims to address the lack of parasite information by contributing to the taxonomic description of some parasites and providing evidence for debate on whether there exist general laws in parasite ecology.

Introduction to the Myxozoa

Over the past two decades parasites belonging to Phylum Myxozoa Grasse, 1970 have garnered greater attention from the scientific community (Fig. 1) due to the discovery of new species and life cycles, the intense controversy over myxosporean phylogeny and origin, and the threats virulent myxozoans pose to fisheries (Lom and Dykova, 2006). This section will review what myxozoans are and their roles in the ecosystem and fisheries.

Biology of Myxozoa

Myxozoans are microscopic metazoans that most commonly parasitize invertebrates, typically oligochaetes, polychaetes and bryozoans; and poikilotherms, primarily fishes but also reptiles and amphibians (Kent et al., 2001; Canning and Okamura, 2004; Lom and Dykova, 2006). They have also been documented in homeotherms including moles, birds and humans (Boreham et al., 1998; Friedrich et al., 2000; Moncada et al., 2001; Lowenstine et al., 2002,

Dyková et al., 2007; Prunescu et al., 2007; Bartholomew et al., 2008). The most recent myxozoan census estimated the number of described species to be 2184 species assigned to 62 genera (Lom and Dykova, 2006).

Morphology of Myxozoa

Myxozoans exhibit a highly simplified body form as a result of their parasitic nature (Canning and Okamura, 2004). They can be coelozoic, where they occur in organ cavities, or histozoic, where they occur inter- or intracellularly (Canning and Okamura, 2004; Lom and Dykova, 2006). Currently, the identification of myxozoans is based primarily on the morphology of the spore (Lom and Arthur, 1989; Lom et al., 1997), which acts as the vehicle by which myxozoans transmit themselves from one host to the next (Canning and Okamura, 2004; Lom and Dykova, 2006). Spores are characterized by three types of cells: valvogenic cells, capsulogenic cells, and sporoplasmic cells. Valvogenic cells form the spore shell valves, which envelop and protect the interior of the spore. Capsulogenic cells form nematocyst-like polar capsules, which house coiled, extrudible polar filaments (Fig. 2). Sporoplasmic cells form at least one multinucleate, amoeboid infective germ. Together, these three cell types form one of two kinds of spores: myxospores (Figs. 3-5) or actinospores (Fig. 6-7). Myxospores possess two to seven shell valves encompassing one to four polar capsules and one or two sporoplasms. Contrastingly, actinospores have three shell valves encapsulating three polar capsules, a sporoplasm, and many infectious cells. They may possess caudal projections, which are extensions of the shell valves believed to aid in flotation and anchorage intracellularly (Canning and Okamura, 2004; Lom and Dykova, 2006).

After spores, the next most identifiable form of myxozoans is that of the sporogonic, or spore-producing stage, which can be plasmodia or pseudoplasmodia when in vertebrates, or

pansporocysts when in invertebrates intracellularly (Canning and Okamura, 2004; Lom and Dykova, 2006). Plasmodia have many vegetative nuclei that can produce many spores while pseudoplasmodia are uninucleate and can only develop one or two spores (Canning and Okamura, 2004; Lom and Dykova, 2006).

Systematics of Myxozoa

When myxozoans were first discovered they were assigned to Protista in Class Sporozoa Leukart, 1879 by Btitschli (1882), a direct result of their deceptively simple body plan. However, assertions that myxozoans were actually metazoans were made as early as 1899 by Stolc and subsequently by Emery (1909) and Ikeda (1912), all of whom drew attention to the multicellularity of spores. In 1938, the protistan assignment was again questioned by Weill, who noted the striking similarity between myxozoan polar capsules and cnidarian nematocysts. This debate continued to simmer until 1994, when Smothers et al. used 18S ribosomal DNA to show conclusively that myxozoans in fact grouped with Metazoa and not with Protista.

The conclusion of the dispute over the cellularity of Myxozoa did not end the complex debate over myxozoan systematics. During the 1980s and early 1990s Myxozoa was comprised of two classes: Class Myxosporea Btitschli, 1881 and Class Actinosporea Noble, 1980. The groundbreaking transmission studies by Wolf and Markiw (1984) again highlighted the problematic classification of Myxozoa when they showed that the life cycle of *Myxobolus cerebralis*, which causes salmonid whirling disease, consisted of both an invertebrate, where actinospores proliferated, and a fish host, where myxospores proliferated. The discovery of the alternating myxospore-actinospore cycle resulted in the eventual eradication of Class Actinosporea (Kent et al., 1994). Today, the classification of Myxozoa remains hotly debated: are myxozoans more closely related to cnidarians or bilaterians? Morphological evidence derived

from their nematocyst-like polar capsules and genetic evidence from 18S rDNA suggest that myxozoans are more closely related to cnidarians (Siddall et al., 1995; Siddall and Whiting, 1999; Lom and Dykova, 1997). However, other genetic studies, including those using HOX genes, suggest that myxozoans are more closely related to the Bilateria (Anderson et al., 1998; Hanelt et al., 1996; Smothers et al., 1994). Also generating great interest are the results from the analysis of small-subunit RNA gene sequences; several morphological characteristics used to assign a species to a genus were found to be homoplasious (Fiala, 2006), which means the current system of classifying based on spore morphology is inaccurate and requires revision.

Life Cycle

Today, Phylum Myxozoa is composed of two classes—Malacosporea Canning, Curry, Feist, Longshaw et Okamura, 2000, which parasitizes freshwater bryozoans, and Myxosporea, which will be the focus of the remainder of this thesis.

After Wolf and Markiw's discovery (1984) and the subsequent resolution of other myxozoan life cycles (summarized by Kent et al., 2001; Koie et al., 2004; 2007; 2008), myxosporeans are generally accepted to alternate between two hosts, a vertebrate and an invertebrate (Fig. 8). It must be noted, though, that this generalization is based only on evidence from fish and annelid hosts. The cycle begins with infection, when an actinospore encounters a vertebrate through random contact (Kent et al., 2001; Canning and Okamura, 2004; Lom and Dykova, 2006). Unknown factors cause the release of the polar filaments (Kent et al., 2001), which attach the spore to the new host. After attachment, the valves open along the suture line, or line of dehiscence, and the sporoplasm is released. The vertebrate now acts as an intermediate host during the myxospore phase intracellularly (Lom and Dykova, 2006). During post-invasion development, a vegetative, trophic stage migrates to the final site of infection. The next stage is

the sporogonic, or spore-producing phase where a plasmodium or a pseudoplasmodium develops. During this phase myxospores are produced in sporogonial plasmodia, and then released for the purpose of transmission to the definitive host, the invertebrate (Lom and Dykova, 2006). The myxospore is introduced to the definitive host via host ingestion (Lom and Dykova, 2006). As with the actinospore, the polar filaments are discharged and the released sporoplasm migrates to the final site of infection (Canning and Okamura, 2004; Lom and Dykova, 2006). This is where the actinospore phase, a sexual phase during which actinospores are produced, begins. Trophozoites quickly replicate to first make multinucleate then uninucleate daughter cells. These cells then form a pansporocyst, within which gametic cells are formed. The gametes then fuse together to form zygotes. Cell division followed by differentiation of the zygotes results in actinospores. The cycle of infection continues when actinospores are released into the water to infect a new intermediate host (Canning and Okamura, 2004; Lom and Dykova, 2006).

Thus far at least 29 myxosporean life cycles have been described (summarized by Kent et al., 2001; Koie et al., 2004; 2007; 2008), of which only three are marine (Koie et al., 2004; 2007; 2008).

Ecological and Commercial Significance of Myxozoa

In feral populations, infection by Myxozoa generally results in no to mild symptoms, such as inflammation (Lom and Dykova, 1995). Hence, myxozoans are presumed to have minimal impact on host populations in the wild. However, virulent infections, especially in high sea fisheries, may result in tissue hyperplasia, cysts, lesions, and myoliquefaction (Lom and Dykova, 1995). The resultant aesthetically displeasing or degraded fish are thereby rendered unmarketable, causing commercial fishers grave concern (Kent et al., 2001).

Since the expansion of marine fish aquaculture in the 1990s, several myxozoans have been categorized as significant pathogens (Kent et al., 2001). One notorious species known to cause economic havoc is *Kudoa thyrsites* Gilchrist, 1924. *Kudoa thyrsites* was first described from the coast of South Africa in *Thyrsites atun,* snoek, and causes post-mortem myoliquefaction. A cosmopolitan parasite, *K thyrsites* infects the muscles of over twenty different fishes, particularly salmonids raised in netpen aquaculture such as the commercially important Atlantic salmon, *Salmo salar* (Moran and Kent, 1999; Moran et al., 1999).

Other myxozoans that have been deemed nuisances to the fishing industry include species of the genus *Enteromyxum* Palenzuela, Redondo et Alvarez-Pellitero, 2002, such as *E. scophthalmi* Palenzuela, Redondo et Alvarez-Pellitero, 2002 in turbot and *E. leei* Diamant, Lom et Dyková , 1994 in sea bream and other fishes. These species are known to plague mariculture by causing acute enteritis, which results in high rates of mortality (Padrós et al., 2001; Redondo et al., 2004). Two more examples of troublesome marine myxosporeans are *Myxobolus acanthogobii* Hoshina, 1952, a brain parasite causing scoliosis of *Serila quinqueradiata* (Sakaguchi et al., 1987) and *Ceratomyxa sparusaurati* Sitjà-Bobadilla, Palenzuela et Alvarez-Pellitero, 1995, a parasite of the gall bladder of *Sparus aurata* (Palenzuela et al., 1997).

Spatial and Temporal Variations in Parasite Assemblages

As with myxozoans, knowledge and information on parasite communities have greatly increased since the 1990s (Poulin, 2007). However, the bulk of this information is composed of species distributions of parasites from previously unstudied host populations. Though these descriptive studies are undoubtedly useful, they lack theory-based explanations for elucidating the observed distributions and therefore do not contribute significantly to the understanding of

laws in parasite ecology. Some work (reviewed by Poulin, 2007) has been completed in the search for recurring, non-stochastic patterns in the structure of parasite communities. However, few of these studies have been replicated either spatially or temporally, even in the short range or term, despite the possibility that local and seasonal differences can strongly affect the structure of parasite communities (Poulin, 2007).

Nestedness

Community structure is characterized by a departure from randomness, where species occur in a fashion that is more ordered than expected by chance. One model depicting a departure from randomness is nestedness. Nestedness occurs when groups of species are comprised of subsets of increasingly depauperate populations (Fig. 9). The concept of nestedness was introduced independently by Hulten (1937 in Hausdorf and Hennig 2003), Darlington (1957) and Daubenmire (1975). The concept garnered little attention and remained an obscure model of community structure until the 1960s, when the concept began to receive a modest degree of interest. The concept finally began to gain popularity in 1986 when Patterson and Atmar introduced their statistical approach to testing for nestedness. Since then, significant nestedness has been found in various taxa, plant and animal, and environments, and is acknowledged to be a natural, commonly occurring phenomenon (reviewed by Wright et al., 1998).

In a general nestedness analysis, presence-absence data is organized in a matrix (Fig.9); columns represent species and rows sampling sites or times, or vice versa. The matrix is subsequently reorganized by arranging columns and rows in descending order of number of species and species richness, respectively, i.e. the most common species are in the left-hand columns and the species-rich sites are in the upper rows. Nestedness is indicated when there is a

concentration of presences in the upper left corner of the matrix. The presence and quantification of nestedness can be based on various metrics, each of which uses various criteria to order the rows and/or columns of the matrix; a review of existing metrics is presented by Ulrich et al. (2009).

When the presence of nestedness is confirmed, the pattern is attributed primarily to the following mechanisms: i) ordered extinctions mainly related to habitat area (Patterson and Atmar, 1986; Patterson, 1990; Bolger et al., 1991; Worthen, 1996; Wright et al., 1998), ii) differential colonization usually related to habitat isolation (Darlington, 1957; Patterson and Atmar, 1986; Patterson, 1990; Worthen, 1996; Wright et al., 1998), iii) nested habitats or environmental tolerances (Patterson and Brown, 1991; Kodric-Brown and Brown, 1993; Worthen, 1996; Wright et al., 1998), and iv) passive sampling (Worthen, 1996; Wright et al., 1998). The availability of methods to test for nestedness, and, when possible, the mechanism causing the pattern, provides ecologists with an intriguing and insightful measure for understanding species distributions on islands.

Hosts as Islands

Traditionally, nestedness has been studied at very large spatial and temporal scales at which processes operate, e.g. landbridge islands, oceanic islands, and isolated mountains. However, parasite communities can also provide an amenable framework for searching for and understanding non-stochastic patterns. In parasitology, each host individual can be viewed as an island, an insular community where parasites occupy defined niches, or sites of infection, in the ecosystem provided by the host. Viewed in this manner, hosts are replicated habitats that, together, form an archipelago occupying a certain place and time. Though the dynamics of the traditional systems may differ, the relative importance of the processes involved in the

generation of a nested subset pattern in parasite communities is significant, especially for determining if there exist general laws in parasite ecology.

Parasite-specific Terminology

To understand parasite community ecology, certain terminology must first be defined, specifically infrapopulation, infracommunity, and component communities. An infrapopulation is a population that includes all individuals of a species in an individual host at a particular time (sensu Bush et al., 1997). An infracommunity comprises all infrapopulations in a single host (sensu Bush et al., 1997). A component community comprises all the infrapopulations within a subset of one host species associated with some subset of the abiotic environment (sensu Bush et al., 1997), e.g. all the infrapopulations of a host species captured from a specific place at a specific time. Studies of nestedness in parasite communities can be examined at the infracommunity level, to test for and quantify nestedness at a particular place and time, and the component community level, to test for the persistence of nestedness across space or time.

Nestedness in Parasite Communities

The first study on nestedness in parasite infracommunities was conducted by Guégan and Hugueny (1994) on tropical fish from West Africa. The results from this study showed a general pattern of enrichment of gill parasite species with host individual length, and possibly age of host, i.e. a nested subset pattern based on island size and length of time available for colonization. Since then, nestedness has been documented in parasite communities across different environments, terrestrial and aquatic, and host taxa, mostly fish and mammals (e.g. Rohde et al., 1998; Poulin and Valtonen, 2001; Gouy de Bellocq et al., 2003; Fellis et al., 2003; Timi and Poulin, 2003; Zelmer et al., 2004). However, the acceptance of nestedness in parasite communities as a generality remains undecided since other studies have shown conflicting

results (e.g. Poulin, 1996; Worthen and Rohde, 1996; Hayward et al., 1998). To further complicate the matter, the bulk of the studies that did find nestedness failed to consider its persistence through space and time. The repeatability of community structure in both space and time has only been examined in a handful of studies (Carney and Dick, 2000; Poulin and Valtonen, 2001; Vidal-Martinez and Poulin, 2003; Timi and Poulin, 2003; Gonzalez and Poulin, 2005; Gonzalez and Oliva, 2009); of these studies, only Carney and Dick (2000), Gonzalez and Poulin (2005), and Gonzalez and Oliva (2009) found repeatability. Hence, more evidence needs to be collected to either accept or refute the existence of a law in parasitology that dictates community structure, in this case a nested subset pattern.

The Host Fish: *Muraenoclinus dorsalis* Bleeker, 1860

***Muraencolinus dorsalis* is an intertidal fish belonging to Family Clinidae. Also known as the nosestripe klipfish, *M dorsalis* is one of 39 clinid species dominating the intertidal ichthyofauna of Southern Africa's west and south-western coast (Prochazka and Griffiths, 1992). *Muraenoclinus dorsalis* is endemic to Southern Africa, ranging from Swakopmund, Namibia to the Natal Coast of South Africa (Smith, 1986) where it rarely exceeds lengths of 70 mm (Penrith, 1965). *Muraenoclinus dorsalis* is abundant throughout the intertidal, but is most easily found outside of tidepools under damp rocks in the upper intertidal zone (personal observation). In fact, it may at times display a stranding behavior during the low tide, where it lies under damp rocks outside of water (personal observation).**

***Muraenoclinus dorsalis* is a viviparous fish, giving birth to live young. Though abundant, only a handful of studies have focused on *M dorsalis.* These studies focused on the reproduction (Cornish, 1985; Veith and Cornish, 1986; Cornish and Veith, 1986; Cornish and Veith, 1987; Prochazka, 1994), diet (Bennett et al., 1983), morphology (Gon et al., 2007), ecology (Prochazka**

and Griffiths, 1992), and phylogeography (von der Heyden et al., unpublished data) of *M dorsalis.* The present study is the first to examine the parasites of *M dorsalis.*

Objectives and structure of dissertation

The aims of this investigation are to: i) provide preliminary observations on the myxozoans of *Muraenoclinus dorsalis* in preparation for the description of new species, ii) compare the parasite infracommunities of *M dorsalis,* pairwise between summer and winter, across three different localities, and iii) determine if these communities are structured non-randomly, specifically whether or not they exhibit nestedness.

The first objective will be covered in Chapter 2, while the two ecological objectives will be addressed in Chapter 3.

Figure Captions

Figure 1. Histogram showing the number of publications found from using the search term "Myxozoa" using ISI Web of Knowledge.

Figure 2. Light micrograph of a live *Ceratomyxa* sp. spore discharging its polar filaments, indicated by arrows. Scale bar = 5 µm.

Figures 3-5. Stylized drawings of representative myxospores. Key to abbreviations: V-valve; PC-polar capsule; S-sporoplasm. Scale bars = 5 µm.

Figure 3. *Myxobolus* myxospore.

Figure 4. *Kudoa* myxospore.

Figure 5. *Ceratomyxa* myxospore.

Figures 6-7. Stylized drawings of representative actinospores. Key to abbreviations: V-valve; PC-polar capsule; 5-sporoplasm; CA-caudal appendage.

Figure 6. Neoactinomyxid actinospore. Scale bar = 10 µm.

Figure 7. Triactinomyxid actinospore. Scale bar = 50 [tin.

Figure 8. Stages in the generalized life cycle of a myxosporean based on information provided by Canning and Okamura (2004) and Lom and Dyková (2006). The dashed line indicates separation of hosts during the life cycle.

Figure 9. Illustration of two component communities consisting of three infracommunities with presence/absence matrix. Size of square indicates species richness; overlap indicates shared species.

A) A nested component community.

B) A non-nested component community

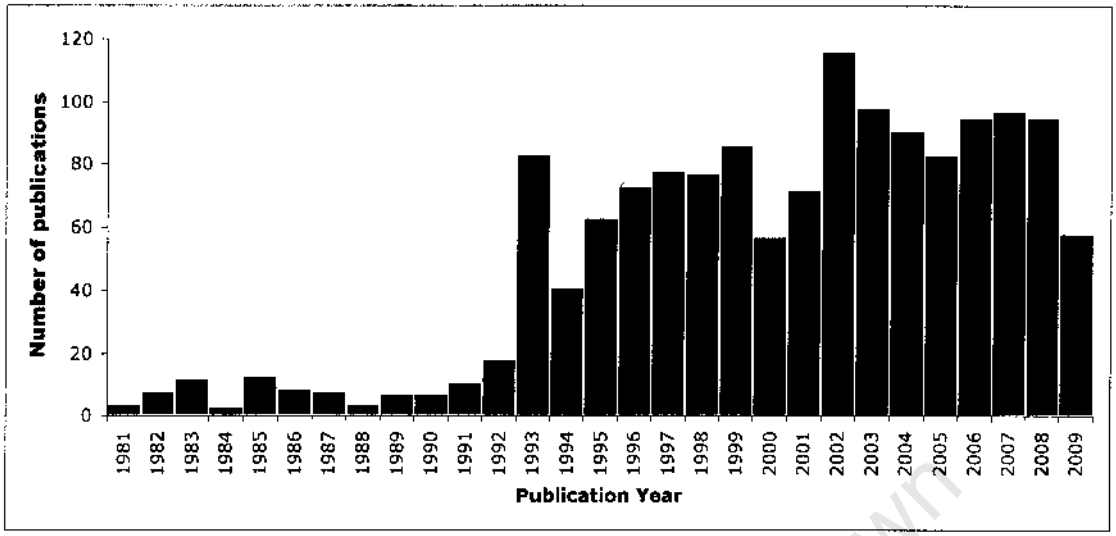
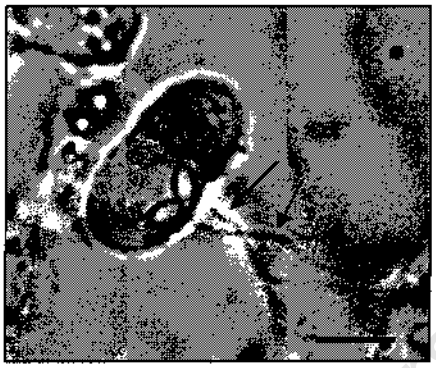


Figure 1



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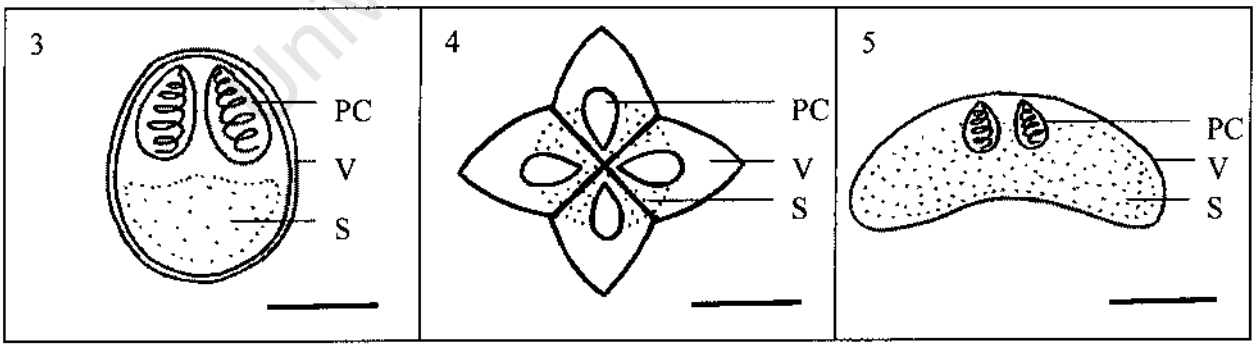

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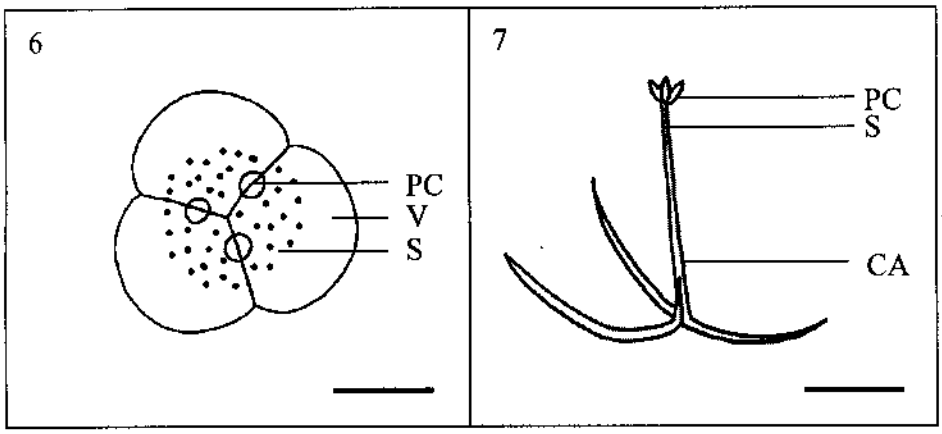

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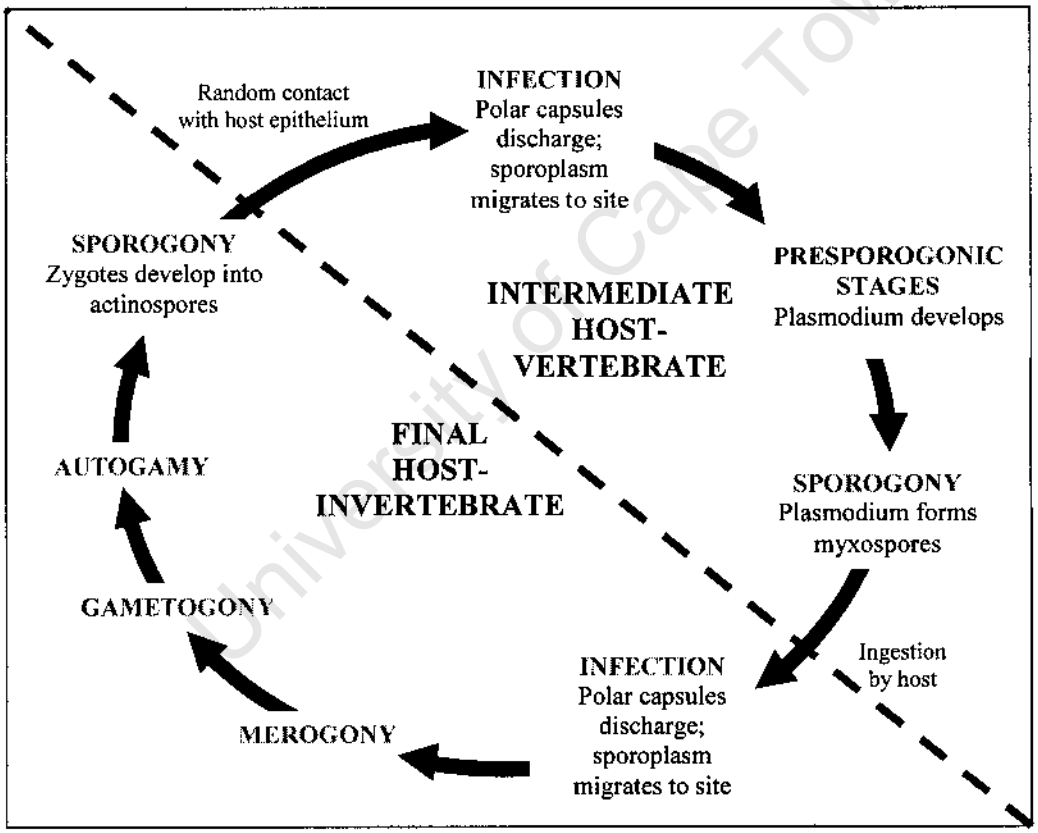

Figure 8

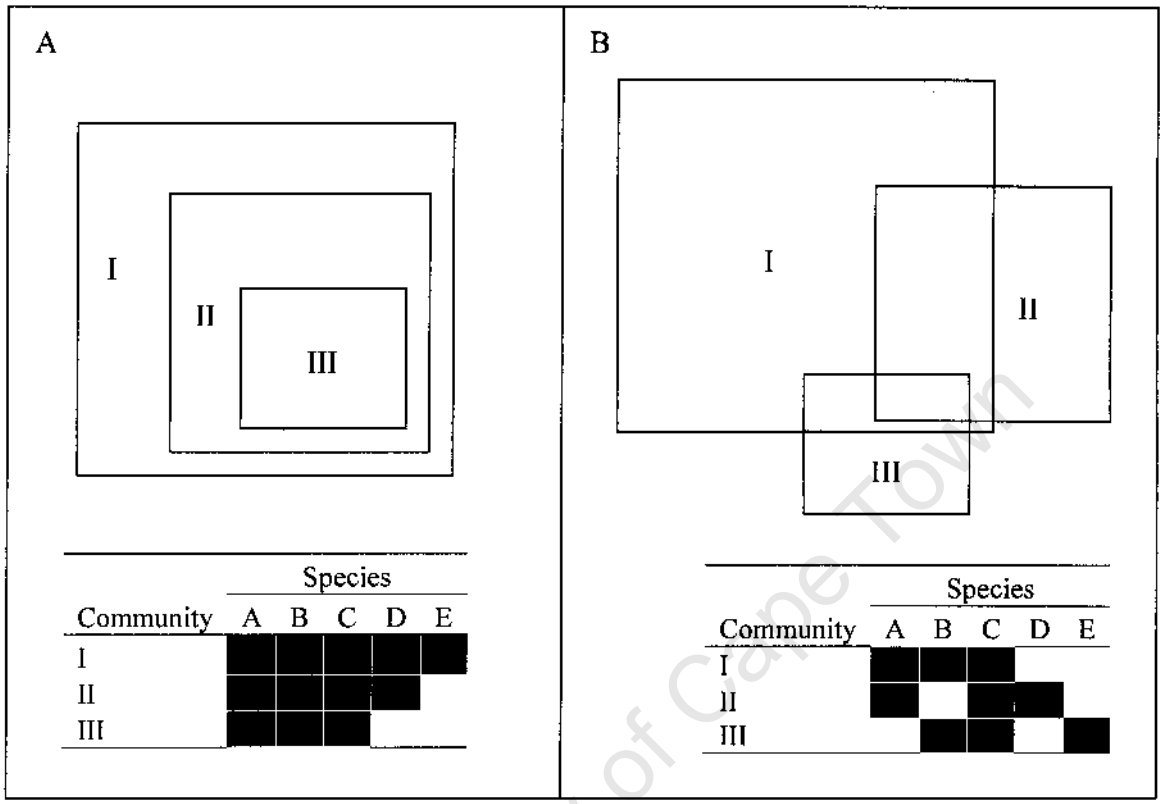

Figure 9

CHAPTER 2: Observations on myxozoans (Myxozoa: Myxosporea) infecting an intertidal fish, *Muraenoclinus dorsalis* Bleeker, 1860 (Perciformes: Clinidae)

Abstract

Muraenoclinus dorsalis **is a highly abundant fish species endemic to the coast of Southern Africa. This fish is infected with five myxozoan species, all possibly new to science. Two hundred and seven fish captured from Granger Bay, Jacobsbaai, and Kommetjie, South Africa revealed *Ceratomyxa* sp. and *Sphaeromyxa* sp. 1 spores in the gall bladder and plasmodia in the bile ducts. Nearly all fish captured from the three localities were infected with *Ceratomyxa* sp. The mean spore measurements were 7.3 gm in length and 16.3 p.m in width while mean polar capsule measurements were 2.7 µm in length and 2.2 µm in width. *Sphaeromyxa* sp. 1 was only found at Granger Bay. Mean spore length measured 20.7 gm and mean spore width was 6.1 µm while the polar capsules were on average 6.9 pm long and 3.0 µm wide. Histological sections showed *Kudoa* sp. infections in the skeletal muscle in fish from Granger Bay and Kommetjie; fish from Jacobsbaai were not examined for this myxozoan. The mean spore measurements were 5.1 pm in length, 5.9 µm in width, and 5.4 pm in thickness while the mean polar capsule measurements were 2.5 m in length and 1.8 µm in width. *Myxobolus* sp. plasmodia were discovered on the sclera of the eyes, but only on fish from Kommetjie and Jacobsbaai. Mean spore length of this species was 12.8 µm and mean spore width was 13.7 gm; the polar capsules averaged 5.1 pm in length and 4.0 µm in width. Spores of *Ortholinea* sp. were also detected in gall bladder squashes from Kommetjie and Granger Bay but the actual location of infection remains unknown; fish from Jacobsbaai were not examined for this species. The spores were an average of 8.8 pm in diameter, and the single, visible polar capsules were on average 2.9 µm**

long and 2.4 wide. This study is the first documentation of myxozoans in *M dorsalis* and almost doubles the number of myxozoans documented in South Africa.

Key words: **Myxozoa; *Muraenoclinus dorsalis; Ceratomyxa; Sphaeromyxa; Kudoa; Myxobolus; Ortholinea;* South Africa**

Introduction

The past two decades have seen a rise in research on myxozoans due to increasing interest in their pathogenicity and systematics. Despite this, myxozoans remain greatly understudied, especially for marine myxosporeans from the African continent. To date, only 75 marine myxosporeans have been described from the African coast (Table 1), mostly from the coasts of Senegal and Egypt. Only eight have been described from South Africa despite South Africa having one of the most extensive coastlines on the African continent and being a hub for marine studies.

Species of the Phylum Myxozoa Grassé, 1970 were first documented in South Africa by Gilchrist in 1924, when he noted the infection of *Thyrsites atun,* snoek, by *Kudoa thyrsites* Gilchrist, 1924 (previously named *Chloromyxum thyrsites).* Fantham (1919; 1930) described several new species, all of which have been deemed *nomina nuda* since the paucity of detail provided by the descriptions and illustrations was insufficient for distinguishing between species. The most recent progress in describing South African myxozoans was by Reed et al. (2007; 2009), who examined various intertidal fishes.

The purpose of this study is to provide preliminary observations on the myxozoan species from the nosestripe klipfish, *Muraenoclinus dorsalis* Bleeker, 1860, which is a common

intertidal clinid endemic to Southern Africa. These observations will provide the basis upon which descriptions of species will be made.

Materials and Methods

Collection of Fish

Muraenoclinus dorsalis **was collected from Granger Bay, Jacobsbaai, and Kommetjie, South Africa (Fig. 1) from November 2008 to November 2009. The fish were collected from the intertidal zone, primarily under damp rocks with little to no seawater, during low tide using small handnets. Fish were euthanized by neural pithing, measured, and examined for the presence of myxosporean infections.**

Detection of Infection

Infections were detected by visual examination of all organs using a stereomicroscope and by squashing fresh gall bladders between a microscope slide and a coverslip and examining the contents using a light microscope at 100-400x magnification. No other tissues were squashed. These preliminary scans were used to establish temporal variations in infection, specifically between the summer (November 2008-February 2009) and the winter (May 2009-July 2009).

Morphological Examination of Spores

The morphological examination of spores was performed from August 2009 to November 2009. Live spores were examined by squashing the gall bladders of 40 fish captured from Granger Bay and Kommetjie onto a thin layer of 0.5% non-nutritive agar. The spores were photographed using a Leica DM750 Microscope equipped with a Leica ICC50 camera or a Zeiss Axiovert 200M Fluorescence microscope equipped with a Zeiss AxioCam HRm, then measured according to the guidelines provided by Lom and Arthur (1989) using the software Leica LAS

EZ or AxioVision 4.7 software, respectively. All measurements are presented as mean ± standard deviation followed by the range in parentheses.

Histology

Plasmodia were detected by examining histological sections of the liver, kidney and gall bladder. Fish were fixed in modified Davidson's Fixative (see Appendix A for reagent protocols) for 24 h, transferred to modified Davidson's Solution for 24 h — 48 h then stored in 70% ethanol. Subsequent work was performed at the Histology Lab in the Department of Human Biology, University of Cape Town where the tissues were embedded in paraffin wax (see Appendix B for detailed methodology for histology). Sections 5 thick were cut from the wax blocks using a Reichert Jung 2040 rotary microtome and stained with haemotoxylin and eosin/phloxine.

Statistical Analyses

Statistical significances between prevalences were determined using Fisher's Exact Test. In cases where the spore and polar capsule shape and measurements from the current study and described species were approximately the same, an unpaired t-test was used to determine significant differences in mean measurements. P-values of less than 0.05 were considered significant. All statistical analyses were performed using SPSS for Windows Release 18.0.0 (2009).

Results

Capture offish

Observations on the capture of fish are not relevant to the description of myxozoans. They are provided here as a resource for future researchers on how to catch *M dorsalis.* Fishing conditions were ideal on cool, wet, overcast days with wave heights below 4 m and when the tide

was at most 0.35 m above standard datum at Granger Bay and Kommetjie and 0.45 m at Jacobsbaai. *Muraenoclinus dorsalis* proved difficult to catch on warmer days, which were characterized by low humidity coupled with clear, sunny skies. As a result, the number of fish collected from Granger Bay and Kommetjie during the summer was relatively low. Furthermore, when it was time to collect fish to study spore morphology, the return of warmer weather resulted in the availability of fewer fish. Jacobsbaai was consistently an excellent location for capturing *M dorsalis* regardless of weather conditions, although personal observations suggest that fish here are generally larger than those from Granger Bay and Kommetjie. The capture of *M dorsalis* was easier using a large, empty shell, such as that of a mussel, than with a hand net, which *M dorsalis* consistently avoided.

Observations on the myxozoans of Muraenoclinus dorsalis

This is the first report of species from Phylum Myxozoa infecting *M dorsalis.* Five myxozoans, representing five genera, were found: *Ceratomyxa* sp. , *Sphaeromyxa* sp.1, *Myxobolus* sp. , *Kudoa* sp. , and *Ortholinea* sp.

***Ceratomyxa* sp.**

***Ceratomyxa* sp. is a coelozoic species that was found in the gall bladders and bile ducts of fish from Kommetjie, Granger Bay and Jacobsbaai. Prevalences of *Ceratomyxa* sp. during summer and winter at each locality are given in Table 2. Mature spores were found floating in the bile (Figs. 2-4). They exhibited high morphological plasticity, ranging from arcuate to ellipsoidal, with the anterior margin often convex, though sometimes flat, and the posterior margin concave to flat. Often, ellipsoidal spores possessed shell valves of different lengths and widths, resulting in a tapered appearance in frontal view (Fig. 4). Abnormal spores were detected regularly. Some abnormal spores exhibited normal shell valves, but possessed polar capsules that**

were distant from each other along the width axis (Fig. 5). Other abnormal spores possessed triradiate forms with three shell valves and three polar capsules typically located in the center (Fig. 6) but occasionally at the ends of the shell valve (Fig. 7).

Eighty-eight spores were measured. They measured 7.3 ± 1.5 (4.4-10.6) µm long and 16.3 ± 2.1 (9.6-22.3) m wide. The sutural line was straight, crossing the spore medially. The equal-sized polar capsules were pyriform to subspherical, measuring 2.7 ± 0.4 (1.8-3.6) µm in length and 2.2 ± 0.3 (1.5-2.9) µm in width. The polar filaments were coiled, but the number of turns remains undetermined, with a possibility of there being only one coil making an incomplete turn (Fig. 8).

Disporic pansporoblasts with developing and mature spores were observed lining the gall bladder epithelium. Wisp- or tuft-like plasmodia were also found developing within the bile ducts (Fig. 9).

***Sphaeromyxa* sp. 1**

***Sphaeromyxa* sp. 1 is a coelozoic species that was found in the gall bladders and bile ducts of fish from only Granger Bay. Prevalence was 18% (N = 22) during the summer and 45% (N = 31) in the winter. Mature spores were found floating in the bile. In frontal view, they were slightly arched, rounded obtuse on one side and almost flat on the other, with valves tapering to bluntly rounded ends (Fig. 10, 12). In sutural view, the spores were mildly sinusoidal (Fig. 11, 13). Sixty-four spores were measured with length of 20.7f 1.8 (18.4-25.0) pm and width of 6.1 ± 0.3 (5.4-6.8) m. The suture line crossed the spore obliquely in sutural view. Polar capsules were elongated ovoid, measuring 6.9 ± 0.4 (5.7-8.4) µm in length and 3.0 ± 0.3 (2.3-3.6) lam in width. Polar filaments folded over themselves at least once.**

Polysporic plasmodia with developing and mature spores were seen lining the gall bladder epithelium. Plasmodia were also found developing within the bile ducts. Some plasmodia were small and thin, almost worm-like, while larger plasmodia took on various distended, globular shapes that curled up, almost completely obstructing the bile duct (Fig. 14).

***Kudoa* sp.**

***Kudoa* sp. is a histozoic species that was found in the muscles of fish from Granger Bay and Kommetjie. This species was first detected in bile from gall bladder squashes examined at 1000x under oil immersion using the thin-layer agar technique; *Kudoa* sp. spores were not detected during the preliminary scans from November 2008 - July 2009. Therefore, summer and winter prevalences are unknown. Fish from Jacobsbaai were not examined for this species as *Kudoa* sp. was only detected after the period allocated for catching *M dorsal is* from Jacobsbaai. Based on the presence of mature spores in bile, the prevalence of *Kudoa* sp. was 32% (N = 34) from Granger Bay and 17% (N = 6) from Kommetjie. When present in the bile, mature spores were exceptionally rare and difficult to find.**

Mature spores were very simple: rounded quadrate and asymmetrical in apical view (Figs. 15, 18) and subspherical to ovoid in lateral view (Figs. 16, 19). Sometimes, one valve was larger than the other equally-sized three (Figs. 17, 20). As there were only a few spores available for measurement, most of which were in lateral view, making it difficult to distinguish the larger polar capsule, an overall average is provided. The spores (N = 13) measured 5.1 ± 0.4 (4.5-5.8) gm long, 5.9 ± 0.7 (4.5-7.5) gm wide, and 5.4 ± 0.2 (5.2-5.6) gm thick. Polar capsules were pyriform. In some cases, spores possessed three equally-sized polar capsules and a fourth, thicker polar capsule situated within the larger valve. They measured 2.5 ± 0.5 (1.6-3.7) gm long and 1.8 ± 0.4 (1.3-2.6) gm wide.

Histological sections revealed that *Kudoa* sp. formed numerous plasmodia within the skeletal muscles of the host (Fig. 21). Only muscles from the thorax were examined. Plasmodia were round to rounded polygonal in shape and often occurred singularly in muscle fibers. However, pairs of plasmodia were occasionally observed within a single muscle fiber (Fig. 22). There appeared to be no tissue reaction, with neither alteration of either myomeres nor muscle fibers, as a result of infection.

***Myxobolus* sp.**

***Myxobolus* sp. is a histozoic species infecting the eye of *M dorsalis.* This species only infected fish from Jacobsbaai and Kommetjie. Summer and winter prevalences are presented in Table 3. *Myxobolus* sp. formed white, round to elongated oval plasmodia on the sclera along the anterior margin of the eye (Fig. 23). Plasmodia were approximately 0.25-2.0 mm in diameter and occurred either singularly or in clusters of up to 13 on a single eye. Infections could occur on only one eye of the host or on both. Numbers of plasmodia on each eye typically differed for hosts with both eyes infected. The maximum number of plasmodia recorded on a single host was 27.**

Mature spores were ovoid in valvular view (Figs. 24-26). Due to the rareness of *Myxobolus* sp. after July 2009, only a few live spores were available for measurement. Of these, only two spore photographs were clear enough for measurement. They measured 12.8 ± 0.4 (12.6-13.3) µm in length and 13.7 ± 2.1 (11.4-15.4) µm in width. The suture line was not observed. The polar capsules were pyriform and equal, measuring 5.1f 0.5 (4.4-5.8) in length and 4.0 ± 0.5 (3.2-4.5) pm in width. A single spherical iodinophilous vacuole was present in the sporoplasm of each spore. In addition to the mature spores, disporic pansporoblasts were present in the plasmodia (Fig. 25).

***Ortholinea* sp.**

***Ortholinea* sp. (Figs. 27, 28) was found floating freely in the bile during morphological examinations of live spores. As with *Kudoa* sp., *Ortholinea* sp. was not detected during the preliminary scans, hence summer and winter prevalences are unknown. *Ortholinea* sp. was detected from fish from Granger Bay and Kommetjie; fish from Jacobsbaai were not examined for this species. Prevalence was 30% (N = 6) at Kommetjie and 12% (N = 34) at Granger Bay. When present in the bile, mature spores were exceptionally rare and difficult to find. Ten spores were measured. Spores were spherical, measuring in diameter 8.8 ± 0.8 (7.2-10.2)1.1m. Only one subspherical polar capsule was visible in each spore, measuring 2.9 ± 0.5 (2.4-3.4) m long and 2.4 ± 0.3 (2.0-2.9) µm wide.**

Mixed Infections

Mixed infections, where two or more species share the same infection site, were observed in the gall bladder and bile ducts. The most prevalent and noticeable mixed infection was that of *Ceratomyxa* sp. and *Sphaeromyxa* sp. 1 at Granger Bay. Thirteen percent (N = 22) of the fish had *Ceratomyxa-Sphaeromyxa* mixed infections during the summer compared to 42% (N = 31) in the winter. Examinations of gall bladders infected concurrently by the two species revealed simultaneous development of pansporoblasts along the gall bladder epithelium. Histological sections also revealed co-development of *Ceratomyxa* sp. and *Sphaeromyxa* sp.1 plasmodia in the bile ducts (Fig. 29).

Mature *Kudoa* sp. spores were always found in conjunction with either *Ceratomyxa* sp. or *Sphaeromyxa* sp. 1, or both. *Ortholinea* sp. was always found in conjunction with *Ceratomyxa* sp. but also occurred with a mixed *Ceratomyxa-Sphaeromyxa* infection, resulting in a triple infection (Fig. 30). In two instances, the bile of two fish contained the spores of all four species.

Discussion

The following remarks compare the species from the current study to described species from around the world to deduce whether or not those from the current study can be identified as already described species. Spore and plasmodia morphology, site of infection, host locality, and host taxonomy are all factors used for comparisons. Only marine species are compared, as marine and freshwater myxosporeans are split into two phylogenetic lineages (Fiala, 2006). Pathogenicity is also discussed.

Remarks on Ceratomyxa sp.

Genus *Ceratomyxa* Thélohan, 1892 boasts at least 172 documented species (Lom and Dykova, 2006), 17 of which have been described from the coast of Africa (Table 4). The ceratomyxid of the present study exhibits great morphological plasticity and is similar in appearance to a few of its African congenerics. *Ceratomyxa australis* Gaevskaya et Kovaleva, 1979, which infects the gall bladder of *Trachurus trachurus capensis* in Namibia, possesses similar physical attributes to some morphs of *Ceratomyxa sp*. However, *C. australis* is smaller, with spore and polar capsule measurements overlapping with only the lower ends of the ranges. Also similar to *Ceratomyxa* sp. is *C. honckenii* Reed, Basson, Van As et Dykova., 2007, which infects the gall bladder of *Amblyrhynchotes honckenii.* Though the spore and polar capsule measurements are similar, t-tests reveal that the spore widths and polar capsule lengths and widths of *C. honckenii* are significantly different (p = 0.0001, 0.001, 0.001, respectively), which strongly suggests that *Ceratomyxa* sp. is indeed not *C. honckenii.*

A more likely candidate for *Ceratomyxa* sp. is *C. cottoidii* Reed, Basson, Van As et Dykova, 2007, which infects the gall bladder of *Clinus cottoides,* because its spores are morphologically similar to those of *Ceratomyxa* sp. and because *C. cottoidii* infects a clinid that

is sympatric with *M dorsalis.* However, t-tests show that though spore and polar capsule lengths are not statistically different (p = 0.45 and 0.58, respectively), spore and polar capsule widths are (p = 0.0004 and 0.0035, respectively). Reed et al. (2007) did not note *C. cottoidii* plasmodia in the bile ducts of *C. cottoides,* so their presence or absence in that site is unknown. The significant differences in spore and polar capsule widths suggest that *Ceratomyxa* sp. and *C. cottoidii* are not the same species, but more evidence, such as DNA sequences and comparisons of plasmodia, are recommended to verify this.

Spore anomalies, which include anomalies in the position and number of the polar capsules, were documented as early as 1895 by Thélohan. The presence of abnormal, trivalvular spores is not uncommon for *Ceratomyxa* spores (Auerbach, 1912; Meglitsch, 1960; Sitjà-Bobadilla et al., 1995; Yokohama and Fukuda, 2001; Aseeva, 2003; Maillo-Bellón and Gracia-Royo, 2007), nor is the current documentation of abnormal spores the first in Africa. *Ceratomyxa bassoni* Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar 2008, described from the gall bladder of *Plectorhinchus gaterinus* in Egypt, has also been documented as having abnormal, triradiate spores.

The infection of *M dorsalis* by *Ceratomyxa* sp. appears to have no consequences for the fish, as with the case for many cases of gall bladder infection by myxosporeans (Lom and Dyková, 1995). However, some cases involving coelozoic plasmodia may result in inflammation and eventually jaundice. Infection of bile ducts may result in lesions, resulting in pericholangitis or complete blockage of the bile ducts (Lom and Dykova, 1995).

Remarks on *Sphaeromyxa* sp. 1

Myxozoan species from Genus *Sphaeromyxa* Thélohan, 1892 are coelozoic parasites of marine fish gall bladders (Lom, 2004). Species from this genus have been recorded four times in

Africa; first *S. balbianii* Thélohan, 1892 in *Abudefduf marginatus* and *Sardinella maderensis* off the coast of Senegal by Kpatcha et al. (1996a), then *S. arcuata* Fantham, 1930 from *Argyrozona argyrozona* and *S. curvula* Fantham, 1930 from *Pachymetopon blochii*, both from the coast of South Africa (Fantham, 1930), and finally *Sphaeromyxa* Thélohan, 1892 sp. in *Pavoclinus graminis* off the coast of South Africa by Reed et al. (2009). Both species described by Fantham have been deemed *nomina nuda.* The present study is the most recent record of a species from the genus *Sphaeromyxa* from the African coast, raising the number of recorded *Sphaeromyxa* species in the world to 40 (see Diamant et al., 2004; Lom, 2004; Reed et al., 2009).

Genus *Sphaeromyxa* is divided into two morphological groups: the Incurvata group, composed of species with arcuate spores and pyriform polar capsules, and the Balbiani group, composed of species with straight or slightly curved, fusiform or ovoid spores with ovoid polar capsules (Laird, 1953). Only 11 species belong to the Incurvata group (Table 5).

When compared to the described species from the Incurvata group, *Sphaeromyxa* sp. 1 emerges as a morphologically distinct species. *Sphaeromyxa parva* Dogiel, 1948, described from *Pholis pictus*, and *S. hexagrammi* Dogiel, 1948, which infects a variety of scorpaenids from the Japan Sea, produce spores that are considerably shorter than those of *Sphaeromyxa* sp. 1. Contrastingly, *S. cottidarum* Dogiel, 1948, known to infect various scorpaenid fishes from the Japan Sea as well as the Bering Sea and Atlantic Ocean; *S. solomoni* Aseeva, 2002, which parasitizes *Gymnocanthus pistilliger* and *Enophrys dicerus* in the Japan Sea; and *S. exneri* Awerinzew, 1913, which has been found *Thrysanophrys japonicus* in the Japan Sea and *Sarritor leptorhynchus* in the Indian Ocean; produce spores which are markedly longer than those of *Sphaeromyxa* sp. 1. *Sphaeromyxa hellandi* Auerbach 1909, described from *Molva molva* off the coast of Norway, though very similar in shape to *Sphaeromyxa* sp. 1, is slightly larger with

longer spores and polar capsules. *Sphaeromyxa noblei* Lom, 2004, described from *Heteroclinus whiteleggii* captured from the Australian coast, produces spores that are approximately the same size as *Sphaeromyxa* sp. 1, but they are slightly more arched than those of *Sphaeromyxa* sp 1. Furthermore, the spores of *S. noblei* are less sinusoidal in sutural view. Finally, the polar capsules of *S. noblei* are slightly smaller than those of *Sphaeromyxa* sp. 1. *Sphaeromyxa sabrazesi* Laveran et Mesnil, 1900, found in *Hippocampus brevirostris, H. guttulatus, Syngnathus acus,* and *Motella tricirrata* in the Mediterranean Sea and the Atlantic Ocean, and *S. tripterygii* Laird, 1953, which was described from *Forsterygion varium* and *Bellapiscis medius* from New Zealand's intertidal zone, also produce spores comparable in length to that of *Sphaeromyxa* sp. 1. However, the spores and polar capsules of both species are narrower than those of *Sphaeromyxa* sp. 1. Furthermore, the spore ends of *S. tripterygii* are not as rounded. *Sphaeromyxa* sp. , which also infects an intertidal clinid, possesses spores that are much more arched than those of *Sphaeromyxa* sp. 1. Also, the polar capsules of *Sphaeromyxa* sp. appear more elongated than those of *Sphaeromyxa* sp. 1.

The arched spores of *Sphaeromyxa* sp. 1 are unique in comparison to those of the others because of their obliquely-crossing suture line. All the spores in the Incurvata group possess sutures lines that bisect the spore longitudinally following the shape of the spore. Hence, *Sphaeromyxa* sp. 1 is likely a new species.

Thus far, *Sphaeromyxa* spp. have yet to be proven to be pathogenic (Lom and Dykova, 2006).

Remarks on *Kudoa* sp.

Genus *Kudoa* Meglitsch, 1947, with its four shell valves and four polar capsules, has been assigned to the order Multivalvulida Shulman, 1959 (Lom and Dykova, 2006). Only four

species of *Kudoa* are known from the African coast (Table 6). Of these, only *K boopsi* Kpatcha, Diebekate, Faye et Toguebaye, 1999, described from the gills of Senegalese *Boops hoops*, bears much resemblance to *Kudoa* sp. The two share similar spore and polar capsule shapes in apical view. However, in lateral view, *K. boopsi* is much more ellipsoidal than *Kudoa* sp. Also, *K. boopsi* infects the gill filaments, unlike *Kudoa* sp. *Kudoa cynoglossi* Obiekezie et Lick, 1994, described from the muscles of *Cynoglossus senegalensis* in Nigeria, has spores that are much longer and adorned with an apical projection while the spores of *K. thyrsites* Gilchrist, 1924, described from the muscles of South African *Thyrsites atun*, and *K pagrusi* Al Quraishy, Koura, Abdel-Baki, Bashtar, El Deed, Al-Rasheid et Ghaffar, 2008, which infects the heart of Egyptian *Pagrus pagrus*, are larger and stellate.

The very simple, rounded spores of *Kudoa* sp. are not common amongst its congenerics; many of those that are rounded quadrate in apical view (Table 6) have protrusions or thickened valves at the apical end, such as *K inornata* Dykova, de Buron, Fiala et Roumillat, 2009, which infects the muscles of *Cynoscion nebulosus* from the United States, or *K. dianae* Dykova, Avila et Fiala, 2002, which infects the connective tissue and mesentery of *Sphoeroides annulatus* from Mexico. *Kudoa paralichthys* Cho et Kim, 2003, described from the brain of *Paralichthys olivaceus* in South Korea, lacks protrusions. However, in lateral view, it is more vertically compressed than *Kudoa* sp. Furthermore, *K paralichthys* has relatively small polar capsules; *Kudoa* sp. has polar capsules that occupy a greater portion of the space in the valves than its most morphologically similar congenerics. *Kudoa paniformis* also possesses inornate, rounded quadrate spores. The spore dimensions of this species are approximately the same as those of *Kudoa* sp. As insufficient data was provided for the measurements of *K. paniformis*, spore and polar capsules could not be compared statistically. Though *K. paniformis* and *Kudoa* sp. infect

fishes from different families, they cannot be deemed different species based on hosts, but possibly based on locality, as some species of *Kudoa* are considered to be habitat-, not host-, specific (Whipps et al., 2003; Whipps and Kent, 2006; Burger et al., 2008).

The detection of mature spores in the bile is highly unusual as *Kudoa* is commonly histozoic in muscles (Lom and Dykova, 2006) and very rarely coelozoic, especially in gall bladders (see Sarkar and Mazumder, 1983; Sarkar and Ghosh, 1991; Campbell, 2005). The presence of spores in the bile could be the result of contamination from the removal of the gall bladder.

Despite the presence of many plasmodia in fish fixed for histology, the presence of plasmodia was not obvious to the naked eye. Longitudinal sections would be useful for examining the shape of plasmodia along the length of the fish and the depth of plasmodial infection within a muscle fiber. Though the fish that were examined did not exhibit soft flesh, it is possible that *Kudoa* sp. plasmodia cause muscle degradation since species from the genus *Kudoa* are well known to cause tissue degradation in a broad range of hosts (Egusa, 1986; Moran et al., 1999). The intracellular localization of developmental stages can result in hypertrophy, hyperplasia and tissue death (Lom and Dykova, 1995).

***Remarks on Myxobolus* sp.**

Ten species of *Myxobolus* have been described from fishes from the coast of Africa (Table 7). None of the species documented in Africa have spores that are wider than they are long. This may simply be the artifact of measuring only two spores. More spores need to be measured to obtain more precise values of spore measurements.

When compared to African myxobolids, *Myxobolus* sp. looks similar to *M miilleri* Btitschli, 1882, described from the gills of *Mugil cephalus* off the coast of Senegal, with

approximately the same spore and polar capsule lengths. However, the spores and polar capsules of *M mulleri* appear more elongated than those of *Myxobolus* sp. *Myxobolus mulleri* also lacks iodinophilous vacuoles. *Myxobolus* sp. looks similar to *M ichkeulensis* Bahri et Marques, 1996, described from the gills of *Mugil cephalus* in Tunisia, since the spores and polar capsules of the two are approximately the same size and shape. However, *M ichkeulensis* possesses two iodinopholous vacuoles, as opposed to the one of *Myxobolus* sp., and the polar capsules appear slightly more distant from one another. Furthermore, the polar capsules of *M ichkeulensis* sit more vertically upright while *Myxobolus* sp. has polar capsules that sit angled with the apices leaning toward one another. Finally, both *M mfilleri* and *M ichkeulensis* infect the gill, not the eye.

Though no African myxobolid species is documented as infecting the eye, there are at least 17 described species from other parts or the world infecting this site. Of these, only one is marine: *Myxobolus lairdi* Moser et Noble, 1977 from *Coryphaenoides rupestris* off the coast of Norway. This is the second record of *Myxobolus* infecting the eye of a marine fish. The unique infection site of *Myxobolus* sp. as well as its size and presence of one iodinopholous vacuole are sufficient evidence to warrant description as a new species.

Infection by *Myxobolus* sp. is likely to lead to minimal problems for *M dorsalis* as the site of infection is unlikely to directly interfere with vision. However, it may cause the host other problems, as *Myxobolus* spp. may cause pressure atrophy on surrounding tissue and lead to deleterious effects on the organs of the host from the growth of large plasmodia (Lom and Dykova, 1995). For example, *M petenensis* Frey, Cone et Duobinis-Gray 1998, described from *Dorosoma petenense* in the United States, is a freshwater species that also infects the eye. It causes stretching of the circumorbital integument when plasmodia are numerous and occur in

large aggregates (Frey et al., 1998).

Remarks on *Ortholinea* sp.

Genus *Ortholinea* Shulman, 1962 consists of approximately 12 species (Lom and Dykova, 2006), of which nine are marine (Table 8). The marine species infect the urinary system, except *0. australis,* which infects the gall bladder and biliary ducts. To date, only one species of *Ortholinea* has been described off the coast of Africa: *0. basma* Ali, 2000 from South African *Clinus agilis.* Though *0. basma* infects a clinid host that is sympatric with *M dorsalis,* its spores, which taper anteriorly and posteriorly in sutural view, differ from those of *Ortholinea* sp. Spores of *Ortholinea* sp. also differ from those of *0. orientalis* Shulman et Shulman-Albova, 1953, described from clupeids in the Northern Pacific, and *0. gobiusi* Naidenova, 1968, found in *Gobius ophiocephahis* in the Black Sea, which taper posteriorly to a sharp point. *Ortholinea australis* Lom, Rohde et Dykova, 1992, found in *Acanthopagrus australis* and *Caulopsetta scapha* from Australia, and *0. polymorpha* Davis, 1917, found in *Opsanus tau* and *0. beta* from the Atlantic Ocean of the United States, can be distinguished from the present species by their polar capsules. The polar capsules of *0. australis* occupy a greater proportion of the spore than those of *Ortholinea* sp., while those of *0. polymorpha* are elongated. *Ortholinea* sp. spores differ in shape from those of *0. irregularis* Kabata, 1962, described from *Drepanopsetta platessoides* in the North Sea, which are rounded triangular, and those of *0. divergens* Thélohan, 1895, described from *Hippoglossoides platessoides* in the North Atlantic, which are markedly larger and contain polar capsules that occupy a smaller proportion of the spore. *Ortholinea undulans* Meglisch, 1970, which infects *Peltorhamphns novaezelandiae* in New Zealand, produces spores that are ovoid, not round like those of *Ortholinea* sp., and *0. striateculus* Su et White, 1994,

found in *Leptatherina presbyteroides* in Australia, produces spores that are much larger than those of the present species. Based on these comparisons, *Ortholinea* sp. is a new species.

Pathogenicity has only been documented for *0. australis.* Fish infected by *0. australis* exhibited enlarged gall bladders with thickened walls, hepatic lesions, and distended biliary ducts (Lom et al., 1992). Hepatic disorder or stagnation of the bile out flow was associated with heavy infection (Lom et al., 1992).

Since *Ortholinea* is coelozoic and generally infects the urinary system of marine fishes (Lom and Dykova, 2006), squashes of the urinary system of more fish examined under high magnification are recommended for determining whether the urinary system is the infection site of *Ortholinea* sp.

Remarks on Mixed Infections

Mixed infections by myxozoans in one site are not uncommon and have been documented three times from Africa's coast: *Ceratomyxa entzerothi* and and *Ortholinea* species in the gall bladder of *Valamugil seheli* (Abdel-Ghaffar et al., 2008a), *Myxidium elmatboulii* with *C. ghaffari* in the gall bladder of *Tylosurus choram* (Ali et al., 2006), and *Zschokkella helmii* mixed with a species of *Ceratomyxa* in the gall bladder of *Siganus rivulatus* (Abdel-Ghaffar et al., 2008b). However, what is rare is a mixed infection composed of more than two species. In this study, a mixed infection was found with four species floating in the bile. This is very unusual, although it is possible that the bile had been contaminated by the spores of *Kudoa* sp. and *Ortholinea* sp.

Figure Captions

Figure 1. Map of the western coast of South Africa (insert) and the locations of the three sampling localities.

Figures 2-7. Light micrographs of live *Ceratomyxa* sp. spores infecting the gall bladder of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa. Scale bars = 5 m.

Figure 2. Typical arcuate spore of *Ceratomyxa* sp.

Figure 3. Spore of *Ceratomyxa* sp. with flatter anterior end.

Figure 4. Spore of *Ceratomyxa* sp. with unequal shell valves. Photo courtesy of C.C. Reed.

Figure 5. Abnormal spore with polar capsules located opposite each other.

Figure 6. Abnormal spore with three shell valves and three polar capsules medially located.

Figure 7. Abnormal spore with three shell valves and two medially located polar capsules with a third at the tip of a shell valve.

Figure 8. Line drawing of *Ceratomyxa* sp. spore infecting the gall bladder of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa. Polar filaments are estimates based on what could be seen under a light microscope. Scale bar = 5µm

Figure 9. Light micrograph of a transverse histological section through the liver of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa showing *Ceratamyxa* sp. plasmodia infecting a bile duct, some of which are indicated by arrows. Photo courtesy of C.C. Reed. Scale bar = 20 gm.

Figures 10-11. Light micrographs of live *Sphaeromyxa* sp. 1 spores infecting the gall bladder of

Muraenoclinus dorsalis **Bleeker, 1860 collected from the western coast of South Africa. Scale bars = 5 µm**

Figure 10. ***Sphaeromyxa*** **sp. 1 spore in frontal view.**

Figure 11. ***Sphaeromyxa*** **sp. 1 spore in sutural view.**

Figures 12-13. Line drawings of ***Sphaeromyxa*** **sp. 1 spores infecting the gall bladder of** ***Muraenoclinus dorsalis*** **Bleeker, 1860 collected from the western coast of South Africa. Scale bars = 5 m.**

Figure 12. ***Sphaeromyxa*** **sp. 1 spore in frontal view. Polar filaments are estimates based on what could be seen under a light microscope.**

Figure 13. ***Sphaeromyxa*** **sp. 1 spore in sutural view. Polar capsules are blank because polar filaments could not be observed when spores were in sutural view.**

Figure 14. Light micrograph of a transverse histological section through the liver of ***Muraenoclinus dorsalis*** **Bleeker, 1860 collected from the western coast of South Africa showing** ***Sphaeromyxa*** **sp. 1 plasmodia infecting bile ducts. Photo courtesy of C.C. Reed. Scale bar = 20 1.1m.**

Figures 15-17. Light micrographs of live ***Kudoa*** **sp. spores found floating in the bile of** ***Muraenoclinus dorsalis*** **Bleeker, 1860 collected from the western coast of South Africa. Scale bars = 5 µm**

Figure 15. Spore in apical view. Arrow indicates larger polar capsule.

Figure 16. Spore in lateral view. Arrow indicates larger polar capsule.

Figure 17. Spore in oblique view. Arrow indicates larger polar capsule.

Figures 18-20. Line drawings of ***Kudoa*** **sp. spores observed floating in the bile of** ***Muraenoclinus***

dorsalis **Bleeker, 1860 collected from the western coast of South Africa . Polar capsules left blank because polar filaments could not be observed. Scale bars = 5 m.**

Figure 18. ***Kudoa*** **sp. spore in apical view.**

Figure 19. ***Kudoa*** **sp. spore in lateral view.**

Figure 20. ***Kudoa*** **sp. spore in oblique view.**

Figure 21. Light micrograph of a transverse histological section through skeletal muscle in the trunk of ***Muraenoclinus dorsalis*** **Bleeker, 1860 collected from the western coast of South Africa showing infection by numerous** ***Kudoa*** **sp. plasmodia, some of which are indicated by arrows. Scale bar = 20 m.**

Figure 22. Light micrograph of a transverse histological section through skeletal muscle in the trunk of ***Muraenoclinus dorsalis*** **Bleeker, 1860 collected from the western coast of South Africa showing infection by** ***Kudoa*** **sp. plasmodia. Note two plasmodia simultaneously infecting one muscle fiber. Scale bar = 20 m.**

Figure 23. Photograph of an eye from ***Muraenoclinus dorsalis*** **Bleeker, 1860 collected from the western coast of South Africa eye in oblique view showing two** ***Myxobolus*** **sp. plasmodia. Scale bar = 500 m.**

Figures 24-25. Light micrographs of live ***Myxobolus*** **sp. spores infecting the eye of** ***Muraenoclinus dorsalis*** **Bleeker, 1860 collected from the western coast of South Africa. Scale bars = 5µm**

Figure 24. Mature spore with iodinophilous vacuole indicated by arrow.

Figure 25. Disporous pansporoblast with iodinophilous vacuole indicated by arrow.

Figure 26. Line drawing of ***Myxobolus*** **sp. spore in sutural view infecting the eye of**

Muraenoclinus dorsalis **Bleeker, 1860 collected from the western coast of South Africa. Polar capsules left blank because polar filaments could not be observed. Scale bar = 5µm.**

Figure 27. Light micrograph of live *Ortholinea* sp. spore in sutural view found floating in the bile of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa. Scale bar = 5µm.

Figure 28. Line drawing of *Ortholinea* sp. spore in sutural view floating in the bile of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa. Polar capsule left blank because polar filaments could not be observed. Scale bar = 5µm.

Figure 29. Light micrograph of *Ceratomyxa* sp. (C) and *Sphaeromyxa* sp. 1 (S) plasmodia, some of which are indicated by arrows, infecting the bile ducts of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa. Scale bar = 20 µm.

Figure 30. Light micrograph of live spores found in floating in the bile of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa showing the presence of *Ceratomyxa* sp. (C), *Sphaeromyxa* sp. 1 (S), and *Ortholinea* sp. (0). Scale bar = 5µm.

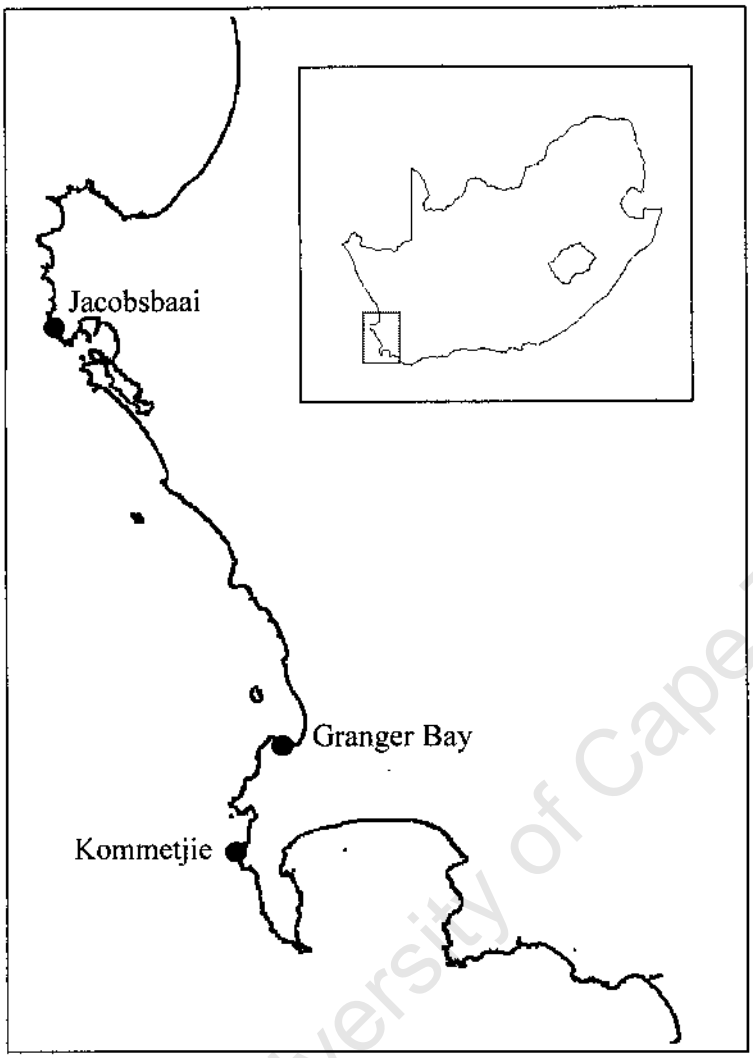

Figure 1

Table 1. Myxozoan species known from marine fishes along the coast of Africa.

Species	Host	Locality	Site of Infection	Reference
Alataspora africana Shulman, Kovaleva et Dubina, 1979	*Callanthias ruber*	Western Sahara	Gall bladder	Shulman et al., 1979
Alataspora contrariocapsularia Shulman, Kovaleva et Dubina, 1979	*Macrorhamphosus gracilis*	Western Sahara	Gall bladder	Shulman et al., 1979
Alataspora subtilis Kovaljova, Velev et Vladev, 1993	*Epinephelus aeneus*	Mauritania	Gall bladder	Kovaljova et al., 1993
Alatospora samaroidea Shulman, Kovaleva et Dubina, 1979	*Chlorophthalmus atlanticus*	Atlantic coast of Africa	Gall bladder	Shulman et al., 1979
Bipteria merluccii Kovaljova, Velev et Vladov, 1993	*Merluccius pollii*	Guinea Bissau	Gall bladder	Kovaljova et al., 1993
Ceratomyxa acanthuri Kpatcha, Diebakate, Faye et Toguebaye, 1996	*Acanthurus monroviae*	Senegal	Gall bladder	Kpatcha et al., 1996b
Ceratomyxa australis Gaevskaya et Kovaleva, 1979	*Trachurus trachurus capensis*	Namibia	Gall bladder	Gaevskaya and Kovaleva, 1979
Ceratomyxa bassoni Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008	*Plectorhinchus gaterinus*	Egypt	Gall bladder	Abdel-Ghaffar et al., 2008a
Ceratomyxa cottoidii Reed, Basson, Van As et Dyková, 2007	*Clinus cottoides*	South Africa	Gall bladder	Reed et al., 2007
Ceratomyxa dehoopi Reed, Basson, Van As et Dyková, 2007	*Clinus superciliosus*	South Africa	Gall bladder and liver	Reed et al., 2007
Ceratomyxa entzerothi Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008	*Valamugil seheli*	Egypt	Gall bladder	Abdel-Ghaffar et al., 2008a
Ceratomyxa fistulariae Kpatcha, Diebakate, Faye et Toguebaye, 1996	*Fistularia petimba*	Senegal	Gall bladder	Kpatcha et al., 1996b
Ceratomyxa ghaffari Ali, Abdel-Baki et Sakran, 2006	*Tylosurus choram*	Egypt	Gall bladder	Ali et al., 2006
Ceratomyxa honckenii Reed, Basson, Van As et Dyková, 2007	*Amblyrhynchotes honckenii*	South Africa	Gall bladder	Reed et al., 2007
Ceratomyxa hurghadensis Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008	*Fistularia commersonii*	Egypt	Gall bladder	Abdel-Ghaffar et al., 2008a
Ceratomyxa lagocephali Kpatcha, Diebakate, Faye et Toguebaye, 1996	*Lagocephalus laevigatus*	Senegal	Gall bladder	Kpatcha et al., 1996b

Table 1 (continued).

Species	Host	Locality	Site of Infection	Reference
Ceratomyxa schulmani Dubina et Isakov, 1976	*Alepocephalus australis*	South Africa	Gall bladder	Dubina and Isakov, 1976
Ceratomyxa swaisi Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008	*Saurida undosquamis*	Egypt	Gall bladder	Abdel-Ghaffar et al., 2008a
Ceratomyxa syacil Kpatcha, Diebakate, Faye et Toguebaye, 1996	*Syacium micrurum*	Senegal	Gall bladder	Kpatcha et al., 1996b
Ceratomyxa trachinocephali Kpatcha, Diebakate, Faye et Toguebaye, 1996	*Trachinocephalus myops*	Senegal	Gall bladder	Kpatcha et al., 1996b
Ceratomyxa trichiuri Kpatcha, Diebakate, Faye et Toguebaye, 1996	*Trichiurus lepturus*	Senegal	Gall bladder	Kpatcha et al., 1996b
Ceratomyxa truncota Kpatcha, Diebakate, Faye et Toguebaye, 1996	*Sardinella maderensis, S. aurila*	Senegal	Gall bladder	Kpatcha et al., 1996b
Chloromyxum dogieli Kovaleva, 1988	*Raja miraletus*	Guinea Bissau	Gall bladder	Kovaleva, 1988
Chloromyxum lissosporum Kovaleva, 1988	*Squatina oculata*	Guinea Bissau	Gall bladder	Kovaleva, 1988
Chloromyxum schulmani Kovaleva, 1988	*Raja streleni*	Western Sahara	Gall bladder	Kovaleva, 1988
Chloromyxum striatellus Kovaleva, 1988	*Scyliorhinus canicula*	Western Sahara	Gall bladder	Kovaleva, 1988
Davisia donecae Gaevskaya et Kovaleva, 1979	*Trachurus trachurus capensis*	Namibia	Urinary bladder	Gaevskaya and Kovaleva, 1979
Henneguya brachydeuteri Kpatcha, Faye, Diebakate, Fall et Toguebaye, 1997	*Brachydeuterus auritus*	Senegal	Heart	Kpatcha et al., 1997
Henneguya clini Reed, Basson, Van As et Dyková, 2007	*Clinus superciliosus, C. cottoides*	South Africa	Gills, gill arches	Reed et al., 2007
Henneguya joalensis Kpatcha, Faye, Diebakate, Fall et Toguebaye, 1997	*Cephalopholis taeniops*	Senegal	Kidney	Kpatcha et al., 1997
Henneguya kayarensis Kpatcha, Faye, Diebakate, Fall et Toguebaye, 1997	*Galeoides decadactylus*	Senegal	Liver	Kpatcha et al., 1997
Henneguya lutjani Kpatcha, Faye, Diebakate, Fall et Toguebaye, 1997	*Lutjanus agennes*	Senegal	Gills	Kpatcha et al., 1997
Henneguya mbourensis Kpatcha, Faye, Diebakate, Fall et Toguebaye, 1997	*Dentex canariensis*	Senegal	Kidney	Kpatcha et al., 1997
Henneguya priacanthi Kpatcha, Faye, Diebakate, Fall et Toguebaye, 1997	*Priacanthus arenatus*	Senegal	Gills	Kpatcha et al., 1997

Table 1 (continued).

Species	Host	Locality	Site of Infection	Reference
Henneguya Thélohan, 1892 sp. 1	*Brachydeuterus auritus*	Senegal	Heart	Faye et al., 1997
Henneguya Thélohan, 1892 sp. 2	*Pagrus caeruleostictus*	Senegal	Heart	Faye et al., 1997
Henneguya Thélohan, 1892 sp.3	*Mugil cephalus*	Senegal	Heart	Faye et al., 1997
Henneguya yoffensis Kpatcha, Faye, Diebakate, Fall et Toguebaye, 1997	*Pagrus caeruleostictus*	Senegal	Gills, heart	Kpatcha et al., 1997
Henneguya ouakamensis Kpatcha, Faye, Diebakate, Fall et Toguebaye, 1997	*Mugil cephalus*	Senegal	Gills, heart	Kpatcha et al., 1997
Kentmoseria asymmetrica Kovaljova, Velev et Vladev, 1993	*Decapterus rhonchus*	Sierra Leone	Gall bladder	Kovaljova et al., 1993
Kentmoseria macruri Kovaljova, Velev et Vladev, 1993	*Macrourus fasciatus*	Namibia	Urinary bladder	Kovaljova et al., 1993
Kudoa boopsi Kpatcha, Diebakate, Faye et Toguebaye, 1999	*Boops boops*	Senegal	Gills	Kpatcha et al., 1999
Kudoa cynoglossi Obiekezie et Lick, 1994	*Cynoglossus senegalensis*	Nigeria	Muscle	Obiekezie and Lick, 1994
Kudoa pagrusi Al Quraishy, Koura, Abdel-Baki, Bashtar, El Deed, Al Rasheid et Abdel Ghaffar, 2008	*Pagrus pagrus*	Egypt	Heart	Al Quraishy et al., 2008
Kudoa thyrsites Gilchrist, 1924	*Thyrsites atun*	South Africa	Muscle	Gilchrist, 1924
Leptotheca lutjani Kpatcha, Diebakate et Toguebaye, 1996	*Lutjanus fulgens*	Senegal	Gall baldder	Kpatcha et al., 1996a
Leptotheca pegusae Kpatcha, Diebakate et Toguebaye, 1996	*Pegusa lascaris*	Senegal	Gall baldder	Kpatcha et al., 1996a
Myxidium abudefdufi Kpatcha, Diebakate et Toguebaye, 1996	*Abudefduf marginatus*	Senegal	Gall baldder	Kpatcha et al., 1996a
Myxidium aydai Abdel-Baki, 2009	*Caesio suevicus*	Egypt	Gall baldder	Abdel-Baki, 2009
Myxidium elmatboulii Ali, Abdel-Baki, Sakran, 2006	*Tylosurus choram*	Egypt	Gall bladder	Ali et al., 2006
Myxidium elopsi Kpatcha, Diebakate et Toguebaye, 1996	*Elops sengalensis*	Senegal	Intestine	Kpatcha et al., 1996a
Myxidium maamouni Abdel-Baki, 2009	*Cheilopogon nigricans*	Egypt	Gall bladder	Abdel-Baki, 2009
Myxidium ovale Kovaljova, Velev et Vladev, 1993	*Auxis thazard*	Sierra Leone	Gall bladder	Kovaljova et al., 1993
Myxobolus bizerti Bahri et Marqués, 1996	*Mugil cephalus*	Tunisia	Gill filaments	Bahri and Marqués, 1996

Table 1 (continued).

Species	Host	Locality	Site of Infection	Reference
Myxobolus exiguus Thélohan, 1895	*Mugil cephalus*	Senegal	Gills	Bahri et al., 2005
Myxobolus goreensis Fall, Kpatcha, Diebakate, Faye et Toguebaye, 1997	*Mugil cephalus*	Senegal	Gills	Fall et al., 1997
Myxobolus hani Faye, Kpatcha et Diebakate, 1999	*Mugil curema*	Senegal	Gills	Faye et al.,1999
Myxobolus hannensis Fall, Kpatcha, Diebakate, Faye et Toguebaye, 1997	*Mugil cephalus*	Senegal	Gills	Fall et al., 1997
Myxobolus ichkeulensis Bahri et Marqués, 1996	*Mugil cephalus*	Tunisia	Gill Arches	Bahri and Marqués, 1996
Myxobolus lubati Ali, Abdel-Baki, Sakran, Entzeroth et Abdel-Ghaffar, 2007	*Rhabdosargus haffara*	Egypt	Intestine	Ali et al., 2007b
Myxobolus muelleri Butschli, 1882	*Mugil cephalus*	Senegal	Gills	Bahri et al., 2005
Myxobolus raibauti Fall, Kpatcha, Diebakate, Faye et Toguebaye, 1997	*Mugil cephalus*	Senegal	Liver	Fall et al., 1997
Myxobolus stomum Ali, Abdel-Baki, Sakran, Entzeroth et Abdel-Ghaffar, 2003	*Plectorhynicus gaterinus*	Egypt	Oral cavity, lips	Ali et al., 2003
Ortholinea basma Ali, 2000	*Clinus agilis*	South Africa	Gall bladder	Ali, 2000
Palliatus indecorus Shulman, Kovaleva et Dubina, 1979	*Alepocephalus rostratus*	Guinea Bissau	Gall bladder	Shulman et al., 1979
Palliatus mirabilis Shulman, Kovaleva et Dubina, 1979	*Xenodermichthys copei*	Guinea-Bissau	Gall bladder	Shulman et al., 1979
Pseudalataspora atlantica Kovaljova, Velev et Vladev, 1993	*Chlorophthalmus agazizi*	Angola	Gall bladder	Kovaljova et al., 1993
Pseudalataspora indecora Kovaljova, Velev et Vladev, 1993	*Dentex angolensis*	Sierra Leone	Gall bladder	Kovaljova et al., 1993
Pseudalataspora insolita Kovaljova, Velev et Vladev, 1993	*Decapterus rhonchus*	Sierra Leone	Gall bladder	Kovaljova et al., 1993
Sphaeromyxa Thélohan, 1892 sp.	*Pavoclinus graminis*	South Africa	Gall bladder	Reed et al., 2009
Unicapsula marquesi Diebakate, Fall, Faye et Toguebaye, 1999	*Polydactylus quadrifilis*	Senegal	Gall bladder	Diebakate et al., 1999
Zschokkella egyptica Ali, Abdel-Bakii et Abdel-Ghaffar, 2007	*Plotosus lineatus, Upeneus tragula*	Egypt	Gall bladder	Ali et al., 2007a
Zschokkella helmii Abdel-Ghaffar, Ali, Al Quraishy, Entzeroth, Abdel-Baki, Al Farraj et Bashtar, 2008	*Siganus rivulatus*	Egypt	Gall bladder	Abdel-Ghaffar et al., 2008b
Zschokkella mugilidae Kpatcha, Diebakate et Toguebaye, 1996	*Mugil cupurii*	Senegal	Gall bladder	Kpatcha et al., 1996a

Table 2. Summer 2008/2009 and Winter 2009 prevalences of *Ceratomyxa* sp. infecting the gall bladder of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa.

Locality	Season	N	Prevalence (%)
Granger Bay	Summer	22	91
	Winter	31	100
Kommetjie	Summer	31	84
	Winter	30	100
Jacobsbaai	Summer	21	86
	Winter	32	97

Table 3. Summer 2008/2009 and Winter 2009 prevalences of *Myxobolus* sp. infecting the eye of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa.

Locality	Season	N	Prevalence (%)
Kommetjie	Summer	31	16
	Winter	30	17
Jacobsbaai	Summer	21	24
	Winter	32	28

Table 4. Spore measurements of *Ceratomyxa* Thélohan, 1892 species described from marine fishes along the coast of Africa, including the one from the present study. Mean ± SD (Range) in µm. PC-polar capsules.

Species	Spore length	Spore width	PC length	PC width	Reference
Ceratomyxa acanthuri Kpatcha, Diebakate, Faye et Toguebaye, 1996	10.54±0.8 (10.0-12.0)	16.57±0.9 (16.0-18.0)	2.75±0.6 (2.0-3.20)	-	Kpatcha et al., 1996b
Ceratomyxa australis Gaevskaya et Kovaleva, 1979	4.0-5.3	13.3-15.0	2.0-2.6	1.3	Gaevskaya and Kovaleva, 1979
Ceratomyxa bassoni Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008	6.1±0.6 (5.0-7.0)	18.0±1.7 (15.0-20.0)	3.2±0.3 (3.0-4.0)	2.4±0.3 (2.0-3.0)	Abdel-Ghaffar et al., 2008a
Ceratomyxa cottoidii Reed, Basson, Van As et Dyková, 2007	7.1±0.6 (6.5-8.0)	18.2±1.7 (17.0-22.0)	2.7±0.4 (2.3-3.0)	2.4±0.4 (2.0-3.0)	Reed et al., 2007
Ceratomyxa dehoopi Reed, Basson, Van As et Dyková, 2007	4.5±0.5 (4.0-5.5)	15.4±1.4 (12.0-17.5)	2.7±0.3 (2.0-3.0)	2.1±0.2 (2.0-2.5)	Reed et al., 2007
Ceratomyxa entzerothi Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008	10.2 ±0.7 (9.0-11.0)	36.5±4.3 (30.0-46.0)	5.0±0.6 (4.0-6.0)	3.1±0.2 (3.0-3.5)	Abdel-Ghaffar et al., 2008a
Ceratomyxa fistulariae Kpatcha, Diebakate, Faye et Toguebaye, 1996	10.25±0.6 (10.0-12.0)	39.64±0.56 (38.8-40.0)	5.21±0.5 (4.5-5.5) in diameter	-	Kpatcha et al., 1996b
Ceratomyxa ghaffari Ali, Abdel-Baki et Sakran, 2006	7.6±1.1 (6.0-9.0)	29.9±3.6 (25.0-33.0)	3.3±0.4 (3.0-4.0) in diameter	-	Ali et al., 2006
Ceratomyxa honckenii Reed, Basson, Van As et Dyková, 2007	7.8±0.3 (7.5-8.0)	19.0±1.4 (18.0-21.0)	3.1±0.08 (3.0-3.2)	3.0±0.04 (3.0-3.1)	Reed et al., 2007
Ceratomyxa hurghadensis Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008	9.0±0.7 (8.0-11.0)	48.0±0.9 (46.0-52.0)	4.5±0.4 (4.0-50)	2.5±0.3 (2.0-3.0)	Abdel-Ghaffar et al., 2008a
Ceratomyxa lagocephali Kpatcha, Diebakate, Faye et Toguebaye, 1996	21.68±0.6 (20.0-22.5)	9.28±0.4 (9.0-10.50)	4.29±0.48 (3.5-4.5) in diameter	-	Kpatcha et al., 1996b
Ceratomyxa schulmani Dubina et Isakov, 1976	17	120	11	10	Dubina and Isakov, 1976
Ceratomyxa swaisi Abdel-Ghaffar, Ali, Al Quraishy, Al Rasheid, Al Farraj, Abdel-Baki et Bashtar, 2008	7.8±0.8 (7.0-9.0)	51.5±1.1 (43.0-55.0)	3.2±0.3 (3.0-4.0)	1.8±0.3 (1.5-2.0)	Abdel-Ghaffar et al., 2008a

Table 4 (continued).

Species	Spore length	Spore width	PC length	PC width	Reference
Ceratomyxa syacil Kpatcha, Diebakate, Faye et Toguebaye, 1996	23.0±0.9 (22.5-25.0)	23.55±0.9 (22.5-25.0)	1.87±0.1 (1.5-2.0) in diameter	-	Kpatcha et al., 1996b
Ceratomyxa trachinocephali Kpatcha, Diebakate, Faye et Toguebaye, 1996	11.5±0.8 (10-12)	49.66±0.7 (48-50)	2.7±0.4 (2.0-3.0) in diameter	-	Kpatcha et al., 1996b
Ceratomyxa trichiuri Kpatcha, Diebakate, Faye et Toguebaye, 1996	10.62±0.9 (10.0-12.0)	99.2±0.9 (98.0-100.0)	4.85±0.2 (4.5-5.0) in diameter	-	Kpatcha et al., 1996b
Ceratomyxa truncata Kpatcha, Diebakate, Faye et Toguebaye, 1996	6.08±0.16 (5.0-7.0)	26.05±0.7 (25.5-27.0)	2.17±0.1 (2.0-2.25) in diameter	-	Kpatcha et al., 1996b
Ceratomyxa sp.	7.3±1.5 (4.4-10.6)	16.3±2.1 (9.6-22.3)	2.6±0.4 (1.8-3.6)	2.2±0.3 (1.5-2.9)	Present study

Table 5. *Sphaeromyxa* Thélohan, 1892 species of the arcuate Incurvata group, including the one from the present study, and spore measurements. Mean ± SD (Range) in µm. PC-polar capsules; np- not provided.

Species	Host	Locality	Spore length	Spore width	PC length	PC width	References
Sphaeromyxa cottidarum Dogiel, 1948	*Hemitripterus villosus, Enophrys diceraus, Enophrys* sp.	Atlantic Ocean, Bering Sea, Japan Sea	33-35	np	np	np	Lom, 2004
Sphaeromyxa exneri Awerinzew, 1913	*Thrysanophrys japonicus, Sarritor leptorhynchus*	Indian Ocean, Japan Sea	75-80	18-20	30-35	np	Laird, 1953
Sphaeromyxa hellandi Auerbach, 1909	*Merlangius merlangus*	Norway	27.4 ± 2.1 (22.5-30.0)	5.8 ± 0.9 (4.5-7.5)	9.3 ± 1.4 (8.5-12.5)	2.6 ± 0.1 (2.5-3.5)	Kalavati and MacKenzie, 1999
Sphaeromyxa hexagrammi Dogiel, 1948	*Hexagrammus octogrammus, H. stelleri, Pleurogrammus azonu*	Japan Sea	18	np	np	np	Lom, 2004
Sphaeromyxa incurvata Doflein, 1898	*Blennius ocellaris, Solea lascaris nasuta*	Meditteranean Sea, Black Sea	30-35 (inner side of arch)	8	12-15	4-5	Laird, 1953
Sphaeromyxa noblei Lom, 2004	*Heteroclinus whiteleggii*	Australia	20 (18.5-21.5)	5.6 (5.2-6)	5.9 (5-6.5)	2.6 (2.5-2.7)	Lom, 2004
Sphaeromyxa parva Dogiel, 1948	*Pholis pictus*	Japan Sea	15-17	np	np	np	Lom, 2004
Sphaeromyxa sabrazesi Laveran et Mesnil, 1900	*Hippocampus brevirostris, H. guttulatus, Syngnathus acus, Motella tricirrata*	Mediterranean Sea, Atlantic Ocean	22-28	3-4.3	8-10	2-3	Laird, 1953
Sphaeromyxa solomoni Aseeva, 2002	*Gymnocanthus pistilliger, Enophrys dicerus*	Japan Sea	25-27.5	np	np	np	Lom, 2004
Sphaeromyxa tripterygii Laird, 1953	*Forsterygion varium, Bellapiscis medius*	New Zealand	17.2-21.2	3.5	4.6-5.1	1.4	Laird, 1953
Sphaeromyxa sp.	*Pavoclinus graminis*	South Africa	26.8 ± 0.5 (26.5-27.5)	5±0.08 (5-5.2)	7.8 ± 0.76 (7.0-9.0)	2.5 ±0.3 (2.0-3.0)	Reed et al., 2009
Sphaeromyxa sp. 1	*Muraenoclinus dorsalis*	South Africa	20.7 ± 1.2 (18.4-25.0)	6.1 ± 0.3 (5.4-6.8)	6.9 ± 0.4 (5.7-8.4)	3.0 ± 0.3 (2.3-3.6)	Present study

Table 6. *Kudoa* Meglitsch, 1947 species with rounded quadrate spores described from some marine fishes, including all those from Africa and the one from the present study, and spore measurements. Mean ± SD (Range) in µm. PC-polar capsules; np-not provided.

Species	Host	Locality	Site of Infection	Spore length	Spore width	Spore thickness	PC length	PC width	Reference
Kudoa boopsi Kpatcha, Diebekate, Faye et Toguebaye, 1999	*Boops boops*	Senegal	Gills	5.73±0.13 (4-6)	8.87±0.18 (8-10)	7.61±0.11 (7-8)	2.98±0.09 (2-4)	1.73±0.07 (1-2)	Kpatcha et al., 1999
Kudoa cynoglossi Obiekezie et Lick, 1994	*Cynoglossus senegalensis*	Nigeria	Muscle	14.1 (13.8-14.4)	6.2 (5.8-6.5)	np	2.9-3.2	np	Obiekezie and Lick, 1994
Kudoa dianae Dyková, Avila et Fiala, 2002	*Sphoeroides annulatus*	Mexico	Connective tissue, mesentery	6 (5.5-6.5)	5 (4.5-5.5)	6 (5.5-6.5)	2	1.5	Dyková et al., 2002
Kudoa inornata Dyková, de Buron, Fiala et Roumillat, 2009	*Cynoscion nebulosus*	USA	Muscle	6.0 (5.9-6.1)	5.4 (5.3-5.55)	5.9 (5.8-6.0)	2.7	np	Dyková et al., 2009
Kudoa pagrusi Al Quraishy, Koura, Abdel-Baki, Bashtar, El Deed, Al-Rasheid et Ghaffar, 2008	*Pagrus pagrus*	Egypt	Heart	7.0 (6.5-8.6)	6.4 (5.8-7.2)	np	3.7 (2.6-4.2)	1.5 (1.0-1.8)	Al Quraishy et al., 2008
Kudoa paniformis Kabata et Whitaker, 1981	*Merluccius productus*	Canada	Muscle	5.9 (5.0-6.5)	5.3 (4.5-6.0)	np	2.6 (1.9-3.3)	np	Kabata and Whitaker, 1981
Kudoa paralichthys Cho et Kim, 2003	*Paralichthys olivaceus*	South Korea	Brain	5.19±0.54	8.23±0.50	6.87±0.45	2.2±0.22	1.2±0.14	Cho and Kim, 2003
Kudoa thyrsites Gilchrist, 1924	*Thrysites atun*	South Africa	Muscle	12.7 (10.0-14.0)	7.1 (6.0-8.0)	np	5.5 (4.5-6.2)	np	Kabata and Whitaker, 1981
Kudoa sp.	*Muraenoclinus dorsalis*	South Africa	Muscle	5.1±0.4 (4.5-5.8)	5.9±0.7 (4.5-7.5)	5.4±0.2 (5.2-5.6)	2.5±0.52 (1.6-3.7)	1.8±0.4 (1.3-2.6)	Present study

Table 7. Spore measurements of *Myxobolus* Bütschli, 1882 species described from marine fishes along the coast of Africa, including the one from the present study. Mean ± SD (Range) in µm. PC-polar capsules; np-not provided.

Species	Spore length	Spore width	PC length	PC width	Reference
Myxobolus bizerti Bahri et Marqués, 1996	14.25 (14-14.5) in diameter	-	6.5 (6-7)	5.75 (5.5-6)	Bahri and Marqués, 1996
Myxobolus exiguus Thélohan, 1895	8-9.5	6-7.5	1.5-3	4.4-4.8	Bahri et al., 2005
Myxobolus goreensis Fall, Kpatcha, Diebakate, Faye et Toguebaye, 1997	10.94±0.68 (10-13) in diameter	-	4.14±0.48 (4-5)	3.08±0.22 (2-4)	Fall et al., 1997
Myxobolus hani Faye, Kpatcha et Diebakate, 1999	8.03±0.48 (7-9)	7.13 ± 0.33 (7-8)	np	np	Faye et al.,1999
Myxobolus hannensis Fall, Kpatcha, Diebakate, Faye et Toguebaye, 1997	13.93±0.44 (13-15) in diameter	-	8.96±0.67 (7-9)	5.73±0.44 (5-6)	Fall et al., 1997
Myxobolus ichkeulensis Bahri et Marqués, 1996	13.5 (13-14)	12.5 (12-13)	5.5 (5-6)	4.15 (4-4.3)	Bahri and Marqués, 1996
Myxobolus lubati Ali, Abdel-Baki, Sakran, Entzeroth et Abdel-Ghaffar, 2007	9.8±0.8 (9.0-11.0)	7.2±1.1 (7.0-9.0)	4.2±0.5 (4.0-5.0)	1.6±0.2 (1.5-2.0)	Ali et al., 2007b
Myxobolus müelleri Bütschli, 1882	12.63±0.65 (12-14)	10.91±0.3 (10-11)	5.13±0.53 (4-6)	3.13±0.49 (3-4)	Bahri et al., 2005
Myxobolus raibauti Fall, Kpatcha, Diebakate, Faye et Toguebaye, 1997	15.33±0.42 (14-16)	12.11±0.19 (12-13)	5.96±0.17 (5-6.5)	3.63±0.48 (3-4)	Fall et al., 1997
Myxobolus stomum Ali, Abdel-Baki, Sakran, Entzeroth et Abdel-Ghaffar, 2003	8.5±0.8 (7.0-10.0)	6.5 ± 0.6 (5.5-7.5)	4.4 ± 0.5 (4.0-5.0)	2.4 ± 0.4 (2.0-3.0)	Ali et al., 2003
Myxobolus sp.	13.0 ± 0.5 (12.6-13.3)	14.9 ± 0.7 (14.4-15.4)	5.2 ±0.6 (4.4-5.8)	4.2 ± 0.4 (3.8-4.5)	Present study

Table 8. *Ortholinea* Shulman, 1962 species described from marine fishes, including the one from the present study, and spore measurements. Mean ± SD (Range) in µm. PC-polar capsules.

Species	Host	Locality	Site of Infection	Spore Length	Spore Width	PC Length	PC Width	Reference
Ortholinea australis Lom, Rohde et Dyková, 1992	*Acanthopagrus australis, Caulopsetta scapha*	Australia	Hepatic duct	8.7 (7.8-10.4)	8 (7.3-9.5)	3.7 (2.8-4.4)	2.9 (3.3-3.2)	Lom et al., 1992
Ortholinea basma Ali, 2000	*Clinus agilis*	South Africa	Urinary bladder	13.5±1.0 (12.0-15.0)	12.3±0.5 (11.8-13.0)	4.3±0.3 (4.0-4.8)	3.5±0.5 (3.0-4.3)	Ali, 2000
Ortholinea divergens Thélohan, 1895	*Hippoglossoides platessoides*	North Atlantic	Urinary bladder	9.2	9.4	2	2.4	Abdel-Ghaffar et al., 2008c
Ortholinea gobiusi Naidenova, 1968	*Gobius ophiocephahis*	Black Sea	Urinary bladder	8.8	8.4	1.9	1.9	Abdel-Ghaffar et al., 2008c
Ortholinea irregularis Kabata, 1962	*Drepanopsetta platessoides*	North Sea	Urinary bladder	10.6 (8-11)	7.1 (6.0-9)	2.2	2.2	Abdel-Ghaffar et al., 2008c
Ortholinea orientalis Shulman et Shulman-Albova, 1953	*Clupea spp.*	Northern Pacific	Kidney, urinary bladder, gall bladder	7.8	6	2.5	2.1	Abdel-Ghaffar et al., 2008c
Ortholinea polymorpha Davis, 1917	*Opsanus tau, O. beta*	USA	Urinary bladder	6.5-10 in diameter	-	4.4	2.4	Abdel-Ghaffar et al., 2008c
Ortholinea striateculus Su et White, 1994	*Leptatherina presbyteroides*	Australia	Ureters	10.1	10	3.5	2.9	Su and White, 1994
Ortholinea undulans Meglisch, 1970	*Peltorhamphns novaezelandiae*	New Zealand	Urinary bladder, ureter, oviduct	8.3 (7-10)	7.4 (6-9)	2.9 (2-4)	2.2 (2-3)	Abdel-Ghaffar et al., 2008c
Ortholinea sp.	*Muraenoclinus dorsalis*	South Africa	Spores found in gall bladder; site of infection unknown	8.8±0.8 (7.2-10.2) in diameter	-	2.9±0.5 (2.4-3.4)	2.4±0.3 (2.0-2.9)	Present study

CHAPTER 3: Spatial and temporal variation in parasite assemblages of the nosestripe klipfish, *Muraenoclinus dorsalis* Bleeker, 1860 (Perciformes: Clinidae)

Abstract

The component communities of an intertidal clinid, *Muraenoclinus dorsalis,* were examined to determine if patterns in species composition and structure existed, and if so, whether those patterns persisted across space and time. Data were collected from Granger Bay, Kommetjie, and Jacobsbaai in South Africa during Summer 2008/2009 and Winter 2009. Endoparasite community composition of component communities were compared over space and time using non-metric multidimensional scaling, cluster analysis, and Analysis of Similarity. The multivariate analyses revealed that assemblages from Kommetjie and Jacobsbaai, the most geographically distant localities, consistently showed the greatest dissimilarity in comparison to other within-season pairwise groups, suggesting a decay in similarity across distance. Component communities from Granger Bay showed no dissimilarity between the summer and the winter while component communities from Kommetjie showed little dissimilarity between the two seasons (Global R: 0.105; p-value = 0.002). These results suggest that season or season-associated factors have little effect on endoparasite community composition at these two localities. Contrastingly, the composition of the summer and winter component communities from Jacobsbaai were significantly dissimilar (Global R: 0.201; p-value = 0.003). The higher parasite load of the winter fish population is attributed to greater exposure time to parasite infection during the winter, when storms force the tide higher, causing *M dorsalis* to be submerged for greater lengths of time. Nestedness was found in all endoparasite component communities and was likely a result of differential colonization, passive sampling, or a

combination of both. The persistence of a nested subset structure over space and time in the endoparasitic infracommunities of *M dorsalis* lends credence to the debate on whether or not laws in parasite ecology exist.

Key words: **Nestedness; *Muraenoclinus dorsalis;* South Africa; parasites; community ecology**

Introduction

A major goal in the field of parasite ecology is to determine whether parasite assemblages are the result of stochastic events or predictable processes (Esch et al., 1990). One method to test for a departure from stochasticity is to seek nestedness. With roots in the theory of island biogeography, nestedness of insular communities is a phenomenon in which communities from species-poor islands form non-random subsets of species-rich islands (Lomolino et al., 2006). With parasite communities, each host represents an island; a nested subset pattern occurs when the parasite species found on depauperate islands, or infracommunities, represent non-random subsets of progressively richer ones (Rohde et al., 1998; Poulin and Valtonen, 2001; Gonzalez and Poulin, 2005).

The first study that sought nestedness in parasite infracommunities was conducted by Guégan and Hugueny (1994) on tropical fish from West Africa. This study confirmed the presence of a nested subset pattern in gill monogenean infracommunities. Since then, nestedness has been documented in various parasite assemblages from different environments, terrestrial and aquatic; and host taxa, mostly fish and mammals (e.g. Rohde et al., 1998; Poulin and Valtonen, 2001; Fellis et al., 2003; Gouy de Bellocq et al., 2003; Timi and Poulin, 2003; Zelmer et al., 2004; Krasnov et al., 2005; Patterson et al., 2009). However, the results from these studies failed to provide parasite ecologists with sufficient information to determine if nested subset

patterns in parasite communities are predictable. Some studies failed to find nestedness (e.g. Poulin, 1996; Worthen and Rohde, 1996; Hayward et al., 1998), while most of the early studies only examined assemblages at the infracommunity level rather than at the component community level. By focusing on just infracommunities, the persistence of nestedness through space and time is disregarded, thereby limiting our understanding of how local ecological factors can affect observed patterns.

The issue of persistence was first addressed by Carney and Dick (2000). They examined parasite communities from yellow perch, *Perca flavescens,* captured from five lakes and found that there existed non-random community organization and structure. Since then, several studies have examined the component communities of various hosts across space, time, or both (Poulin and Valtonen, 2002; Gouy de Bellocq et al., 2003; Timi and Poulin, 2003; Vidal-Martinez and Poulin, 2003; Calvete et al., 2004; Zelmer and Arai, 2004; Gonzalez and Poulin, 2005; Gonzalez and Oliva, 2009; Violante-González et al., 2009). Of these studies, only Gonzalez and Poulin (2005), Gonzalez and Oliva (2009), and Violante-González et al. (2009) found the nested subset pattern to be repeatable. The remainder of the studies found structure to be fleeting and wholly unpredictable. Therefore, as a result of the lack of consensus, there is a continued need to explore this topic in order to finally determine whether nestedness is in fact a phenomenon that can also be consistently found in parasite communities.

The aim of this investigation is to i) compare species composition of the parasite component communities of an intertidal fish, *Muraenoclinus dorsalis,* across three localities from the western coast of South Africa during the summer and during the winter, ii) compare species composition of the parasite component communities of *M dorsalis* from each locality

across summer and winter, and iii) determine if these component communities are structured non-randomly, specifically whether or not they exhibit nestedness.

Muraenoclinus dorsalis **is one of 39 species dominating the intertidal ichthyofauna of Southern Africa's west and south-western coast (Prochazka and Griffiths, 1992). This fish is endemic to Southern Africa, ranging from Swakopmund, Namibia to the Natal Coast of South Africa (Smith, 1986) and rarely exceeds lengths of 70 mm (Penrith, 1965). This fish does not undergo ontological diet or habitat shifts (Bennett et al., 1983), hence limiting the effects of those factors on producing apparent nested patterns (see Rohde et al., 1998; Zelmer and Arai, 2004). The utility of analyzing the assemblages of an intertidal host rather than a pelagic host is that one can begin to probe the extent to which local ecological factors can affect community structure. In the intertidal, the host's environment is much more varied and extreme compared to that of pelagic fish from previous studies. Local ecological factors can vary greatly from one locality to the next, e.g. water quality as a result of runoff, substrate type, or wave exposure. Furthermore, *M dorsalis* occasionally exhibits a stranding behavior (personal observation), hence making this fish a fascinating study host because of its exposure to both the marine and terrestrial environment.**

In Southern Africa, only two studies have compared parasite assemblages. Fellis et al. (2003) compared species composition of ectoparasitic assemblages from the greater kudu, *Tragelaphus strepsiceros,* between the Kruger National Park, South Africa, and the Etosha National Park, Namibia and tested each component community for nestedness. Yeld (2009) compared community composition of all parasites, but not structure, of two catshark species, *Haploblepharus pictus* and *H edwardsii* from False Bay and De Hoop Nature Reserve. Neither study made comparisons across time.

Materials and Methods

Study localities

Muraenoclinus dorsalis was collected from Granger Bay, Kommetjie, and Jacobsbaai in South Africa (Fig. 1). Granger Bay is a wave-exposed locality where the rock shelf has eroded into many large boulders and tidepools with sand interspersed between them. Kommetjie, also a wave-exposed locality, consists of a boulder field on a rock shelf. Jacobsbaai, a sheltered locality, is a boulder field atop muddy substrate.

Collection of Fish

Fish were collected during Summer 2008/2009 (November-February) and Winter 2009 (May-July). They were collected from the upper intertidal zone, primarily under damp rocks with little to no seawater, during low tide using small handnets. Only fish measuring 50-60 mm standard length were examined to account for host size and age of fish, which can possibly affect colonization patterns resulting in an apparent nested pattern (Guégan and Hugueny, 1994; Poulin and Valtonen, 2001; Timi and Poulin, 2003; Gonzalez and Poulin, 2005). Fish of this size class were selected because they are mature, which allows for higher species richness and hence maximal matrix fill for nestedness analyses. Fish of this size class were also more common than fish of other size classes, which were arbitrarily determined *a priori* to be 10 mm intervals. Fish were transported live, individually, in aerated aquaria to the University of Cape Town for processing. They were subsequently euthanized by neural pithing, measured, and examined for the presence of parasites.

Detection of Infection

Fish were dissected and examined for macroparasites using a stereomicroscope. Myxozoan infections were detected by visual examination of all organs using a stereomicroscope

and by squashing fresh gall bladders between a microscope slide and a coverslip and examining the contents under a light microscope using the 30x objective. No other tissues were squashed. Blood films were prepared, fixed in absolute methanol, stained with Giemsa's stain then examined using a 10x objective for one minute per slide. A low magnification was used as scanning blood under higher magnification caused the author extreme motion sickness. When found, parasites infecting the various organs were recovered and fixed using standard techniques. Intensity of infection by trichodinids was quantified as infection levels based on three gill smears per host. Infection at Level 1 indicated an average of 10 trichodinids present per slide, Level 2 indicated an average of up to 100 individuals per slide, and Level 3 indicated hundreds of individuals per slide.

Statistical Analyses

Prevalences and mean intensities of infrapopulations (sensu Bush et al., 1997) were calculated and compared using Fisher's Exact Test and a bootstrap t-test based on Welsh's t-statistic respectively, using Quantitative Parasitology 3.0 (Reiczigel and Rózsa, 2005). Similarities between component communities were calculated using the Bray-Curtis similarity index. Differences between assemblages were examined using non-metric multidimensional scaling and cluster analysis. An Analysis of Similarity (ANOSIM) was used to test for statistical differences in species compositions between component communities. For component communities that differed significantly, Simper analyses were used to determine which parasite species contributed most to the observed result. For all analyses, a p-value < 0.05 was considered significant. All multivariate statistical analyses were performed using PRIMER v.6.1.5 for Windows (PRIMER-E Ltd., 2006).

For each component community, the index of nestedness, T, was computed using the Nestedness Temperature Calculator (Atmar and Patterson, 1995). For each component community, the observed matrix temperature was compared with T-values of 1000 randomly generated presence-absence matrices produced with Monte-Carlo simulations. A p-value < 0.05 indicated significant nestedness.

For the multivariate analyses and nestedness analyses, ectoparasitic and endoparasitic component communities were analyzed separately as the generalized nature, particularly the life cycle, of these two parasite types differs greatly.

Results

Twenty-three parasitic species, seven ectoparasites and sixteen endoparasites, were found infecting *M dorsalis* (Table 1). The lack of information on the specific identities of the parasites is due to the host species having never examined for macroparasites before. No parasites were found in the blood. Most of the parasites found were rare, with less than five individuals of each species collected. These species were only considered for nestedness analyses as their rareness is unlikely to provide any other useful information. It is important to note that the two diplostomes may in fact be one species infecting both the brain and the eye. Generally, the brain diplostomes were smaller and ribbon-like, while the eye diplostomes were more rotund. However, there have been occasions where small ribbon-like trematodes have been found in the eye as well as individuals with intermediate forms. For ease of analysis, this study will tentatively consider the trematodes infecting the brain as different from those infecting the eye. Furthermore, the myxozoan *Kudoa* sp. was found only after July 2009. As a result, *Kudoa* sp. is not included in

any analyses but is listed here to provide a comprehensive list of all parasites found to infect *M dorsalis.*

The following parasites were commonly found in *M dorsalis:*

Ectoparasites

Caligus mortis

This species was found exclusively at Jacobsbaai. Due to small sample sizes, prevalence and mean intensity could not be compared statistically between the summer and the winter (Table 2).

***Trichodina* spp.**

For ease of analysis all *Trichodina* species were grouped together and analyzed as one unit. Pairwise comparisons of prevalences failed to show any significant differences between localities in during one season or between seasons at each locality (Table 2). Within-season comparisons of mean intensities showed that all three localities differed significantly from each other (Table 3). In the summer, mean intensity was the highest at Granger Bay and lowest at Jacobsbaai; in the winter, the trend was reversed. Comparisons across seasons within a locality revealed that the summer and winter mean intensities were consistently different, with Granger Bay and Kommetjie showing lower mean intensities in the winter and Jacobsbaai showing the reverse (all p-values < 0.001).

Endoparasites

Intestinal hemiurids

During the summer prevalences at Granger Bay and Kommetjie were approximately six times that at Jacobsbaai ($p<0.001$ for both) (Table 4), with mean intensity at Kommetjie approximately twice as high as those at Granger Bay or Jacobsbaai ($p = 0.004$ and 0.003,

respectively). During the winter, the proportion of fish infected with intestinal hemiurids at Granger Bay and Kommetjie was approximately double that at Jacobsbaai ($p < 0.001$ for both). Mean intensities from all three localities differed significantly (Table 5). Comparisons across seasons within each locality only revealed a greater prevalence during the winter than the summer at Jacobsbaai ($p = 0.046$).

Eye diplostome

Unlike the majority of parasites found to infect *M dorsalis,* the eye diplostomes were not adults; they were metacercariae. During the summer, prevalence at Kommetjie was twice as high as prevalence at either Granger Bay ($p = 0.001$) or Jacobsbaai ($p < 0.001$) (Table 4). In addition to having a higher prevalence, the mean intensity at Kommetjie was almost four times greater than those at Granger Bay ($p = 0.002$) and Jacobsbaai ($p = 0.001$). A similar trend was also observed during the winter (Table 6). Comparisons across seasons within each locality showed that mean intensities at Kommetjie were greater in the summer than in the winter ($p = 0.002$).

Brain diplostome

These trematodes were also metacercariae. During the summer this species infected a higher proportion of fish from Kommetjie than at Granger Bay or Jacobsbaai ($p = 0.002$ and < 0.001, respectively) (Table 4). During the winter prevalence increased at Jacobsbaai, nearing that of Kommetjie's, leaving prevalence at Granger Bay significantly different from those at Jacobsbaai or Kommetjie ($p = 0.011$ and < 0.001, respectively). Comparisons across seasons within each locality only revealed one significant difference, a much higher prevalence at Jacobsbaai during the winter than the summer ($p < 0.001$).

***Ceratomyxa* sp.**

Almost all fish sampled were infected by *Ceratomyxa* sp. There were no significant differences between prevalences across localities during a season or across seasons within each locality (Table 4).

***Sphaeromyxa* sp. 1**

This species was found exclusively at Granger Bay, where prevalence during the winter was greater than in the summer ($p = 0.021$) (Table 4).

***Myxobolus* sp.**

This species was found only at Kommetjie and Jacobsbaai. There were no significant differences between the prevalences of *Myxobolus* sp. at these two localities during the summer or the winter (Table 4). There were also no significant differences between summer and winter prevalences at each locality.

Ovarian hemiurid

This species was found exclusively in pregnant females from Kommetjie and Jacobsbaai. Since it was uncertain whether digenean infected the mother or offspring, all females from each component community were pooled together for analysis (Table 7). The resulting sample size was too small for statistical analyses. Ovarian hemiurids were also excluded from both the multivariate and nestedness analyses as this study is interested in assemblages from the general fish population, both males and females.

Comparing species composition across space

The comparison of ectoparasitic assemblages across localities during a single season could not be carried out as fish from Granger Bay and Kommetjie were infected only by *Trichodina* spp. The use of multivariate analyses would be inappropriate for this situation.

In order to compare endoparasitic assemblages across localities during the summer, three fish from Jacobsbaai were removed from the data set because preliminary MDS plots and cluster analysis revealed them to be outliers. After the removal of the outliers, the MDS plots and cluster analyses showed that there were three well-defined groups: the component communities from each locality (Fig. 2A). An ANOSIM confirmed that the component communities were significantly dissimilar from one another (Global R: 0.453; p = 0.001) with the greatest difference in species composition between communities from Kommetjie and Jacobsbaai (Table 8). A Simper analysis revealed that eye diplostomes and intestinal hemiurids contributed most to the observed dissimilarities between all localities. MDS, cluster analysis (Fig. 2B), and an ANOSIM comparing the winter component communities to one another also showed significant dissimilarity between the three assemblages (Global R: 0.33; p = 0.001), although the dissimilarity is less pronounced than in the summer. Again, the greatest dissimilarity was between Kommetjie and Jacobsbaai with intestinal hemiurids and eye diplostomes contributing most to the dissimilarity (Table 9).

Comparing species composition across time

Comparisons of the summer and winter ectoparasitic component communities could only be undertaken for those from Jacobsbaai because hosts from the other localities were only infected by *Trichodina* spp. MDS and cluster analysis showed that species composition of the winter infracommunities was highly variable (Fig. 3). ANOSIM results showed that species composition of the two component communities differed significantly between the summer and the winter (Global R: 0.158; p = 0.002), with *Trichodina* spp. accounting for 63.1% of the difference observed.

The summer and winter endoparasite component communities from Granger Bay (Fig. 4A) showed no significant difference (Global R: 0.026; p-value = 0.201) while those from Kommetjie (Fig. 2B) showed little dissimilarity between the two seasons (Global R: 0.105; p-value = 0.002). At Jacobsbaai, three outliers from the summer group were removed from analysis (Fig. 2C). The resulting comparison revealed significant dissimilarities in species composition between the two seasons (Global R: 0.201; p-value = 0.003) with intestinal hemiurids and eye diplostomes contributing to 34.0% and 29.7% of the observed dissimilarity respectively.

Nestedness

Nestedness analyses were only conducted for the endoparasites as the ectoparasite component communities consisted of just two species, which would produce colder characteristic temperatures as a result of the mostly empty, small, and highly rectangular matrix (Patterson and Atmar, 1995). Significant nestedness was found in the endoparasite component communities of *M dorsalis* at all localities during both summer and winter (Table 10).

Discussion

***Muraenoclinus dorsalis* is host to many parasitic species despite its relatively small size. Virtually every organ of *M dorsalis* is infected, with the exception of blood. However, this study's failure to find blood parasites may simply be the result of the low magnification at which the blood films were examined. On the other hand, it is entirely possible that *M dorsalis* may not be a host to any blood parasites. Hayes et al. (2006) examined the blood of various intertidal fishes from De Hoop, South Africa and found that of three *M dorsalis* examined, none were infected with blood parasites. Still, it is recommended that the smears be examined again at a higher magnification to confirm that *M dorsalis* does not act as a host to blood parasites.**

The discovery of three parasite taxa in the ovary is "surprising" (MacKenzie', personal communication). The author is confident that these parasites infected the ovary and were not displaced from the intestine, as the ovaries were easy to remove intact and were dissected on a clean Petri dish.

The absence of *Myxobolus* sp. and presence of *Sphaeromyxa* sp. 1 at Granger Bay is unlikely to be the result of ecological factors, but rather the absence and unique presence, respectively, of intermediate hosts. Unlike ectoparasites, which generally have direct life cycles and whose distributions are determined primarily by environmental conditions, endoparasites usually have indirect life cycles, and hence their distribution requires the presence of all developmental stages (MacKenzie and Abaünza, 1998).

The observed parasite richness is expected to be higher, as surveys of less than 40-50 individual hosts can result in failure to detect at least one helminth species (Poulin, 1998). Furthermore, the sampling area was at a relatively small scale. In order to accurately estimate parasite diversity, the sampling area should cover the entire host range (Poulin, 1998). Nonetheless, the results from this study provide valuable insight as an introduction to the parasite community composition and structure of *M dorsalis*.

Though not supported by statistics, there appears to be a general trend where the prevalence of intestinal hemiurids, brain diplostomes, *Ceratomyxa* sp., *Sphaeromyxa* sp. 1 and *Myxobolus* sp. seem to increase from the summer to the winter. This observation warrants further exploration with larger samples sizes, as the lack of statistical significance may just be the result of small sample sizes, as well as a longer term study since annual variations may result in anomalies in infracommunity composition. If these trends are verified, then virtually all of the

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endoparasites of *M dorsalis* are controlled by seasonality or season-associated factors. If this is the case, then the rise in prevalence during the winter may be attributed to increased host stress, which can lead to a reduction in the efficacy of the immune system (Lewis and Hoole, 2003). Winter can be a very stressful time for *M dorsalis* because of exposure to a destabilized environment. During this time, the rocky intertidal is battered by waves and rocks they carry. Furthermore, the water is increasingly polluted as a result of runoff.

The three fish outliers that were excluded from the endoparasite summer sample of Jacobsbaai were unique because they lacked endoparasitic infections. This observation might be attributed to immigration from another population, though immigration from the south is unlikely as a study on the phylogeography of *M dorsalis* showed that the population from Jacobsbaai is genetically isolated from populations to the south (von der Heyden [2], personal communication). If these three fish were indeed migrants, they would have migrated from the north. An alternate and more likely explanation for why these fish were free of endoparasites is that they were relatively young; all measured only 50 mm in length. It may just be that they had not yet been infected by parasites.

The observation of greatest dissimilarity in endoparasite infracommunity composition between Kommetjie and Jacobsbaai compared to other within-season comparisons suggests a decay of similarity over geographical distance. This observation has also been seen in the parasite communities of other vertebrates along their distributional range (Poulin and Morand, 1999; Poulin, 2003; Oliva and Gonzalez, 2005). On a study examining forests, Nekola and White (1999) proposed two plausible explanations for declines in similarity with distance. First, increasing distance equates to a decay of environmental similarity; a reduction in similarity is

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thus the result of the ability of species to adapt to variations in the environment. Regarding parasites, the environment can control which parasites could be available according to the parasites' varying tolerances. As a result, neighboring localities expose a host species to the same local pool of parasites. With almost 200 km of coastline between Jacobsbaai and Kommetjie, it is highly likely that there would be differences in environmental factors. Secondly, geographical barriers impede dispersal. This reason is not likely to apply to the current study. The range of the current study's localities is not impeded by an oceanographic barrier; all three localities are well within the boundaries of the cool, temperate Namaqua marine biogeographic zone, unlike with the previous studies where the subject fishes occupied different biogeographic zones (Poulin and Morand, 1999; Oliva and Gonzalez, 2005).

The lack of dissimilarity between summer and winter endoparasite component communities from Granger Bay and the low level of dissimilarity between those from Kommetjie suggest that the factors that control community structure remained relatively stable at those two localities during the study period. These observations are contrary to expectations for wave-exposed localities, where winter waves can often be destructive. However, it may be that though waves during winter are often larger than those during summer, the winter waves exhibited in 2009 were no more destructive to these wave-exposed sites than the ones exhibited during the summer. Exposure to waves can also be an explanation for why the component communities from Jacobsbaai showed significant dissimilarity. During winter, the sampling locality at Jacobsbaai is not battered by waves as at Granger Bay and Kommetjie. Instead, the tide is pushed upwards, which would result in *M dorsalis* being submerged for longer, hence increasing the hosts' exposure time to parasites.

The presence of nestedness at all localities during both the summer and the winter

suggests that there exists order in endoparasite infracommunity structure in *M dorsalis,* whereby rare species occur only in species-rich infracommunities. In free-living communities, nestedness is primarily generated by two processes: differentiated extinction and colonization (Patterson and Atmar, 1986). The ordered extinction of species is not assumed to be a major determinant of nested subset patterns in most endoparasite infracommunities because parasites generally do not establish reproductive populations on the host (Guégan and Hugueny, 1994; Zelmer et al., 2004). Instead, the survival probability of a single parasitic species is dependent on recolonization by the same species. Therefore, endoparasite assemblages composed of species with indirect life cycles are controlled primarily by differentiated colonization probabilities. Colonization can be further differentiated by positive interactions, where early colonizers depress the immune system of the host, thereby facilitating the colonization by subsequent species (Guégan and Hugueny, 1994).

Alternate hypotheses on how the nested subset pattern could arise include: i) passive sampling of parasites by the host, whereby the probability that a species colonizes a site is dependent on its abundance, i.e. abundant species are predicted to occur in many hosts while rare species occur in only a few and ii) habitat heterogeneity, where host size and age are positively associated with niche diversification (Guégan and Hugueny, 1994). As this study examined fish of only one size class, habitat heterogeneity cannot be considered in explaining the observed nestedness in all the component communities. Though this study cannot clearly implicate the exact processes that produced the observed results, the results indicate that species composition remains relatively constant over time but not so much over space, which will assist in further understanding the principals of parasite ecology.

Figure Captions

Figure 1. Map of the western coast of South Africa (insert) and the locations of the three sampling localities.

Figure 2. Multidimensional scaling plots and dendrograms from cluster analyses comparing endoparasitic component communities infecting *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa across localities during Summer 2008/2009 and Winter 2009.

A) Comparison of component communities from Granger Bay, Kommetjie, and Jacobsbaai during Summer 2008/2009. Three outliers from Jacobsbaai were removed.

B) Comparison of component communities from Granger Bay, Kommetjie, and Jacobsbaai during Winter 2009.

Figure 3. Multidimensional scaling plots and dendrograms from cluster analyses comparing Summer 2008/2009 and Winter 2009 ectoparasitic component communities infecting *Muraenoclinus dorsalis* Bleeker, 1860 from Jacobsbaai, South Africa.

Figure 4. Multidimensional scaling plots and dendrograms from cluster analyses comparing endoparasitic component communities infecting *Muraenoclinus dorsalis* Bleeker, 1860 collected during Summer 2008/2009 and Winter 2009 from the western coast of South Africa across seasons at each locality.

A). Comparison of Summer 2008/2009 and Winter 2009 component communities from Granger Bay.

B) Comparison of Summer 2008/2009 and Winter 2009 component communities from Kommetjie.

C) Comparison of Summer 2008/2009 and Winter 2009 component communities from Jacobsbaai. Three outliers from the summer group were removed.

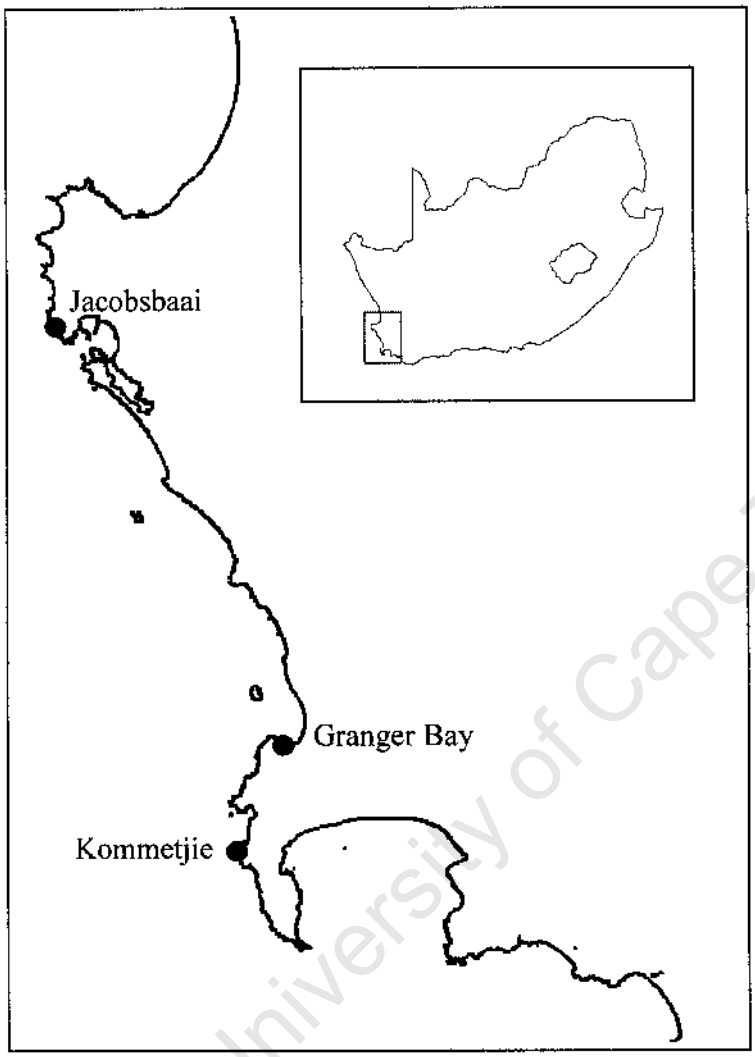

Figure 1

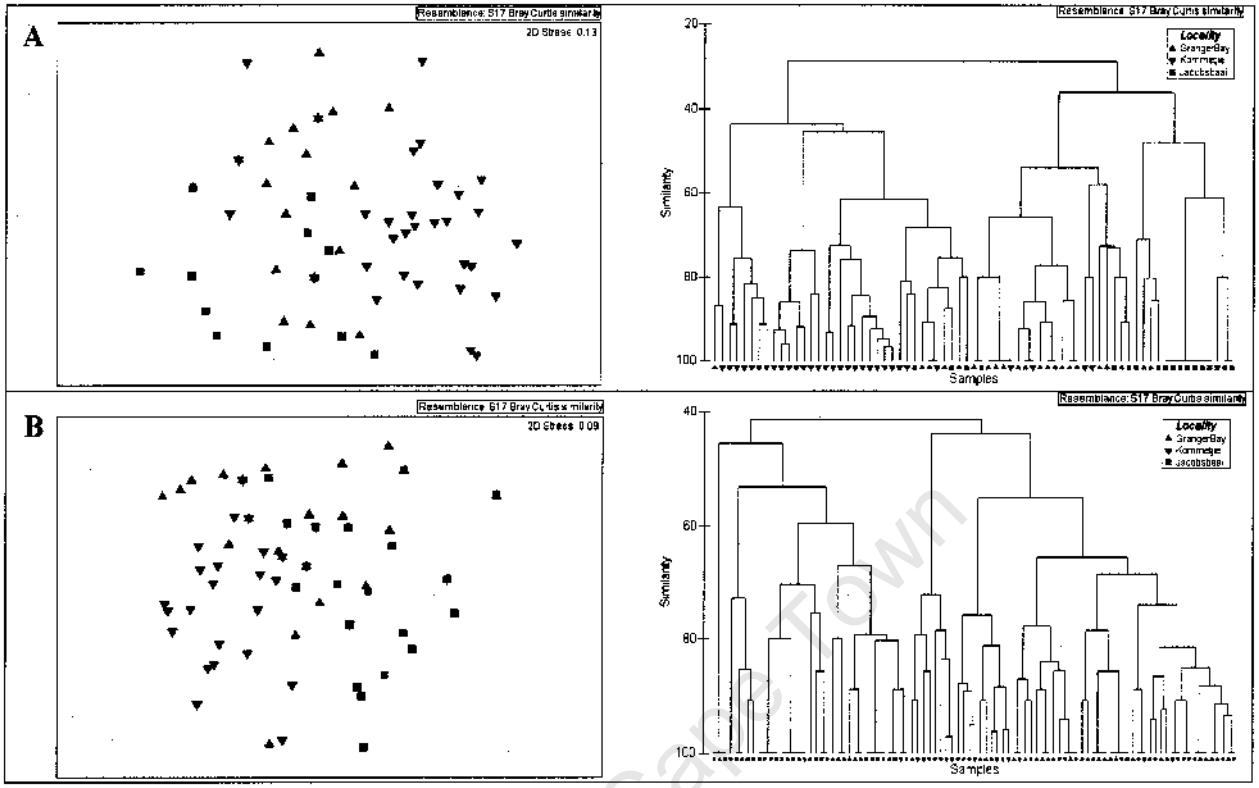

Figure 2

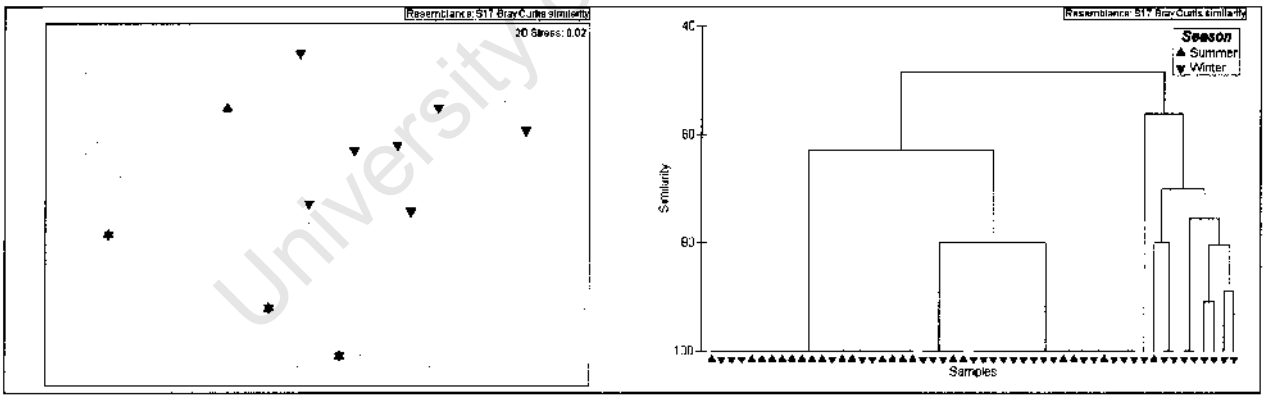

Figure 3

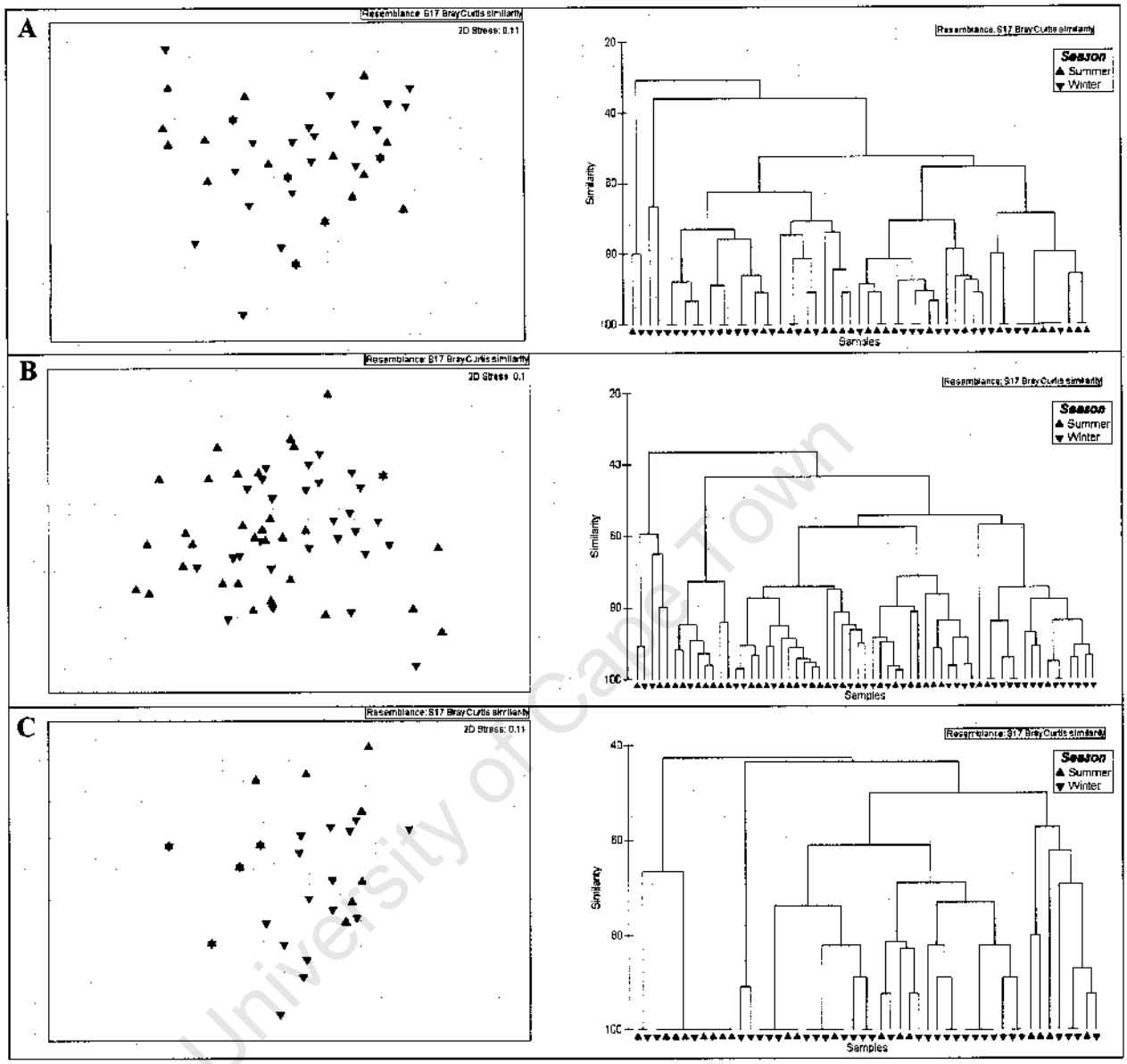

Figure 4

Table 1. Parasitic species found infecting *Muraenoclinus dorsalis* Bleeker, 1860 collected during Summer 2008/2009 and Winter 2009 from the western coast of South Africa and their sites of infection.

	Parasite Taxon			
Category	Common Name	Phylum	Species	Site of Infection
Ectoparasite	Copepod	Arthropoda	*Caligus mortis*	Skin
	Copepod	Arthropoda	Unknown	Skin
	Leech	Annelida	Unknown	Skin
	Trichodinid	Ciliophora	*Trichodina* sp. A	Gills
	Trichodinid	Ciliophora	*Trichodina* sp. B	Gills
	Trichodinid	Ciliophora	*Trichodina* sp. C	Gills
	Trichodinid	Ciliophora	*Trichodina* sp. D	Gills
Endoparasite	Acanthocephalan	Acanthocephala	Unknown	Ovary
	Acanthocephalan	Acanthocephala	Unknown	Intestine
	Acanthocephalan	Acanthocephala	Unknown	Urinary bladder
	Coccidian	Apicomplexa	Unknown	Gall bladder
	Myxozoan	Myxozoa	*Myxobolus* sp.	Eye (Sclera)
	Myxozoan	Myxozoa	*Ceratomyxa* sp.	Gall bladder
	Myxozoan	Myxozoa	*Sphaeromyxa* sp.	Gall bladder
	Myxozoan	Myxozoa	*Kudoa* sp.	Muscles
	Nematode	Nematoda	Unknown	Abdominal Cavity
	Nematode	Nematoda	Unknown	Ovary
	Nematode	Nematoda	Unknown	Intestine
	Trematode	Platyhelminthes	Unknown	Intestine (Hindgut)
	Trematode	Platyhelminthes	Unknown	Brain
	Trematode	Platyhelminthes	Unknown	Ovary
	Trematode	Platyhelminthes	Unknown	Eye (Vitreous chamber)
	Trematode	Platyhelminthes	Unknown	Testes

Table 2. Prevalences and mean intensities, respectively, of common ectoparasites infecting *Muraenoclinus dorsalis* Bleeker, 1860 collected during Summer 2008/2009 and Winter 2009 from the western coast of South Africa. Bolded numbers indicate significant differences between summer and winter infrapopulations at each locality; capital letters indicate significant differences between summer infrapopulations; lower case letters indicate significant differences between winter infrapopulations. n/a = not applicable; this was used for species whose intensities could not be quantified.

Locality	Season	N	Parasite species	
			Caligus mortis	*Trichodina* spp.
Granger Bay	Summer	22	0; n/a	0.95; **2.09**^{A}
	Winter	31	0; n/a	1; **1.40**^{a}
Kommetjie	Summer	31	0; n/a	1; **1.80**^{B}
	Winter	30	0; n/a	1; **1.13**^{b}
Jacobsbaai	Summer	21	0.05; 1	1; **1.33**^{C}
	Winter	32	0.28; 0.75	1; **1.88**^{c}

Table 3. P-values for bootstrap t-test comparing mean intensities of *Trichodina* spp. infecting the gills of *Muraenoclinus dorsalis* Bleeker, 1860 collected during Summer 2008/2009 and Winter 2009 from the western coast of South Africa.

Paired localities	Summer	Winter
Granger Bay, Kommetjie	0.05	0.01
Granger Bay, Jacobsbaai	<0.001	0.003
Kommetjie, Jacobsbaai	0.02	<0.001

Table 4. Prevalences and mean intensities, respectively, of common endoparasites infecting *Muraenoclinus dorsalis* Bleeker, 1860 collected during Summer 2008/2009 and Winter 2009 from the western coast of South Africa. Bolded numbers indicate significant differences between summer and winter infrapopulations at each locality; capital letters indicate significant differences between summer infrapopulations; lower case letters indicate significant differences between winter infrapopulations. n/a = not applicable; this was used for species whose intensities could not be quantified.

Locality	Season	N	Parasite species					
			Intestinal Hemiurid	Eye diplostome	Brain diplostome	*Ceratomyxa* sp.	*Sphaeromyxa* sp.	*Myxobolus* sp.
Granger Bay	Summer	22	0.86^{A}; 3.1^{A}	0.36^{A}; 2.75^{A}	0.45^{A}; n/a	0.91; n/a	$\mathbf{0.18}^{A}$; n/a	0^{A}; n/a
	Winter	31	0.90^{a}; 3.71^{a}	0.35^{a}; 2.09^{a}	0.58^{a}; n/a	1.00; n/a	$\mathbf{0.45}^{a}$; n/a	0^{a}; n/a
Kommetjie	Summer	31	0.90^{A}; 6.96^{B}	0.87^{B}; $\mathbf{11.19}^{B}$	0.87^{B}; n/a	0.84; n/a	0^{B}; n/a	0.16^{AB}; n/a
	Winter	30	0.93^{a}; 5.79^{b}	0.73^{b}; $\mathbf{5.41}^{b}$	0.97^{b}; n/a	1.00; n/a	0^{b}; n/a	0.17^{a}; n/a
Jacobsbaai	Summer	21	$\mathbf{0.14}^{B}$; 2.67^{A}	0.33^{A}; 2.71^{A}	$\mathbf{0.33}^{A}$; n/a	0.86; n/a	0^{AB}; n/a	0.24^{B}; n/a
	Winter	32	$\mathbf{0.47}^{b}$; 1.87^{c}	0.43^{a}; 1.64^{a}	$\mathbf{0.88}^{b}$; n/a	0.97; n/a	0^{b}; n/a	0.28^{b}; n/a

Table 5. P-values from bootstrap t-test comparing Winter 2009 mean intensities of intestinal hemiurids infecting *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa.

Pairwise Groups	p
Granger Bay, Kommetjie	0.012
Granger Bay, Jacobsbaai	0.004
Kommetjie, Jacobsbaai	<0.001

Table 6. P-values from Fisher's Exact test and bootstrap t-test comparing Winter 2009 prevalences and mean intensities of eye diplostomes infecting *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa.

Pairwise Groups	Measure	p
Granger Bay, Kommetjie	Prevalence	0.002
Jacobsbaai, Kommetjie	Prevalence	0.005
Granger Bay, Kommetjie	Mean intensity	0.001
Jacobsbaai, Kommetjie	Mean intensity	0.005

Table 7. Prevalences and mean intensities of ovarian hemiurids in female *Muraenoclinus dorsalis* Bleeker, 1860 collected during Summer 2008/2009 and Winter 2009 from the western coast of South Africa. Due to small sample sizes, the data could not be analyzed statistically.

Locality	Season	N	Prevalence	Mean Intensity
Granger Bay	Summer	9	0	--
	Winter	15	0	--
Kommetjie	Summer	17	0.47	2.25
	Winter	12	0.33	1.25
Jacobsbaai	Summer	6	0.17	1
	Winter	15	0.13	1

Table 8. Summary of the results from SIMPER analysis comparing Summer 2008/2009 endoparasitic component communities of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa. Species listed are the two species that contributed most and second most to the observed dissimilarity with their respective contributions.

Pairwise Groups	R	p	Species	Contribution (%)
Granger Bay, Kommetjie	0.375	0.001	Eye diplostome, Intestinal hemiurid	55.6, 34.5
Granger Bay, Jacobsbaai	0.352	0.001	Intestinal hemiurid, Eye diplostome	52.7, 27.5
Kommetjie, Jacobsbaai	0.629	0.001	Eye diplostome, Intestinal hemiurid	50.6, 39.3

Table 9. Summary of the results from SIMPER analysis comparing Winter 2009 endoparasite component communities of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa. Species listed are the two species that contributed most and second most to the observed dissimilarity with their respective contributions.

Pairwise Groups	R	p	Species	Contribution (%)
Granger Bay, Kommetjie	0.216	0.001	Intestinal hemiurid, Eye diplostome	46.8, 40.85
Granger Bay, Jacobsbaai	0.277	0.001	Intestinal hemiurid, Eye diplostome	53.6, 20.3
Kommetjie, Jacobsbaai	0.496	0.001	Intestinal hemiurid, Eye diplostome	55.3, 37.9

Table 10. Summary of the results of nestedness analyses on Summer 2008/2009 and Winter 2009 endoparasitic component communities of *Muraenoclinus dorsalis* Bleeker, 1860 collected from the western coast of South Africa. Matrix Temp: Matrix temperature; AverageTemp: Average matrix temperature; SD: Standard deviation; P: probability that the observed pattern is randomly produced. $P < 0.05$ indicates nested communities. Average temperatures and SD were derived from 1000 randomly generated Monte-Carlo simulated matrices.

Locality	Season	Matrix Temp	Matrix Fill (%)	AverageTemp (SD)	P
Granger Bay	Summer	18.02°	56.1	39.75° (8.68°)	0.006
	Winter	12.61°	62.3	40.49°(8.51°)	<0.001
Kommetjie	Summer	13.22°	71.8	37.19°(9.76°)	0.007
	Winter	0.26°	72.8	37.34°(10.05°)	<0.001
Jacobsbaai	Summer	9.96°	45.8	42.68°(9.55°)	<0.001
	Winter	16.82°	60.6	43.48°(8.36°)	<0.001

CHAPTER 4: Conclusions

With one of the most biologically diverse coastlines in the world, South Africa has great potential to dramatically increase the number of known species along its coast just by studying parasites since high free-living species diversity is correlated with high parasite species diversity (Hechinger and Lafferty, 2005; Hechinger et al., 2007; Hechinger et al., 2008). Considering that every organism has a unique set of parasites infecting it, of which at least one should be species-specific, the number of parasites in the world would, at the very least, equal that of free-living species. This would greatly increase the number of described species from South Africa. This estimate is well supported by the survey conducted on the parasites of *Muraenoclinus dorsalis.* Generally of a length of no more than 70 mm (Penrith, 1965), *M. dorsalis* is host to twenty-three parasite species, most of which have yet to be described. One can only imagine the numbers of parasites to be found in larger hosts where there are more niches to be occupied.

Included in this study's parasite species list are five myxozoans, of which *Sphaeromyxa* sp. 1, *Myxobolus* sp., and *Ortholinea* sp. are undoubtedly new species. However, *Ceratomyxa* sp. and *Kudoa* sp. cannot be deemed new species conclusively because of the complexities that arise from spore plasticity. These two myxozoans are morphologically similar enough to known species that they may in fact be described species. To verify the unique identity of each species, genetic analyses should be performed comparing the sequences of morphologically similar species to those observed in the current study. In fact, the DNA of all the species in the current study should be sequenced and submitted to GenBank as a resource for future descriptions and to assist in the accurate classification of myxozoans. It would, additionally, be beneficial to provide details of ultrastructural features of spores to aid in determining how reliable spore morphology

can be in classification since descriptions based on spore morphology alone, as observed under the light microscope are insufficient.

Results from the ecological study also warrant additional study. The apparent increase of intestinal hemiurids, brain diplostomes, *Ceratomyxa* sp., *Sphaeromyxa* sp. 1 and *Myxobolus* sp. prevalences from the summer to the winter, though not supported by statistics, could be verified with a study with a larger sample size. The lack of statistical significance may just be the result of small sample sizes. The fact that there are only three localities in this study does not allow for the definite conclusion of decay of similarity. Further study with more sampling localities is strongly recommended since this preliminary examination provides a positive outlook. Though the deterioration of similarity over space in this study is debatable, it nevertheless provides sufficient grounds to suggest that a comprehensive study with more localities should be undertaken. It is important to note that the previous studies that found such a pattern in parasite communities of marine fish (Poulin and Morand, 1999; Oliva and Gonzalez, 2005) examined fishes from a much larger geographic range than the present study. A decline in community composition similarity with respect to increasing distance over a relatively small geographic range further warrants future studies. A decay of similarity over a short distance, in this case, may be attributed to the varied nature of the intertidal zone compared with the open ocean; i.e. the lack of homogeneity over geographical distance of the intertidal, which is affected by both terrestrial and oceanic processes, is greater than that of the open ocean. Hence, the overlap of the pool of parasites present is effectively reduced. This study reinforces the need for more studies examining the infracommunity composition of intertidal hosts.

This study was designed to be completed within the timeframe of an MSc degree; the abbreviated duration of this study hence limits the influence the observations made in this survey

has on various topics in parasitology, especially in the search for laws in parasite ecology. Collecting samples during only one year rather than over several years does not take into account the life spans of the parasites, which can accumulate with time and complicate the results of this study. For example, the diplostome metacercariae are likely to have life spans of greater than one year while the intestinal hemiurids probably have life spans of less than one year (MacKenzie[3], personal communication). The possible effect of accumulation in hosts was minimized by utilizing fish of only one size class; accumulation would be significant if fish of various lengths, and hence age, were compared. Collecting samples from only one year also brings up the issue of possibly sampling from an anomalous year. This study, nevertheless, has dramatically increased what is known about parasite ecology in South Africa and has provided preliminary results that support the undertaking of larger-scale studies.

[3] **Dr. Ken MacKenzie is a Research Fellow in the Zoology Department at Aberdeen University, Scotland. His research interests lie in the use of parasites as biological tags and as indicators of marine pollution.**

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APPENDIX A: Protocols for Reagents used for fixing fish for histology and for histology

Modified Davidson's Fixative

Formalin (40% formaldehyde in solution)	**200 mL**
Ethanol, 95%	**300 mL**
Glycerol	**100 mL**
Distilled Water	**100 mL**
Glacial Acetic Acid	**78 mL**

Modified Davidson's Solution

Formalin (40% formaldehyde in solution)	**200 mL**
Ethanol, 95%	**300 mL**
Glycerol	**100 mL**
Distilled Water	**100 mL**

Mayer's Haemalum

Haematoxyl in	**1 g**
Sodium Iodate	**0.2 g**
Potassium Alum	**50 g (anhydrous) / 95 g (hydrated)**
Chloral Hydrate	**50 g**
Citric Acid	**2 g**
Distilled Water	**1000 mL**

1% Acid Alcohol

70% Ethanol	**990 mL**

Concentrated HCl		**10 mL**

Scott's Tap Water

Sodium hydrogen carbonate		**3.5 g**
Magnesium sulphate		**20.0g**
Distilled/Tap water		**1000mL**
Pinch of thymol (preservative)		

Eosin/Phloxine stain

1% Eosin Y, aqueous	**CL45380**	**100 mL**
1% Phloxine B, aqueous	**CL 45410**	**10 mL**

APPENDIX B: Histology Methodology

To prepare the fixed samples for sectioning, the samples were first dehydrated, cleared and infiltrated with paraffin wax. The schedule was:

70% ethanol	**1 h**
70% ethanol	**1 h**
90% ethanol	**1 h**
90% ethanol	**2 h**
Absolute ethanol	**2 h**
Absolute ethanol	**2 h**
Absolute ethanol	**2 h**
Xylene	**2 h**
Xylene	**2 h**
Paraffin wax, 58°C - 60°C	**2 h**
Paraffin wax, 58°C - 60°C	**2 h**

Tissues were then embedded in paraffin wax heated to 58°C - 60°C then cooled at room temperature until solid. Prior to sectioning, wax blocks were chilled in the freezer overnight. Sim sections were cut from the wax blocks using a Reichert Jung 2040 rotary microtome. The sections were subsequently placed in 30% ethanol, moved to a 50°C - 52°C water bath, then mounted onto slides. Sections were allowed to dry for 30min at room temperature then baked at 58°C - 60°C for 2h. The slides were transferred to a 42°C oven and left overnight.

To stain, the slides were first deparaffinized. The schedule was:

Xylene	**10 min**

Xylene	**10 min**
Absolute ethanol	**1 min**
Absolute ethanol	**1 min**
Absolute ethanol	**1 min**
90% ethanol	**1 min**
90% ethanol	**1 min**
70% ethanol	**1 min**

After that, the slides were stained with haematoxylin, differentiated, then stained again with eosin/phloxine. The schedule was:

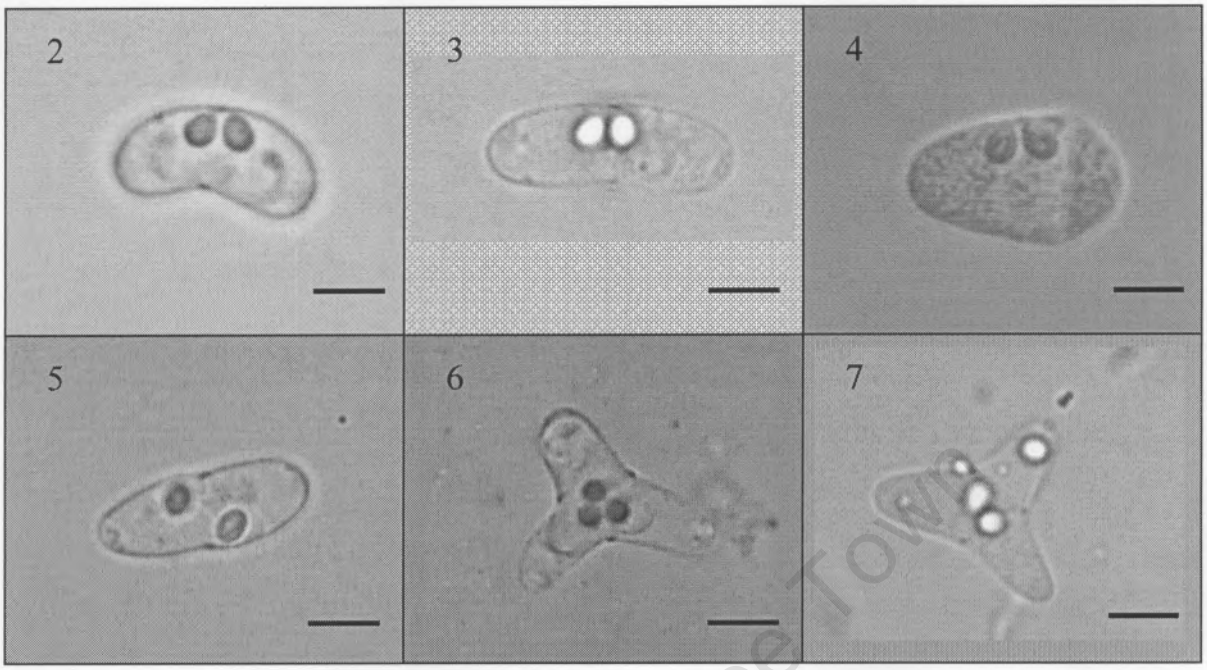
Rinse under running tap water	**1 min**
Stain in Mayer's Haematoxylin	**10 minutes**
Rinse under running tap water	**1 min**
Differentiate in 1% acid alcohol	**3 dips**
Blue in Scott's Tap Water	**2min**
Rinse for under running tap water	**1 min**
Stain in eosin/phloxine stain	**4 minutes**

Dehydrate through graded alcohols

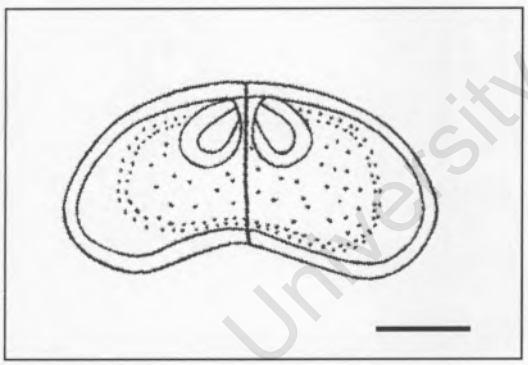
96% ethanol	**2-3 dips**
96% ethanol	**2-3 dips**
96% ethanol	**2-3 dips**
Absolute ethanol	**2-3 dips**
Absolute ethanol	**2-3 dips**
Absolute ethanol	**2-3 dips**

Clear in xylene	**3 dips**
Clear in xylene	**3 dips**

Finally, the slides were mounted using Entallan™.



Figures 2-7



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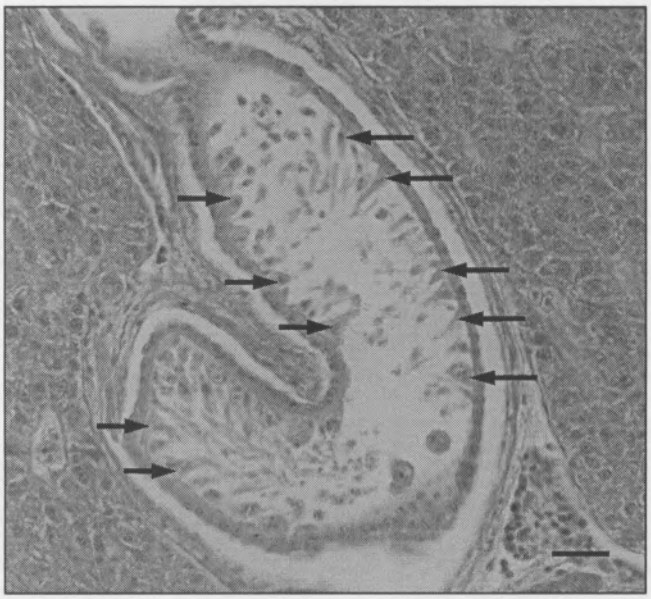

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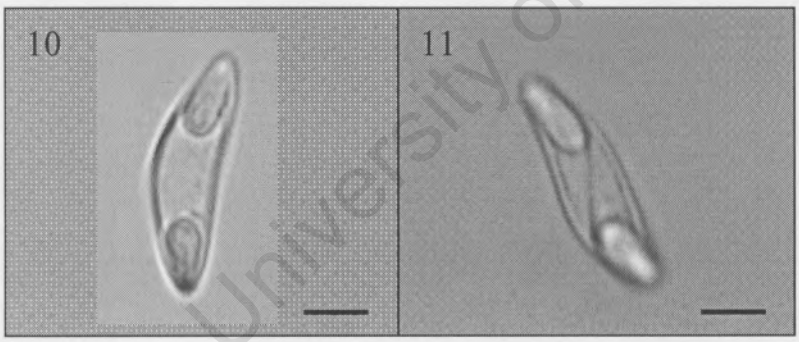

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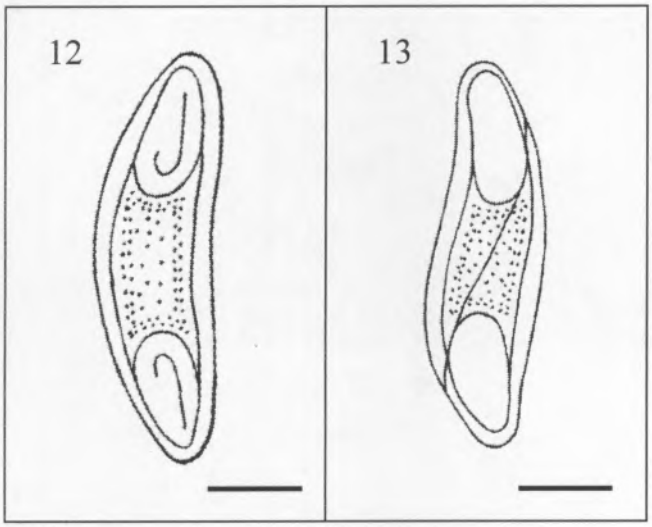

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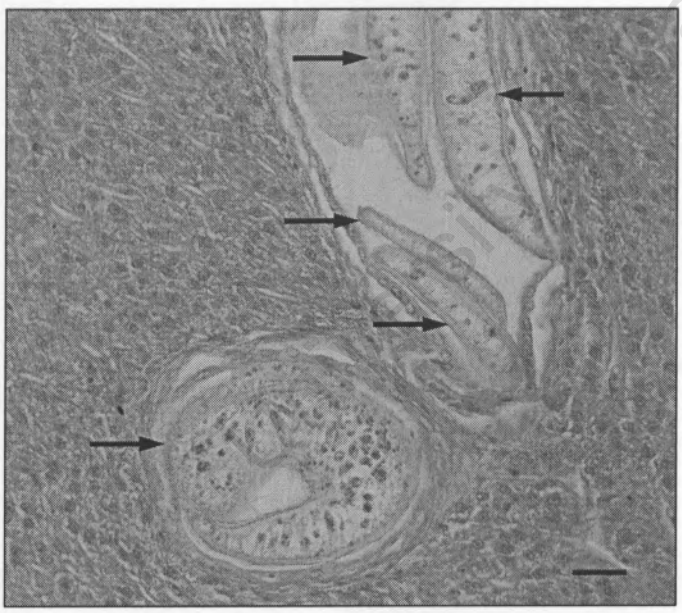

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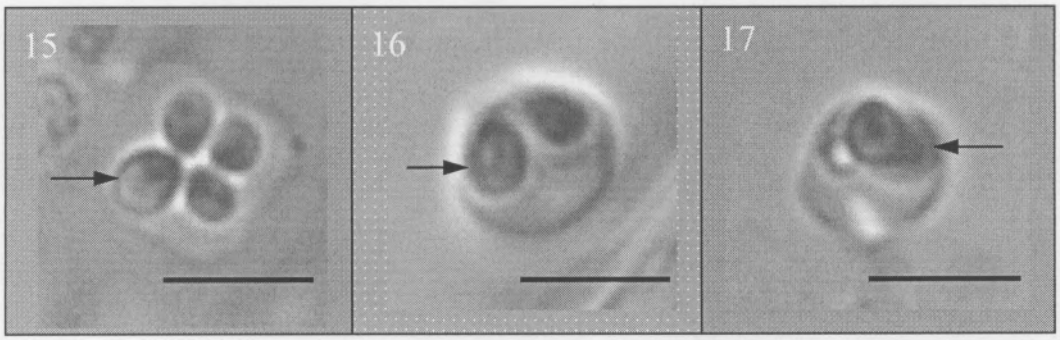

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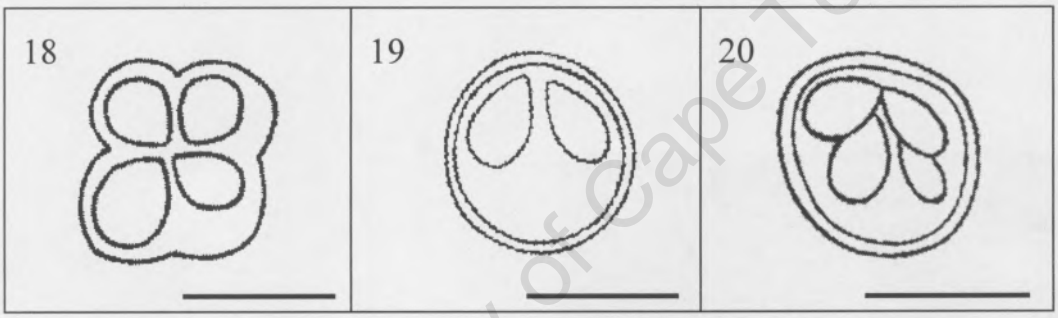

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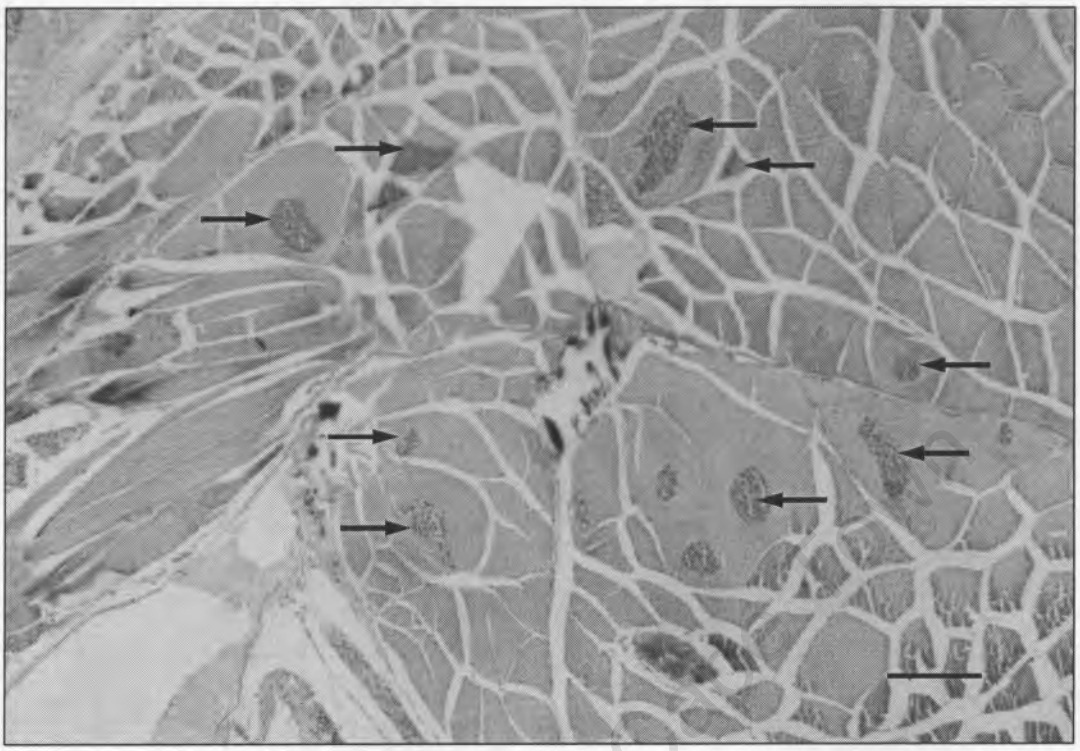

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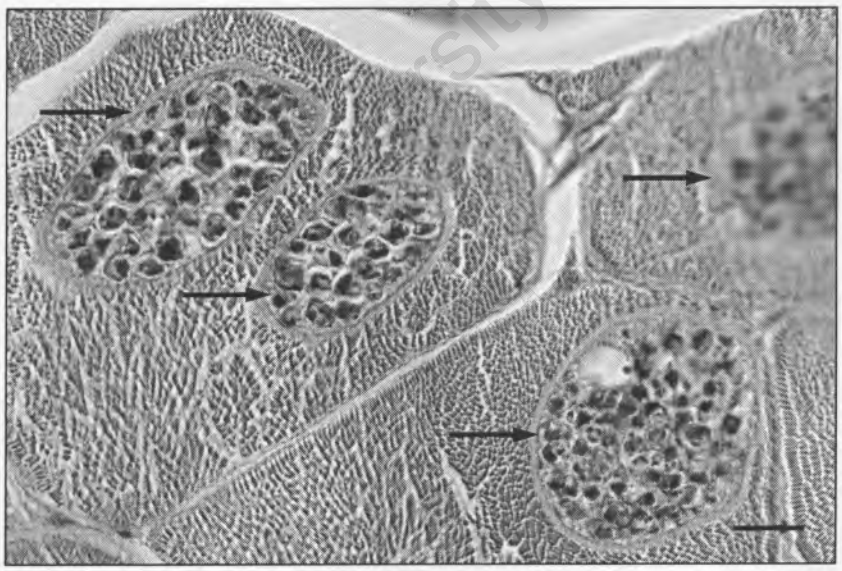

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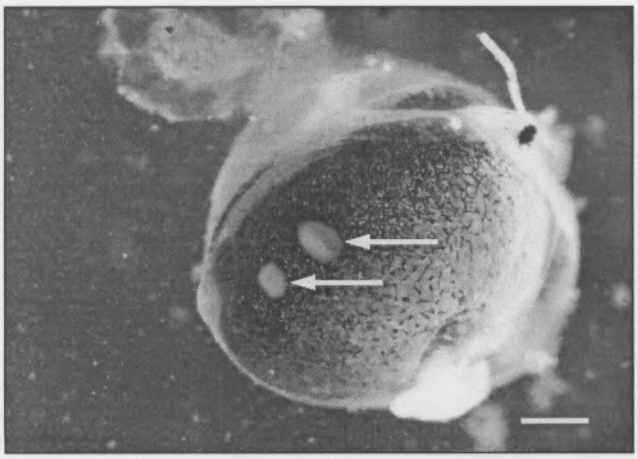

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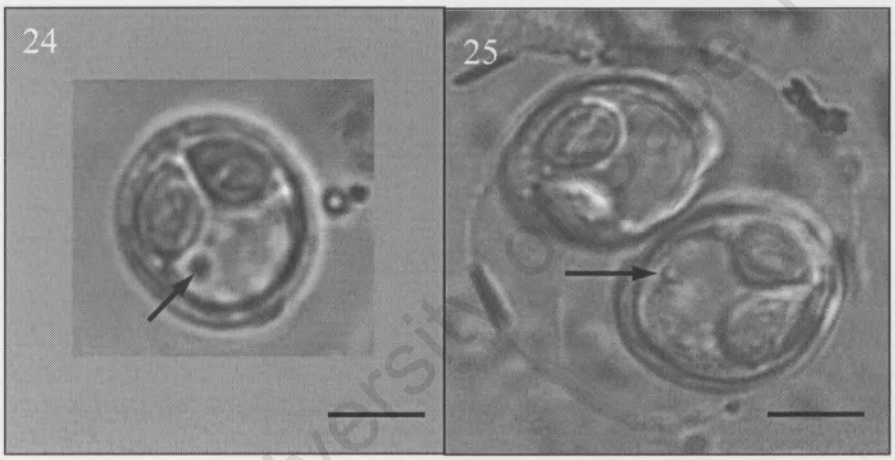

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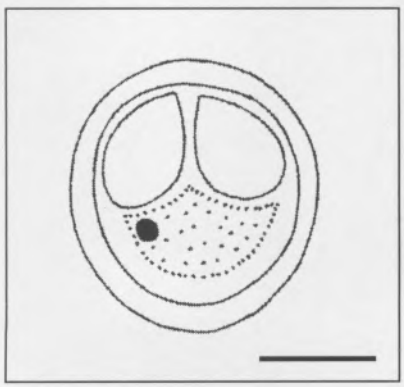

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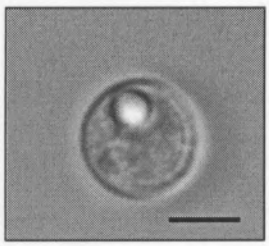

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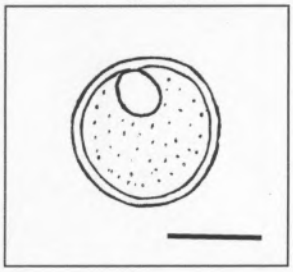

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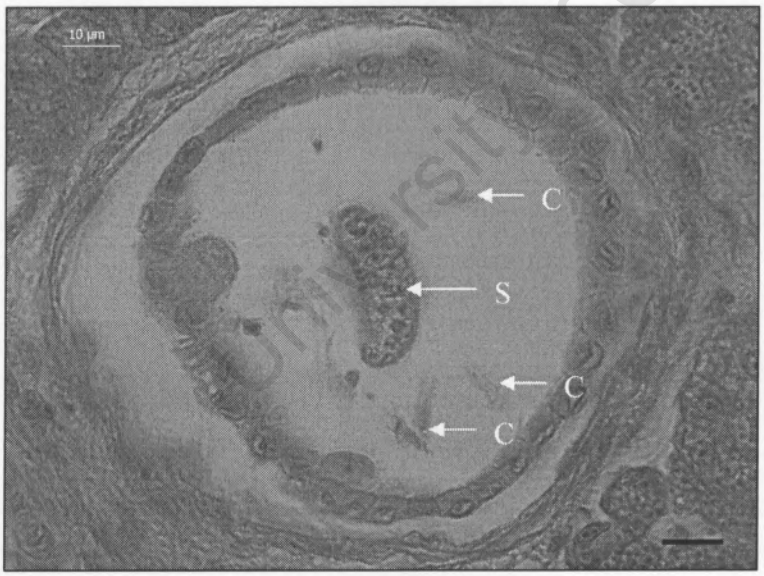

Figure 29

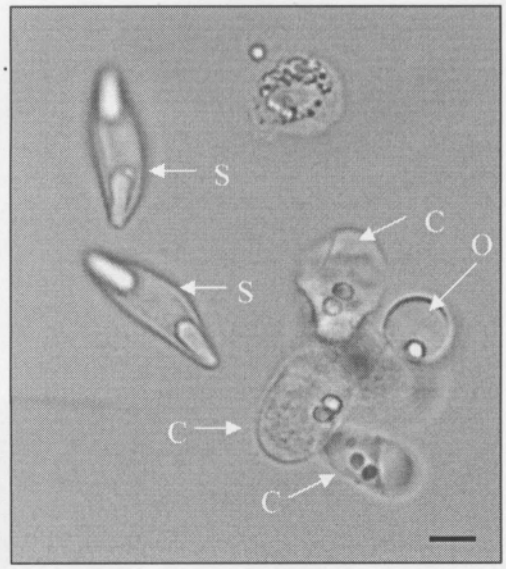

Figure 30