PARASITE ASSEMBLAGES OF THREE ENDEMIC CATSHARK SPECIES FROM THE WEST AND SOUTH COASTS OF SOUTH AFRICA

by
ELEANOR MARGARET YELD

Thesis presented for the degree of
DOCTOR OF PHILOSOPHY
In the Department of Zoology
UNIVERSITY OF CAPE TOWN

February 2009

Supervisors: Prof. Charles L. Griffiths
Dr Nico J. Smit

"You had no right to be born; for you make no use of life. Instead of living for, in, and with yourself, as a reasonable being ought, you seek only to fasten your feebleness on some other person's strength."

Novelist Eliza Reed to her sister Georgiana in Charlotte Brontë's Jane Eyre

For the generations of inspiration and example before me –

Yelds, Segalls, Nobles and Hutchings

DECLARATION

The work presented in this thesis is the sole responsibility of the candidate, although four core chapters (Chapters 3, 5–7) are to be submitted for publication with one or more co-authors. The candidate is principle author of all four. Co-authorship is usually held by my supervisors, who provided guidance during the research and made valuable comments on the draft manuscripts, with two exceptions. Chapter 5: where transmission electron microscopy (TEM) was undertaken at the Electron Microscope Unit at the University of Limpopo (MEDUNSA campus). The candidate spent some time visiting the laboratory, gained instruction in the methodology and instrument use, and collected and prepared samples for TEM. However the majority of this laboratory work was conducted by C. Baker, and although the candidate was solely responsible for the conceptualization, and all interpretation of results and conclusions, this must be considered a collaborative effort. Chapter 6: where scanning electron microscopy (SEM) was undertaken at the Electron Microscope Unit at the University of Limpopo (MEDUNSA campus) by C. Baker, in addition to SEM performed by the candidate as in the remainder of the thesis. Some supplementary laboratory work (clearing, staining and measuring) of samples was carried out by P.H. King. Although the candidate was solely responsible for the conceptualization, and all interpretation of results and conclusions, this too must be considered a collaborative effort. The findings of Chapter 4 have recently been published (*Journal of the Marine Biological Association of the United Kingdom*, **86**, 829-833), in a shortened version.

This work has not been submitted for a degree at any other university and any assistance the candidate received is fully acknowledged.

……………………………………………..   …………………………….

Eleanor Margaret Yeld   Date

ACKNOWLEDGEMENTS

I would like to thank my supervisors, Professor Charles Griffiths and Dr Nico Smit, for their support and constructive comments on draft chapters of this thesis. I am very grateful to Charles for his interest, input, logistical and financial project support, and for giving me this opportunity. I am extremely grateful to Nico for getting me started on fish (and particularly shark) parasitology – and for his enthusiasm, knowledge, guidance and assistance with the practical and theoretical aspects of this research. I could not have started without his help, let alone finished! For help with collecting samples on SCUBA, huge thanks go to Andrea Plos, Karl Reinecke, Debbie Robertson-Andersson, Laura Blamey, Ken Hutchings, the Research Diving Unit at UCT and all the UCT divers. The Marine & Coastal Management De Hoop tagging team, particularly Willie and the shark anglers, went out of their way to help me with catching, carrying and processing sharks, for which I am extremely grateful. Special mention must go to Colin Attwood and Lieze Swart for their support, assistance and interest and to Lieze for the coffee, chats, runs and recipes! For their empathy, and for sharing the early mornings and late nights in the laboratory, field trips, coffee breaks, chocolate, grapes and general all-round support, I wish to thank Tammy Robinson, Robyn Scott, Hannah Medd, Paula de Coito, Kate Watermeyer, Laura Blamey, and the marine postgraduate students. Tammy Robinson, Natasha Kruger and Ken Hutchings were all indispensable when it came to helping with statistics and multivariate analysis and I am very grateful. I wish to thank everyone at the University of Johannesburg, who were all so helpful and gave unstintingly of their time and knowledge: Tannie Edie Lutsch and Yolandi Cloete in the histology lab, the SPECTRAU staff and Maryke Coetzee for assistance with SEM, and all of the fish parasitology students. At the University of Limpopo, MEDUNSA campus, I wish to thank Piet King and Chantelle Baker for their enormous efforts, interest and willingness to help – and for the laboratory work, TEM and SEM. Polly Hayes must be thanked for the inspiration right at the beginning of this project, and for her thesis layout and structure ideas. Gilly Smith, Andrea Plos and all the administrative and support staff at UCT, thank you so much for all the support – from computers and printers to fund numbers, field trip financing, conferences and aquarium functioning! The International Academic Programmes Office at UCT granted me an international exchange in which I spent a semester at the Scripps Institution of Oceanography at the University of California, San Diego. I am extremely grateful for this opportunity, and to Dr Jeff Graham and the shark laboratory (Dan Cartamil, Nick Wegner, Noah Ben-Aderet and Dovi Kacev) for hosting me during my time at Scripps – I learnt so much and had an amazing experience. Angela Russell is gratefully acknowledged for her valuable contribution to the interpretation of TEM images and assistance in the screening of histological sections. Rod Bray, David Gibson and Robin Overstreet are thanked for the original confirmation of the identification of the digenean trematodes (Chapter 6) as belonging to the Anaporrhutinae. Thanks also to David Gibson for allowing me early access to the relevant chapter of the "Keys to the Trematodes", and for sending this to me. This project was funded by a National Research Foundation SEAChange grant to Prof. Griffiths and a SASOL Research Grant to Dr Smit. The South African Biosystematics Initiative Travel Award 2007 allowed me to attend the 7th International Symposium on Fish Parasites in Italy, for which I am very grateful. Thank you to my family and friends for moral support and all the encouragement. To the Yelds, Segalls, Nobles and Hutchings – with so much motivation, and leading by example – how could I not be inspired? Thanks also to my father-in-law, Larry Hutchings, whose motivational tactics of encouragement, threats and competition eventually paid off. To my parents, John Yeld and Nan Yeld, and my step-parents Martine Barker and Robert Segall, for your unswerving support of all kinds, encouragement, tolerance and love, thank you, thank you thank you – I could not have done it without you. Finally, to my husband Ken, thank you for everything. Throughout it all your belief in me never wavered.

ABSTRACT

Parasite assemblages of three endemic catshark species from the west and south coasts of South Africa.

Eleanor Margaret Yeld, February 2009

Key words: Fish parasites, elasmobranchs, biodiversity, taxonomy, Crustacea, Isopoda, Nematoda, *Trypanosoma*, *Haemogregarina*, Digenea, parasite community, host ecology.

This study focused on the parasite assemblages of three catshark (Elasmobranchii: Scyliorhinidae) species: the dark shyshark, *Haploblepharus pictus*, the puffadder shyshark, *H. edwardsii* and the pyjama shark, *Poroderma africanum*, all endemic to Southern Africa. These sharks are found from Namibia to Agulhas (*H. pictus*), Cape Point to Northern KwaZulu-Natal (*H. edwardsii*) and St Helena Bay to KwaZulu-Natal (*P. africanum*), and reach maximum total lengths of 60, 60 and 105 cm respectively. Sharks were collected by SCUBA divers and rod and line fishing from four sites between Saldanha Bay and De Hoop Nature Reserve. Parasites from the skin, gills, body cavity, spleen, stomach and intestine were counted, removed, and fixed as appropriate. Blood smears were collected, fixed, stained with Giemsa and examined. A total of 145 sharks were examined.

The sharks were found to host representatives of a large variety of different parasitic taxa i.e. Crustacea, Trematoda, Nematoda, Hirudinea, Cestoda and Protozoa. Some of these species had previously been recorded parasitising South African elasmobranchs, but little data is available on these other than the original taxonomic description. Others were new to science. The physalopteroid nematode *Proleptus obtusus*, pandarid copepod *Perissopus oblongus* and gnathiid isopod *Gnathia pantherina* have previously been described from the region but additional prevalence and infestation data were recorded from the largest number of sampled hosts to date, host and geographic records were updated, and additional biological notes were presented for these three species, using

light and scanning electron microscope observations. A trypanosome new to science, *Trypanosoma* sp. A, was observed in the blood of all sharks examined and is characterised by its large size, particularly its width, the shape and orientation of the cell nucleus, standard morphometric indices, and its geographic location. Two species of intraerythrocytic apicomplexan haemogregarine new to science, *Haemogregarina* sp. A and *H*. sp. B, were found in the blood of all sharks examined. These are characterised and differentiated by their large size, the shape and orientation of the cell nucleus, standard morphometric indices, staining properties, and cell ultrastructure. *T*. sp. A is the first species of trypanosome, and *Haemogregarina* sp. A and *H*. sp. B the first two haemogregarine species, to be described from Southern African elasmobranchs. These three species also represent the first descriptions of blood parasites of any kind from South African sharks. The digenean trematode *Probolitrema richiardii* was found parasitising the coelom of sharks sampled from the west coast. Although already described, this species was very poorly known. A thorough, detailed redescription was done, using light and scanning electron microscopy, from the largest collection of specimens to date. The tegument structure was also described. It was recommended that this species be reinstated as *Probolitrema capense* and that the current synonymy with *P. richiardii* be disregarded.

Infection sites, prevalence and infestation rates, and standard indicative parasitic indices of the parasites and the assemblage as a whole, were collated. These data were used to elucidate some aspects of the host shark ecology using univariate and multivariate analyses. Host body size did not correlate with parasite load or assemblage, and there was a general lack of significant relationships between parasite load and community composition. Geographic zone of occurrence was of more importance in determining the parasite assemblage than host shark species. The parasite community of South African catsharks seems to be relatively species-poor. There is a notable paucity of research into marine parasite assemblages, particularly on elasmobranch host species, in South Africa. This is the first study to address this shortcoming by investigating a subset of the whole parasite assemblage of marine fish hosts in South Africa, from macro- to micro-parasites.

TABLE OF CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Parasitism is defined as “a relationship in which one species, the parasite, uses the other one, the host, as its habitat and source of nourishment” (Grabda, 1991). In other words, the body of the host forms the primary living environment for the parasite, which is obligated on the host for survival for at least part of its life cycle. This is, however, a form of symbiosis, which literally means “living together” (Roberts & Janovy, 2000). This semi-permanent relationship has many similarities to predation, but the major differences are that parasites cannot afford to kill their host immediately, unlike in a predator-prey situation (Grabda, 1991) and that parasites also usually have only one host (or one host at each life cycle stage) (Roberts & Janovy, 2000). Parasites are ubiquitous, forming one quarter of the world’s described fauna (Grabda, 1991), although this is thought to be a gross underestimate because parasites have been the subject of significantly less research than free-living species (Griffiths, pers. comm.). No known metazoan animal is free from parasite infection.

In order to successfully parasitise a host, a parasite must have certain morphological and physical adaptations (Grabda, 1991). There are both ecto- and endoparasites, with their own specific adaptations. One adaptation displayed by most ecto- and some endo-parasites is the means of attachment to the host’s body, and the morphology that is necessary to accommodate their attachment. This can often be useful in identification, as well as often indicating some degree of host specificity (Kabata, 1979).

Parasite infestations are inevitably detrimental for the host (Roberts & Janowy, 2000). The parasite has either fed on the host's blood or tissues, or has itself digested the host's food (Grabda, 1991). Attachment organs of the parasite can also cause mechanical damage to the host's skin or tissues. Parasites can cause mechanical damage, stimulate a damaging inflammatory or immune response, or simply rob a host of nutrition, but most parasites will inflict a combination of these conditions on their hosts (Roberts & Janovy, 2000). Often the ill effects are slow to accumulate or become obvious, as the parasite is adapted to minimise negative effects on its host. The severity of the effects of parasites on fish species are affected by various factors: how intimate the association is between the host and the parasite; how long the contact is between the two; the abundance, size and nutritional needs of the parasites in comparison to that of the host; and the extent to which the parasite destroys the tissues of the host (Sindermann, 1990).

Parasite specificity can be broken down into the following categories: species, group, organ, physiological, ecological and geographic specificity (Grabda, 1991). The host specificity of a parasite can be very narrow or rather broad. Host specificity also assists in the identification and description of the parasite species. It is also possible to distinguish between phylogenetic and ecological specificity. Phylogenetic specificity indicates that a particular parasite will probably be found in a range of related host taxa; whereas ecological specificity indicates that an abiotic (e.g. host habitat) or biotic (e.g. host food type) variable might be more important (Bush *et al.*, 2001).

In South Africa there is very limited knowledge about parasites on marine fish, and particularly those on elasmobranchs. Elasmobranchs are a subclass of the

Chondrichthyes: the cartilaginous fishes possessing dermal placoid scales and separate gill slits. This group includes most of the sharks, the skates and the rays (Gilbert *et al.*, 1967). The groundsharks, order Carchariniformes, is the dominant group comprising eight families and over 200 known species (Compagno, 1999). Of these, the Scyliorhinidae (catsharks) is the largest family with at least 15 genera and over 100 species (Compagno *et al.*, 1989). Catsharks are generally small (usually less than 100 cm total length), found in both temperate and tropical waters, and from the intertidal zone to over 2000 m depth (Compagno, 1987). At least 15 catsharks species occur in southern Africa and at least 10 of these are endemic (Compagno *et al.*, 1989). This thesis focussed on the parasites of three endemic catshark species, common on nearshore reefs in the southwestern Cape (Smith & Heemstra, 1986; Branch *et al.*, 1995; Dainty, 2002). These species are:

***Haploblepharus pictus* (Müller & Henle, 1838)**

The endemic dark shyshark, *H. pictus*, ranges from Cape Agulhas (nominally, although this species was never seen further east than False Bay during this study) to the mouth of the Orange River (Branch *et al.*, 1995), living in shallow, coastal waters (Figure 1.1). It is a small species of shark, reaching an approximate maximum total length of only 60 cm.

***Haploblepharus edwardsii* (Voight, 1832)**

H. edwardsii, the puffadder shyshark or “Happy Eddie”, is a small, endemic catshark. It reaches an approximate maximum total length of 60 cm. The range of this species is from Cape Point on the west to KwaZulu-Natal on the east (Branch *et al.*, 1995) (Figure 1.1).

Poroderma africanum (Gmelin, 1789)

P. africanum, the pyjama shark, is a common inshore temperate shark, endemic to South Africa. Larger than the other two host species in this study, it reaches an approximate maximum total length of 105 cm. This species ranges from KwaZulu-Natal on the east coast to around St Helena Bay on the west coast (Branch *et al.*, 1995) (Figure 1.1).

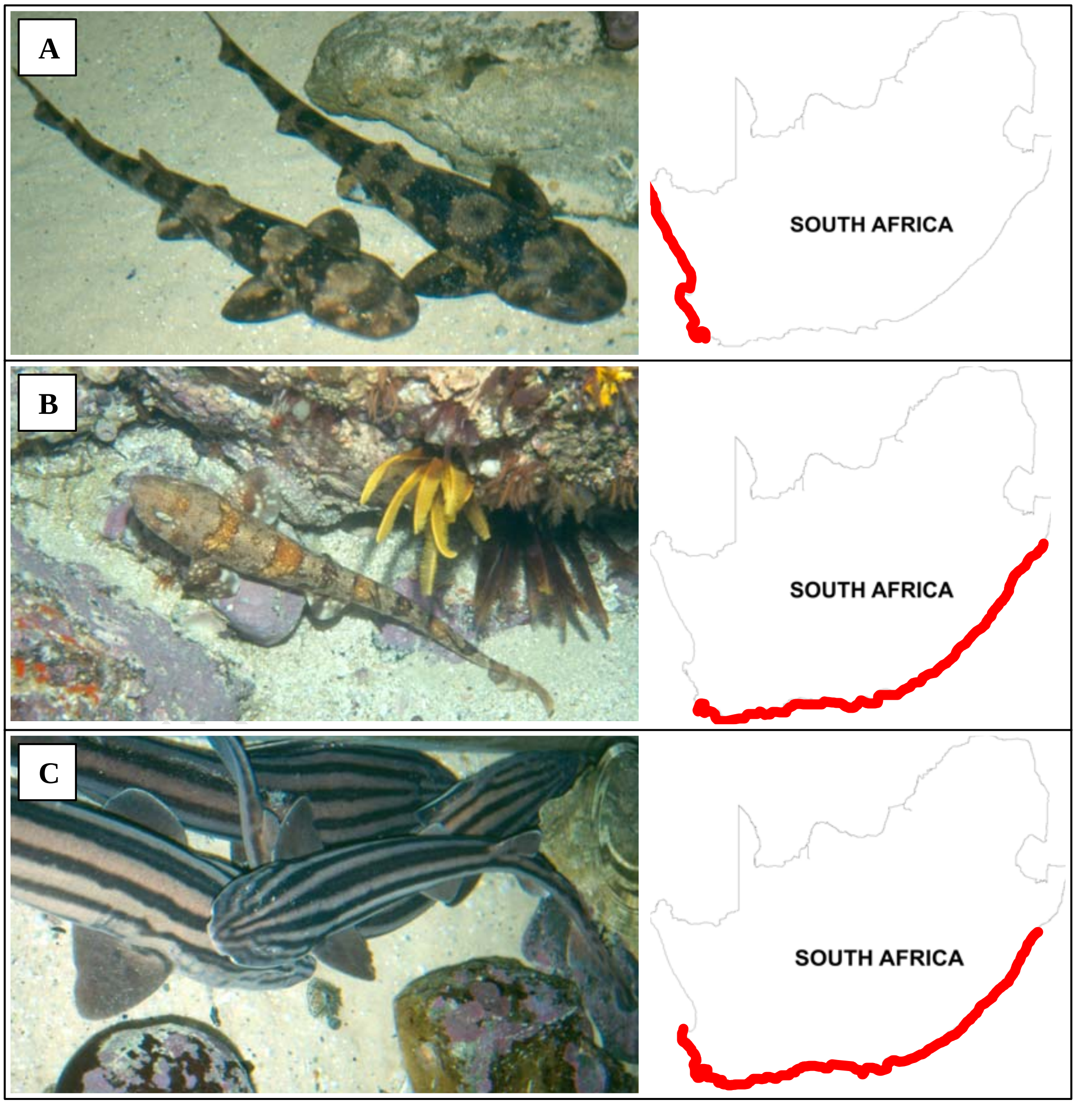

Figure 1.1. Photographs and distributions of the three endemic South African Scyliorhinidae examined as parasite hosts. **A.** The dark shyshark *Haploblepharus pictus*. **B.** The puffadder shyshark *Haploblepharus edwardsii*. **C.** The pyjama shark *Poroderma africanum*.

Relatively little is known about these elasmobranchs, and even less about their parasites. The main groups of marine fish parasites that have been studied in South Africa to date are the copepods (Barnard, 1955; Kensley & Grindley, 1973; Dippenaar & Olivier, 1999; Dippenaar *et al.*, 2001, 2004; Grobler, *et al.*, 2002, 2003a, 2004; Dippenaar, 2004); apicomplexan haemogregarines (Smit, 2000; Davies & Smit, 2001; Smit & Davies, 2001, 2005, 2006; Smit *et al*., 2003a); and the gnathiid isopods (Smit, 2000; Smit & Basson, 2002; Smit *et al*., 2003b; Smit & Davies, 2004; Hayes *et al*., 2007; Hadfield & Smit, 2008; Hadfield *et al*., 2008); with minor studies on the monogeneans (Bullard & Dippenaar, 2003; Grobler *et al*., 2003b; Bullard *et al*., 2004; Vaughan *et al*., 2008); digeneans (Bray, 1984, 1986, 1987); myxozoans (Reed *et al*., 2007); and acanthocephalans (Christison & Amin, 2005). Very little research has been done on the parasites of elasmobranchs, and the majority of this has focused on copepods infecting sharks and rays from the Indian Ocean coast of South Africa (Dippenaar & Olivier, 1999; Dippenaar *et al*., 2001, 2004). Certain parasites are already known from catshark species on the south Coast of South Africa. These are the gnathiid isopod *Gnathia pantherina* Smit & Basson, 2002 from the leopard catshark *Poroderma pantherinum* (Smith, 1838) (Smit & Basson, 2002), and the nematode *Proleptus obtusus* Dujardin, 1845 from the puffadder shyshark *Haploblepharus edwarsii* (Voight, 1832) (Moravec *et al*., 2002). However as hosts, the Scyliorhinidae have been generally neglected, especially those of the west coast (Atlantic Ocean).

The southern African coastline forms one of the world’s 16 marine zoogeographical provinces (Smit, 2000), harbouring over 11000 described species, more than 31 % of which are endemic. The uniqueness of this environment is partly due to its unusual oceanographic properties: namely that it is at the junction of the cold Benguela Upwelling Region and the warm Agulhas Current (Payne & Crawford, 1989). Within the Southern African coastline, five biogeographical provinces are recognised (Branch *et al*., 1995). The study site for this thesis was centered around Cape Point, the junction between the cold temperate Namaqua Province and the warm temperate Agulhas Province. This is an area of high marine productivity and intermediate biodiversity, with the kelp beds found in this region harbouring numerous economically important species and habitats of high conservation value (Payne & Crawford, 1989; Turpie *et al*., 2000).

In South Africa, the few marine fish parasite studies conducted to date have focussed on a particular parasitic taxon or group, and involved a whole number of host species on which that taxon is found (Smit, 2000; Dippenaar & Jordaan, 2006; Hayes *et al*., 2006; Hadfield & Smit, 2008; Hadfield *et al*., 2008). While this allows for excellent systematic work, and adds greatly to the knowledge of that particular group of parasites, both their taxonomy and their ecology, it does not facilitate research into the ecology of the hosts. This thesis aims to look at a group of hosts, and describe the parasite assemblages thereon. It is therefore not be limited to one group of parasite, but rather numerous different groups, even at phylum level. The aim is thus to achieve better understanding of the parasite assemblage infecting these sharks and the possible impacts on the host species, and to use this as indicators of a number of factors of host shark ecology. However, due to the paucity of work on South African elasmobranch parasitology, particularly the catsharks, a large proportion of the parasites are either very poorly known or entirely new to science. This necessitates an in-depth analysis before ecological applications are possible.

There is a gap in knowledge both about the diversity of parasites on elasmobranchs, and the biology and species diversity of these parasites in South Africa. Marine fish parasites have recently been proposed as possible pollution indicators in marine ecosystems for use as an early warning system (MacKenzie, 1999). Other applications of the study of marine fish parasites include the use of parasites as biological indicators of the population biology, migrations, diet and phylogenetics of the host fish (Williams *et al.*, 1992). Parasites as tags in fisheries have some advantages over conventional tagging methods: they do not interfere with host behaviour, they are cheaper and less labour intensive, and less time-consuming. They are being used as tags in population studies as a stock delineation tool, indicators of migratory routes, identifiers of feeding and breeding areas, and to follow juvenile recruitment patterns (Bush *et al*., 2001).

Shark fisheries are of global importance and are generally in decline, due to the life history characteristics of elasmobranchs (Mann, 2000). Research into elasmobranch parasites, their pathogenicity and negative impacts, as well as the ecological and fisheries management applications of parasite studies described above can prove useful for the sustainable management of shark fisheries globally. Although the catsharks in this study are not economically important, the improved taxonomic knowledge and ecological applications developed in this study should be widely applicable.

It is thus important to determine the extent of parasite infestations on elasmobranchs around South Africa, their identity and their effect on the host, whether directly by feeding, or indirectly by acting as vectors. This thesis investigated the parasite assemblages of three endemic catshark species. The relatively small size and ease of capture of these catsharks facilitated their use as study species. These sharks are also keystone species in the nearshore reef and kelp forest environments that they inhabit and are thus integral to the ecosystem functioning in these habitats (Dainty, 2002). Knowledge of the parasites infecting these catsharks is essential before the impacts of the parasites on their hosts and in turn on the broader ecosystem can be understood.

In order to achieve this overall objective, the following key questions were asked:

i. What parasite species are found parasitising the three host catshark species?
ii. Of these parasite species: which are known from South African catsharks, which are known from South Africa although not previously recorded from catsharks, which are known internationally, and which are new to science?
iii. Within each host species, does the parasite assemblage differ over the area sampled?
iv. For areas where host shark species co-occur, does the parasite assemblage differ between host species?
v. Can the subset of the whole parasite assemblage of individual sharks be used to detect these differences?

Thesis outline

Chapter 2 presents the general materials and methods used in this thesis. This is in order to avoid repetition, as the same sampling procedure and sampling areas were used throughout the thesis. Each subsequent chapter details any methodologies specific to that chapter.

Chapter 3 investigates those parasite species already known from South African sharks and provides additional biological notes on each of these. In all cases this study represents a much more thorough sampling of host sharks and a larger number of parasite specimens than previously available. Knowledge of the prevalence, infestation rates, host records and geographical records of these known parasites were all thus substantially increased. Scanning electron microscopy (SEM) and light microscopy were used to elucidate morphological details. In some cases redescription of a poorly described species was done, and in others new aspects of functional morphology and attachment were described.

A trypanosome species new to science is described in Chapter 4.

Chapter 5 presents the description of two intraerythrocytic apicomplexan haemogregarines, both new to science, through the use of light microscopy and transmission electron microscopy (TEM). Possible vectors of these haemogregarine species are investigated by histology.

A digenean trematode species, previously described but poorly known, is detailed in Chapter 6. A complete redescription is presented, using light microscopy, SEM and morphometrics. The systematics of the group is explored, and a taxonomic revision recommended.

Chapter 7 summarises all the information presented in the preceding chapters, in terms of all hosts sampled and all parasites collected. The parasite assemblages are described: infection sites, prevalences and infestation rates are detailed and broken down among host species and sampling locations. Ecological and community study applications of the parasite assemblage are explored via the use of frequency distributions, univariate statistics and multivariate analysis; and the effectiveness of these techniques is discussed. This is placed into context in terms of general parasite community ecology and previous such studies.

Thesis conclusions and syntheses are presented in Chapter 8, which summarises the findings of this thesis and answers the key questions posed in this introductory chapter. Topics for further research are discussed.

CHAPTER 2

GENERAL MATERIALS AND METHODS

The following general methodology applies to Chapters 3–7 and is given here in order to avoid unnecessary repetition in the rest of this thesis. Additional methods specific to individual sections are given in the relevant chapters.

2.1 Collection sites

For the purposes of the main study, fresh material was collected from four locations on the west and south coasts of South Africa (Figure 2.1). A small number of archived samples from previous collections at two of the sites (Granger Bay and De Hoop Nature Reserve) were also available for study. The four locations were chosen to encompass geographic zones where only one of the two congeneric (*Haploblepharus*) host shark species occurred, and also to include zones where the two species co-occurred.

Saldanha Bay (33º02’S 18º02’E) (Figure 2.1) is situated on the west coast of South Africa, between the Berg River mouth in the north and the West Coast National Park (Langebaan) in the south. It is the second largest natural bay on the west coast of South Africa, and site of a naturally sheltered harbour. A temporary field laboratory allowed for examination and processing of the samples on site.

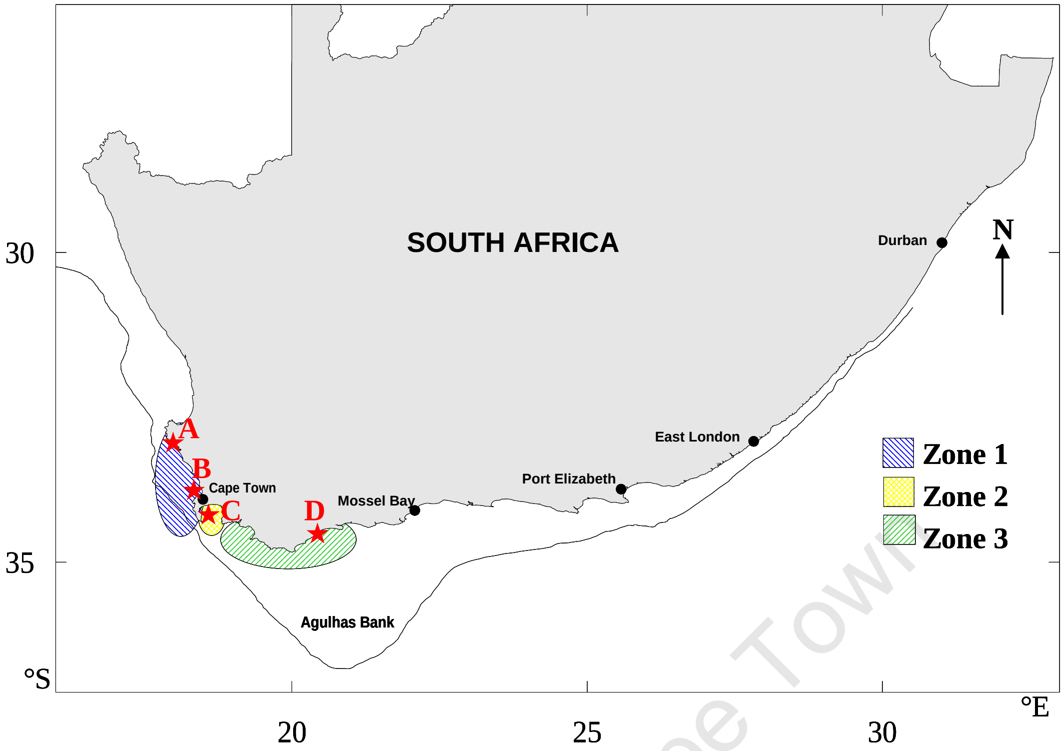

Figure 2.1. Four sampling locations on the west and south coasts of South Africa. **A.** Saldanha Bay. **B.** Granger Bay. **C.** False Bay. **D.** De Hoop Nature Reserve. Occurrence zones of host scyliorhinid catsharks, *Haploblepharus pictus* and *H. edwardsii*, are shown (note that this depicts only those areas sampled and not the complete distributions of these sharks). **Zone 1**. *H. pictus* exclusive occurrence. **Zone 2**. Overlap of both species occurrence. **Zone 3**. *H. edwardsii* exclusive occurrence.

Granger Bay (33º54’S 18º23’E) (Figure 2.1) is situated on the West Coast of South Africa, on the southern edge of Table Bay. It is the site of a busy commercial slipway and a highly built-up suburb of Cape Town, including piers, breakwaters, marinas and hotels. Samples collected at this site were kept in marine aquaria, and examined and processed in the laboratory at the University of Cape Town.

False Bay (34º14’S 18º28’E) (Figure 2.1) is situated just east of Cape Point and the Cape of Good Hope, and is a large bay stretching from Cape Point in the west around to Cape Hangklip in the east. Sampling took place on the western coast of False Bay between Buffels Bay and Simonstown (inclusive). Samples collected at this site were kept in marine aquaria, and examined and processed in the laboratory at the University of Cape Town.

De Hoop Nature Reserve (34^{o}28’S 20^{o}30’E) (Figure 2.1) is situated on the south coast of South Africa and includes a marine protected area (MPA) extending three nautical miles off the coastline. The coastline comprises long stretches of sandy shore, rocky cliffs and a wide, flat rock platform with deep pools that is exposed at low tide. A temporary field laboratory was established at both Koppie Alleen and Lekkerwater (sampling sites within De Hoop) allowing immediate specimen examination.

2.2 Collection and identification of sharks

Sharks were collected from the four sites between February 2004 and November 2006. Two main methods of capture were used. In the first method, sharks were caught by SCUBA divers (Figure 2.2A). Bait and/or chum (sardines or other fish) was placed into mesh bags and taken underwater into suitable habitat. The bait was then manipulated to release the scent. Catsharks are voracious predators and extremely inquisitive, and swiftly respond to bait in the water by approaching the bag and either circling or fiercely biting the bag. Divers were then able to catch the sharks by hand and place them into mesh storage bags. Captured sharks were then transported live to the University of Cape Town, where they were kept in marine aquaria at 14°C.

Alternatively, sharks were collected by rod and line fishing (predominantly at De Hoop Nature Reserve) (Figure 2.2B), and examined immediately in a temporary field laboratory in the reserve (Figure 2.2C).

Sharks were identified using Smith & Heemstra (1986). Following identification, the total length (TL) was measured to the nearest mm from the tip of the snout to the tip of the caudal fin, and the sex of each individual determined (Figure 2.3). Sharks were then anaesthetised with ice and euthenased by severing the spinal cord just posterior to the head (approved by the Animal Ethics Committee of the Zoology Department, University of Cape Town), before being examined for parasites. All sharks were examined for ectoparasites (section 2.3), endoparasites (section 2.4) as well as for blood protozoans (section 2.5) (Figure 2.2D). Parasites from the skin, gills, body cavity, stomach and intestines were counted, removed and fixed in 70% ethanol, 4% or 10% formalin as appropriate.

2.3 Collection, examination and preservation of ectoparasites

Three predominant types of ectoparasites were obtained from the four collection sites, all of which are haematophagous. These were pandarid copepods (found on the skin and fins), juvenile gnathiid isopods (found on the gills and gill arches) and leeches (found on the skin and fins). These were detached with fine brushes or forceps and placed into jars of seawater before being transferred to either 70 or 90 % ethanol, or to formalin, as appropriate. Scanning electron microscopy was performed on some of the specimens (section 2.6).

Figure 2.2. Collection methods and localities. **A.** The author and assistant with a collection bag containing *Haploblepharus pictus* collected on SCUBA at Saldanha Bay. **B.** The author and Marine & Coastal Management Fish Tagging Team rod and line fishing at De Hoop Nature Reserve. **C.** Dissecting the gills of *H. edwardsii* in a temporary field laboratory. **D.** The author making a blood smear slide at De Hoop Nature Reserve.

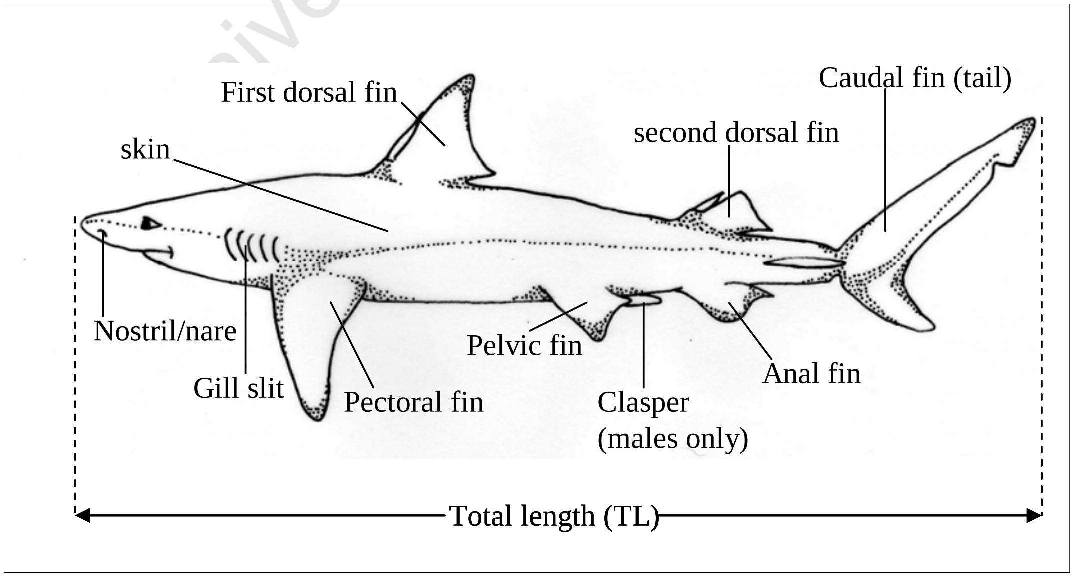

Figure 2.3. Generalised shark (lateral view) showing external features, measurement used, and sites examined for parasites (redrawn from Heemstra & Heemstra, 2004).

2.4 Collection, examination and preservation of metazoan endoparasites

Two predominant types of metazoan endoparasites were found in the host catsharks. These were digenean trematodes, found in the body cavity, and physalopteroid nematodes, found in the stomach and (rarely) intestine. Scanning electron microscopy was performed on some of the specimens (section 2.6). In a few circumstances, tapeworms (Cestoda) were found in the spiral intestine, but these were found in such small numbers and in so few hosts that they were preserved for future study and identification, but not considered further in this thesis.

2.5 Collection, preservation and examination of blood protozoans

Four dragged blood smears of one cell thick were made per shark, and fixed in absolute methanol. A drop of fresh blood was placed onto a clean glass slide and smeared into a thin layer by dragging the edge of an additional slide along the length of the smear slide (Figure 2.2D). Blood smears were stained with phosphate-buffered Giemsa, following standard procedures (Smit & Davies, 2001, 2005), and screened for blood parasites with a Zeiss Axioplan compound microscope. Protozoans were examined, drawn, photographed and measured.

2.6 Scanning Electron Microscopy (SEM)

Scanning electron microscopy was done on specimens using standard techniques (Bozzola & Russell, 1999). Fixed specimens were hydrated from 70 % ethanol to fresh water. They were then dehydrated through a series of ethanol concentrations and critical point dried using a Bio-RAD CPD750. Dried specimens were mounted onto inverted conical stubs with a quick-drying varnish (Japan Gold Size) and then sputter gold-coated with an Emscope SC500. Scanning electron microscopy was done using a JEOL JSM-5600 S.E.M., with Acc volt 15 kV, magnification x 1,800 and a WD (working distance) of 21 mm.

SEM was performed at the University of Johannesburg (SPECTRAU).

2.7 Transmission Electron Microscopy (TEM)

Transmission electron microscopy was performed on specimens using standard techniques (Bozzola & Russell, 1999). Small blocks cut from host shark spleens were fixed in Karnovsky's fixative and rinsed in Sorenson's phosphate buffer (pH 7.4) before being post-fixed in osmium tetroxide. They were then dehydrated through a series of ethanol concentrations and rinsed in transitional fluid at each change, transferred into a mixture of propylene oxide and epoxy resin for 24 hours followed by two changes of freshly prepared resin for 24 hours each. They were finally placed in fresh resin in plastic moulds and polymerised at 60^{o}C for 48 hours.

Semi-thin sections were cut, placed on a slide, and dried before being stained with toluidine blue and examined. Thereafter ultra-thin sections of areas of specific interest were cut, placed on copper grids and stained with uranyl acetate and Reynolds' lead citrate. They were examined with a JEM-1010 transmission electron microscope (JEOL). Digital images were captured using a DualVision® 300W CCD camera and DigitalMicrograph® software system.

TEM was performed at the Electron Microscope Unit at the University of Limpopo (Medunsa campus).

2.8 Histology

Following standard histological techniques (Bancroft & Cook, 1994; Galigher & Kozloff, 1971; Presnell *et al*., 1997). Specimens were dehydrated through a series of increasing concentrations of ethanol (10 % to absolute) before being routinely embedded in paraffin wax, sectioned at 5-7 microns, and stained using haemotoxylin and eosin. Stained sections were mounted on glass slides, cover-slipped using DPX and examined under a microscope. All histology was performed in the Histology Laboratory at the University of Johannesburg.

CHAPTER 3

ADDITIONAL BIOLOGICAL NOTES ON THREE PARASITE SPECIES FROM SOUTH AFRICAN CATSHARKS

3.1 Introduction

During an investigation of the parasite community associated with three species of endemic South African catsharks (family Scyliorhinidae), a variety of parasitic taxa were discovered, comprising well-known and described species, described but poorly known species, and species new to science.

Parasites already known from catshark species on the South Coast of South Africa include the nematode *Proleptus obtusus* Dujardin, 1845 from the puffadder shyshark *Haploblepharus edwarsii* (Voigt, 1832) (see Moravec *et al*., 2002), and the gnathiid isopod *Gnathia pantherina* Smit & Basson, 2002 from the leopard catshark *Poroderma pantherinum* (Smith, 1838) (see Smit & Basson, 2002). Although not recorded from these particular sharks, various copepod species have also been reported from South African elasmobranchs, and are reasonably well known (Dippenaar & Olivier, 1999; Dippenaar *et al.*, 2001; Dippenaar & Jordaan, 2006).

This chapter provides additional biological notes on the morphology, distribution, mean intensity of infection and prevalence of three previously described species – the endo-parasitic nematode *Proleptus obtusus*, the ecto-parasitic copepod *Perissopus oblongus* Wilson, 1908, and the temporarily ecto-parasitic isopod *Gnathia pantherina*, from three catshark species on the west and south coasts of South Africa.

3.2 Materials and methods

As in Chapter 2 of this thesis, with the following additional methods specific to this chapter.

Nematodes were stored in 70 % ethanol, examined using light microscopy, and photographed. Scanning electron microscopy was performed on the specimens.

Copepods were stored in 70 % ethanol before being cleared in 88% lactic acid, and stained in 1 g:10 ml Lignin Pink in lactic acid for one hour. They were then dissected, drawn using a *camera lucida*, photographed and measured. Adapting the methods of Oldewage (1992), measurements were taken of diagnostic features: the width and length of the cephalothorax, the length of the genital segment and the total body length. All measurements presented were taken from a specimen examined under light microscopy. Scanning electron microscopy was performed on four different specimens (two males and two females).

Unfed (zuphea), partially fed (zuphea/praniza) and replete (praniza) juvenile gnathiid isopods were collected from sharks captured in De Hoop Nature Reserve. Archived gnathiid material from Koppie Alleen was also available for examination. Gnathiids were removed with fine forceps from the skin, nares and gills of host sharks. Some live third stage pranizae were kept alive in fresh seawater until moulted into adult stages to confirm species identification using methodology of Smit (2000).

3.3 Results

Table 3.1 lists the three host catshark species examined, the total number of each sampled, and the site of infection, prevalence, mean intensity of infection and total number of collected specimens of each of the three parasites on each shark species. Further biological data on each of these three parasites are given in the following sections.

Table 3.1. Site of infection/infestation, prevalence (percentage), mean intensity of infection/infestation (average number per shark ±SE (range)) and total number of specimens collected (n) of three species of parasite from the catsharks *Haploblepharus pictus*, *Haploblepharus edwardsii* and *Poroderma africanum*. Total number of host catshark specimens collected (N) also shown. Dashes indicate no infection/infestation of a host with that parasite species.

Host catshark	N		*Proleptus obtusus*	*Perissopus oblongus*	*Gnathia pantherinum*
Haploblepharus pictus	91	Site	Stomach	Skin & fins	–
		Prevalence	100 %	12 %	–
		Mean intensity	18.6±2.43(1-117)	0.36±0.12(0-7)	–
		n	1692	33	–
Haploblepharus edwardsii	53	Site	Stomach	Skin & fins	Gills & skin
		Prevalence	100 %	4 %	77 %
		Mean intensity	58.9±6.4(1-162)	0.08±0.05(0-2)	34.1±6.7(0-272)
		n	3061	4	1772
Poroderma africanum	14	Site	Stomach	–	Gills & skin
		Prevalence	100 %	–	64 %
		Mean intensity	37.1±5.6(7-74)	–	65.7±17.6(0-205)
		n	520	–	705

3.3.1 Proleptus obtusus Dujardin, 1845

Systematics[1]

Class SECERNENTEA

Order SPIRURIDA

Superfamily SPIRUROIDEA

Family PHYSALOPTERIDAE

Genus *Proleptus*

Proleptus obtusus Dujardin, 1845

(Figures 3.1 and 3.2)

---

[1] The above represents the classical phylogeny which differs somewhat from the proposed molecular phylogeny used in recent literature.

The physalopteroid nematode, *Proleptus obtusus* Dujardin, 1845, was found in the stomach of every shark examined in this study. This nematode was recorded by Moravec *et al*. (2002) from the puffadder shyshark *Haploblepharus edwarsii* in De Hoop Nature Reserve on the South Coast of South Africa, and a detailed description was given therein. In this study it is also recorded from the catsharks *H. pictus* and *Poroderma africanum*, and the coastal range extended from Saldanha Bay on the West Coast to De Hoop Nature Reserve on the South Coast. The greatest infection of 162 individual nematodes was found in a puffadder shyshark, *H. edwardsii*, from False Bay.

Figure 3.1 shows the nematodes *in situ* in the stomach of a dissected puffadder shyshark, *H. edwardsii*. Further scanning electron micrographs are made available for this species in Figure 3.2.

Figure 3.1 Nematodes *Proleptus obtusus*: *in situ* in the stomach of the puffadder shyshark, *Haploblepharus edwardsii*, from the De Hoop Nature Reserve, South Africa.

Members of the physalopteroid nematode genus *Proleptus* Dujardin, 1845, are for the most part parasites of elasmobranchs (Moravec *et al.*, 2002). However, according to Specian *et al*. (1975), the taxonomy of *Proleptus* spp. is not clear-cut, due to the sparsity of specimens on which many descriptions were based, and also their high level of intraspecific variability (Lloyd, 1920 in Moravec *et al.*, 2002). Dujardin (1845) was the first to describe *P. obtusus*, based on one specimen collected from the shark *Scyllium catulus* from the Mediterranean, but did not give a full description. *P. obtusus* has since been described by various authors (Linstow, 1901; Seurat, 1919; Lloyd, 1920; Müller, 1925; Baylis, 1933; Rodrigues *et al.*, 1975; Petter & Radujkovic, 1989), but there is still inadequate knowledge of its morphological structure, or the relationship between *P. obtusus* and other members of the genus (Moravec *et al.*, 2002).

The site of infection recorded in this study differs from that recorded by Moravec *et al*. (2002) who describe *P. obtusus* from the intestine of the puffadder shyshark *H. edwardsii*. All the nematodes in this study were found in the stomach of the host. However, the presence of the following characteristics: thick, transversely striated cuticle (Figure 3.2A), tapered anterior with rounded cephalic end (Figure 3.2B), inflated cuticle forming cephalic collar in cervical region (Figure 3.2C–E), two extremely large, rounded lateral pseudolabia surrounding oral aperture (Figure 3.2C and E), small conical deirids (Figure 3.2B), conical tail with rounded tip (Figure 3.2F), and in female vulva anterior to anal opening (Figure 3.2F), confirm the identification as *P. obtusus*, as presented by Moravec *et al*. (2002).

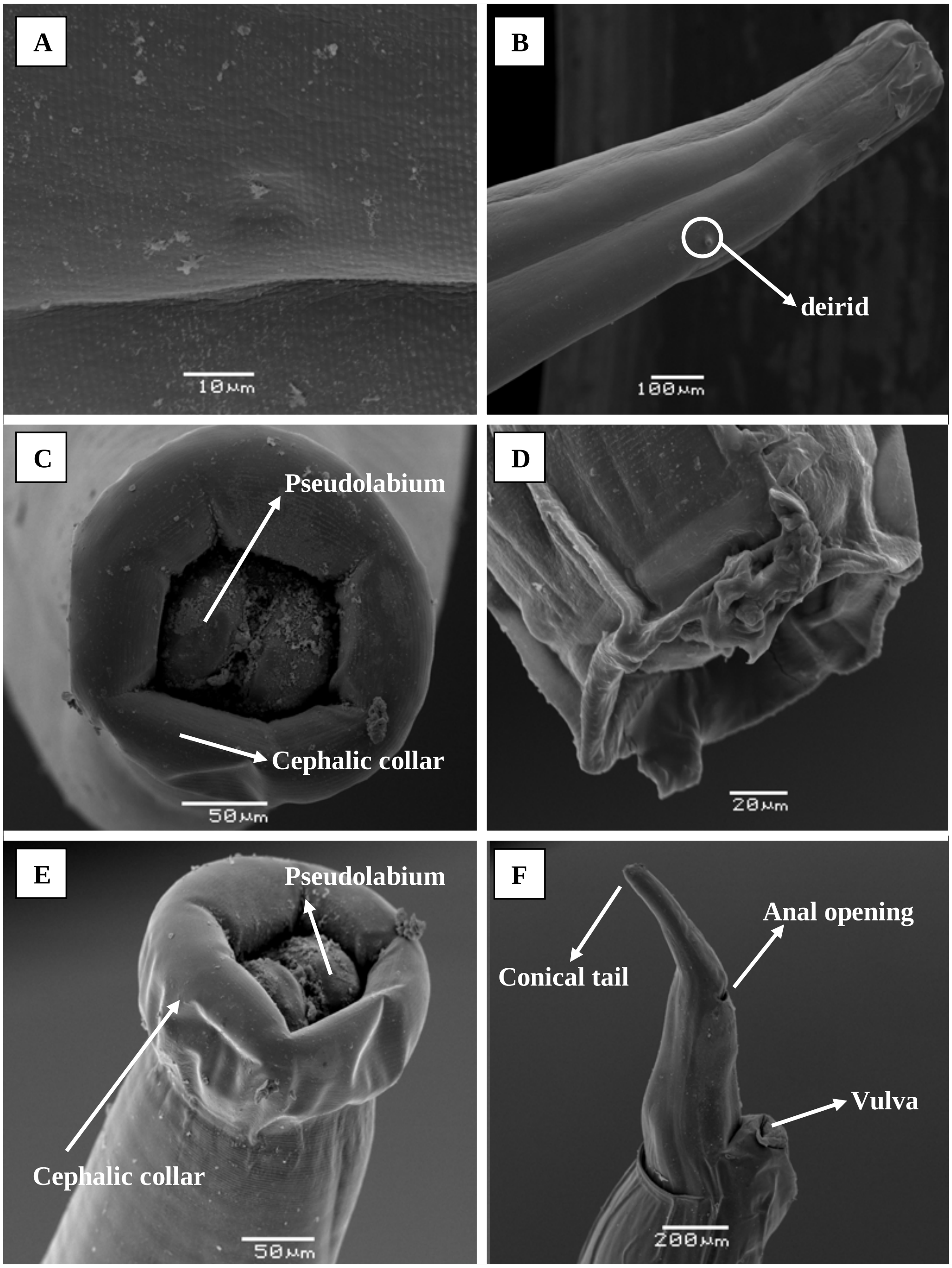

Figure 3.2 Scanning electron micrographs of characteristics of the nematode *Proleptus obtusus*, from scyliorhinid hosts, *Haploblepharus pictus*. *H. edwardsii* and *Poroderma africanum*, from the west and south coasts of South Africa. **A.** Striated cuticle. **B.** Tapering anterior end with deirid. **C.**, **D.**, **E.** Detail of anterior end including cephalic collar and pseudolabium. **F.** Posterior end showing vulva, anal opening and conical tail.

According to Skryabin & Sobolev (1964) and Petter & Radujkovic (1989), *P. obtusus* has been recorded from various species of shark and ray from both the Atlantic and Indian Oceans: *Scyliorhinus stellaris* (type-host), *S. canicula*, *Squalus acanthias*, *Mustelus laevis*, *Aetobatus narinari*, and a "mole-shark" (?*Rhinobatus*) (Table 3.2). In South Africa it has been recorded from a "mole-shark" and an unidentified shark caught off Robben Island (Baylis, 1933), before being reported from *H. edwardsii* off the De Hoop Nature Reserve on the South Coast of South Africa (Moravec *et al.*, 2002). The presence of *P. obtusus* in *H. pictus* and *Poroderma africanum* are new host records for this nematode.

Table 3.2. Descriptions and reports of *Proleptus obtusus* Dujardin, 1845 from various elasmobranchs, detailing host species, location and reference. N/A: not available

Host	**Location**	**Reference**
Scyliorhinus stellaris L.	Mediterranean Sea	Dujardin, 1845
Scyliorhinus canicula (L.)	N/A	N/A
Squalus acanthias L.	N/A	N/A
Mustelus laevis (L.)	N/A	N/A
Aetobatus narinari (Euphrasen)	N/A	N/A
"mole-shark"	South Africa	Baylis, 1933
"shark"	Robben Island, South Africa	Baylis, 1933
Haploblepharus edwardsii	De Hoop Nature Reserve	Moravec *et al*., 2002
Haploblepharus pictus; H. edwardsii; Poroderma africanum	West and South coasts of South Africa	Present study

3.3.2 Perissopus oblongus (Wilson, 1908)

Systematics

Subphylum CRUSTACEA

Class COPEPODA

Order SIPHONOSTOMATOIDA

Family PANDARIDAE Milne-Edwards, 1840

Genus *Perissopus* Steenstrup & Lütken, 1861

Perissopus oblongus

(Figures 3.3–3.7)

Synonyms *Achtheinus dentatus* Wilson, 1911; *A. pinguis* Wilson, 1912; *A. japonicus* Wilson, 1922; *A. parvidens* Wilson, 1923; *A. intermedius* Kurtz, 1924; *A. platensis* Thomsen, 1949; *A. impenderus* Shen & Wang, 1958.

Ectoparasitic copepods found parasitizing the skin, fins and cloacal region of *Haploblepharus pictus* and *H. edwardsii* on the west and south coasts of South Africa, were identified as *Perissopus oblongus*. The majority of the work on copepods done in South Africa has been focused on the Indian Ocean coast (Dippenaar & Olivier, 1999; Dippenaar *et al.*, 2001). A number of parasitic copepod species have been noted on South African sharks, but there has been very little research on these species, other than presence/absence data (Barnard, 1955; Kensley & Grindley, 1973).

The Pandaridae comprises 14 accepted genera, and is further divided into two groups of seven genera each: those with all three thoracic segments with dorsal or dorsolateral plates (*Pandarus*-group, which includes *Perissopus* and *Achtheinus*) and those with the second free thoracic segment without plates (*Dinemoura*-group)

(Kabata, 1979). The taxonomy of this group is based on the characteristics of the female. For the most part pandarid copepods are parasites of elasmobranchs, although there are a few odd records from teleost hosts (Kabata, 1979). They are relatively host and infection-site specific, being found on the body surface, gills, gill-arches, around the cloaca, and in the mouth and nares (Benz, 1981; Rokicki & Bychawska, 1991).

Wilson (1908) originally described various species of the genus *Achtheinus* from sharks off South Africa, and, of particular relevance to this study, *A. dentatus*. Localities of this species included Algoa Bay; Table Bay (on *Acanthias*); Kalk Bay (on *Mustelus* and *Carcharias*); Table Bay (on *Carcharodon*); and Natal (on an unspecified species of dogfish) (Barnard 1955). Cressey (1967) did a complete review of the copepod family Pandaridae, and assigned two species to the genus *Perissopus*: *P. oblongus* and *P. dentatus*. All the previously described species of *Achtheinus* were grouped together under *P. oblongus* (Oldewage, 1992). Recently, however, the systematics of *Perissopus* were again brought into question as *P. galeorhini* (Yamaguti, 1936) was resurrected in an Argentinean study (Etchegoin *et al*., 2001). The genus *Achtheinus* has also been resurrected and is in use by some authors, although there is still debate surrounding this (Benz *et al*., 2003; Dippenaar & Jordaan, 2006). Dippenaar & Jordaan (2006) also questioned the identity of *P. oblongus* described from South African waters. The material collected in this study therefore provided an opportunity to re-examine *P. oblongus* from South Africa.

This chapter presents new host and distribution records, gives a brief, detailed redescription of both male and female *P. oblongus*, and provides additional details of their functional morphology. Also presented are the first scanning electron micrographs of this species, which provide a new perspective and resolution of detail previously lacking in *P. oblongus* studies from South Africa.

Description

Adult female

Cephalothorax rounded anteriorly and approximately as wide (2.60 mm) as long (2.20 mm) (Figure 3.3A). Two pairs of thoracic plates, second pair larger than the first. First pair overlaps the second, although each set does not overlap medially. Genital segment large (3.06 mm long x 2.98 mm wide) in relation to total body length (5.26 mm). A distinctive dorsal keyhole-shaped invagination of genital segment on the posterior edge, above origin of the egg-strings. Two egg-strings originate ventrally from genital segment approximately 25 % from the posterior extremity of the segment. Egg-strings may be up to three times total body length. Abdomen bears two oval caudal rami.

Antennule of two approximately equal segments. Second segment protrudes from frontal plates and bears 10 smooth setae, first segment bears 7–10 setae (Figure 3.3B). Antenna prominent, with terminal segment heavily sclerotized and bearing two loosely arranged rows of proximally-directed triangular hooks (Figure 3.3C). Mouthtube long and slender and typically pandarid in shape (Figure 3.3D). Maxilliped with laterally directed, rounded protuberance at junction with short, terminally hooked segment (Figure 3.3E).

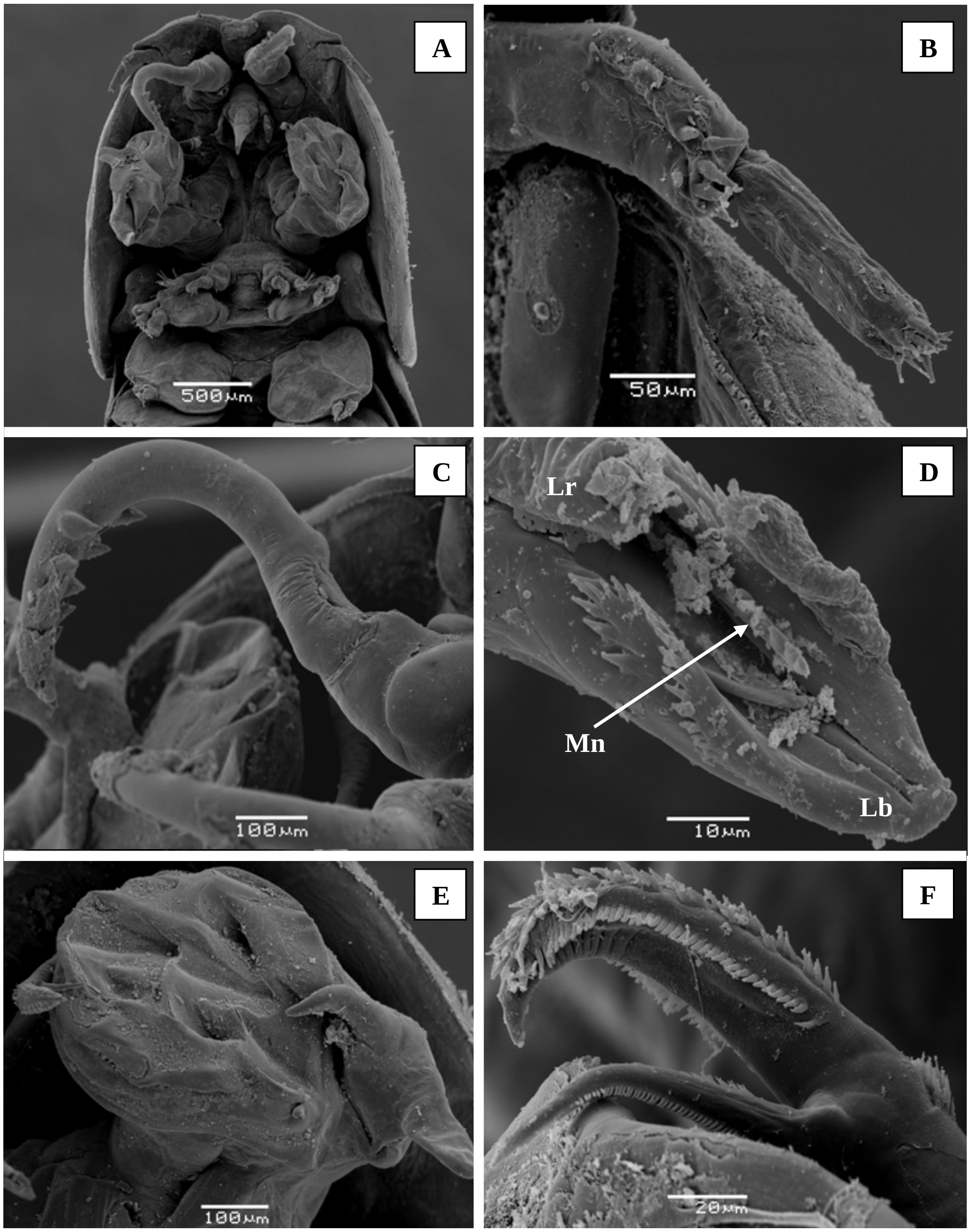

Figure 3.3 Scanning electron micrographs of ventral view of cephalothorax, and cephalic appendages of adult female copepod *Perissopus oblongus*, from scyliorhinid hosts, *Haploblepharus pictus* and *H. edwardsii*, from the west and south coasts of South Africa. **A.** Ventral view of cephalothorax. **B.** Antennule. **C.** Antenna. **D.** Detail of mouthtube showing labrum (Lr), labium (Lb) and mandibles (Mn). **E.** Maxilliped. **F.** Maxilla.

Maxilla with two segments, the first stout and the second slender and bearing a smooth clavus with two short rows of bristles. Four lateral rows of stout bristles on calamus (Figure 3.3F).

Five pairs of swimming legs. First pair have two-segmented exopodite, first segment bearing one and terminal segment six setae, single-segmented endopodite bearing two setae, the tip covered by patch of minute spines (Figure 3.4A). Second leg biramous, both exopodite and endopodite biramous. First segment of exopodite bears a patch of minute spines and terminal segment four stout and two smaller, smooth setae. Both segments of endopodite bear patches of minute spines, and terminal segment two smooth setae (Figure 3.4B). Basipodite of third leg large and round relative to one-segmented exo- and endopodites. Exopodite bears three stout and two smaller, smooth setae. Endopodite bears a lateral and terminal patch of minute spines (Figure 3.4C). Large, rounded basipodite of fourth swimming leg bears one segmented exo- and endopodites. Exopodite bears four stout, short setae of equal length, minute spines present at base and on bulbous tip of endopodite (Figure 3.4D). Fifth swimming leg reduced and bears two large terminal setae (Figure 3.4E).

Caudal rami each have a smooth, oval form and bear four smooth, stout setae and a very small spine on inner surface (Figure 3.4F).

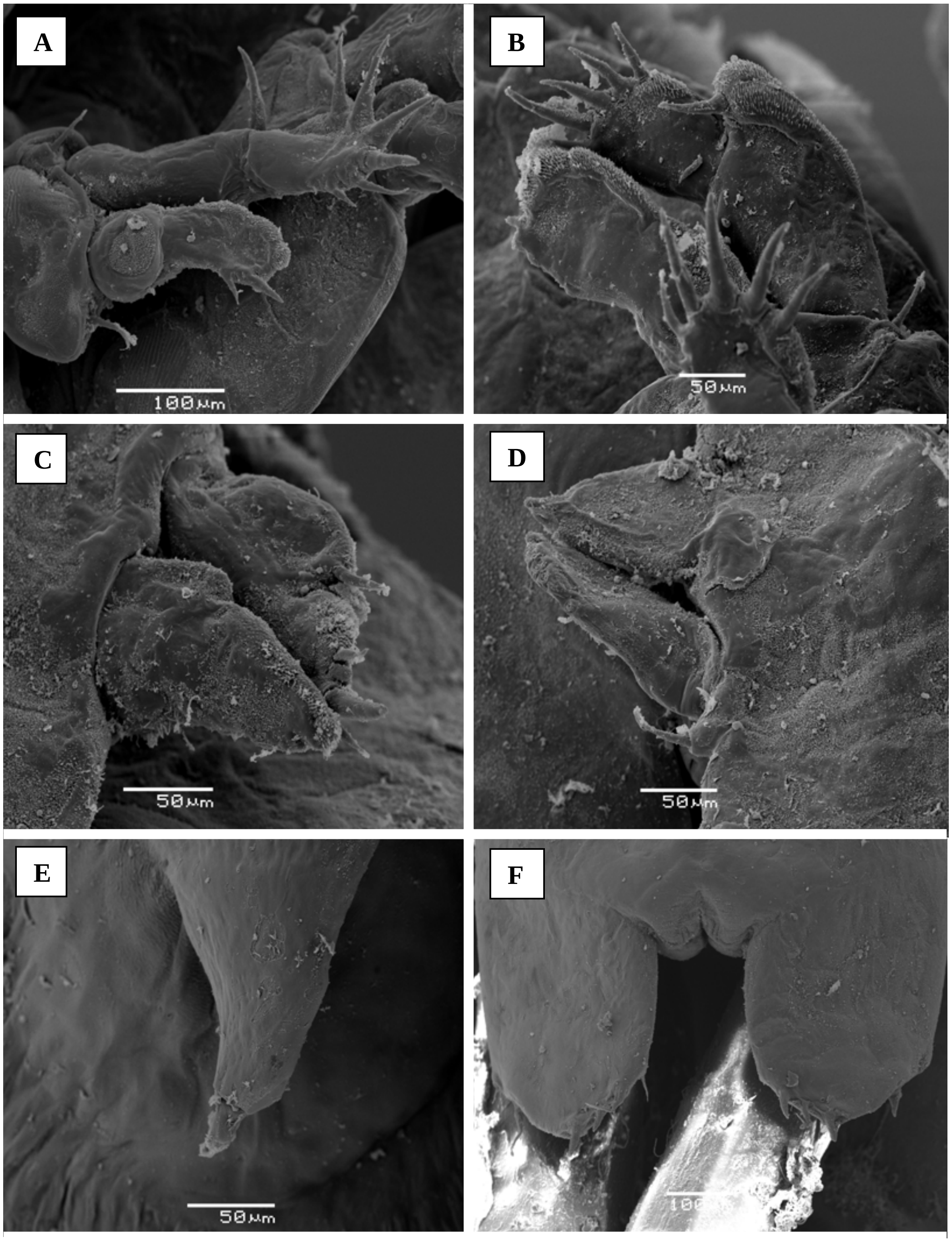

Figure 3.4 Scanning electron micrographs of swimming legs and caudal ramus of adult female copepod *Perissopus oblongus*, from scyliorhinid hosts, *Haploblepharus pictus* and *H. edwardsii*, from the west and south coasts of South Africa. **A.** First swimming leg. **B.** Second swimming leg. **C.** Third swimming leg. **D.** Fourth swimming leg. **E.** Fifth swimming leg. **F.** caudal ramus with egg strings.

Adult male

Cephalothorax slightly wider (1.80 mm) than long (1.50 mm) (Figure 3.5A). Frontal plates quite prominent and extending beyond lateral margin of cephalothorax. Lateral lobes of cephalothorax extending posteriorly to posterior edge of first free thoracic segment. Third thoracic segment slightly rounded posteriorly. Genital segment small (0.70 mm long x 0.85 mm wide) in relation to total body length (3.3 mm). Abdomen bears two oval furcal rami.

Antennule two-segmented. First segment short and bearing 10 setae and a short spine, second segment longer and bearing 10 smooth setae (Figure 3.5B). Antenna prominent and two segmented, terminal segment consisting of a heavily sclerotized curved hook with single small seta on joint with first segment (Figure 3.5C). Mouthtube typically pandarid, as in female (Figure 3.5D). Maxilliped three-segmented, terminal claw of third segment rests embedded in raised, roughly corrugated sclerotized area on second segment (Figure 3.5E). Maxilla as in female, with small patch of spines at base of first segment (Figure 3.5F).

Five pairs of swimming legs. First pair with two-segmented exopodite; the first segment bearing two bifid, pinnate setae and a short spine, and the terminal segment two bifid, pinnate setae, four smooth setae and a longer spine. Endopodite a protuberance bearing a single smooth spine (Figure 3.6A). Second leg biramous, both exopodite and endopodite biramous. Basipodite bears areas of minute spines and single seta. First exopodite segment bears a patch of minute spines and a smooth seta, and the terminal segment two pinnate and four stout, smooth setae.

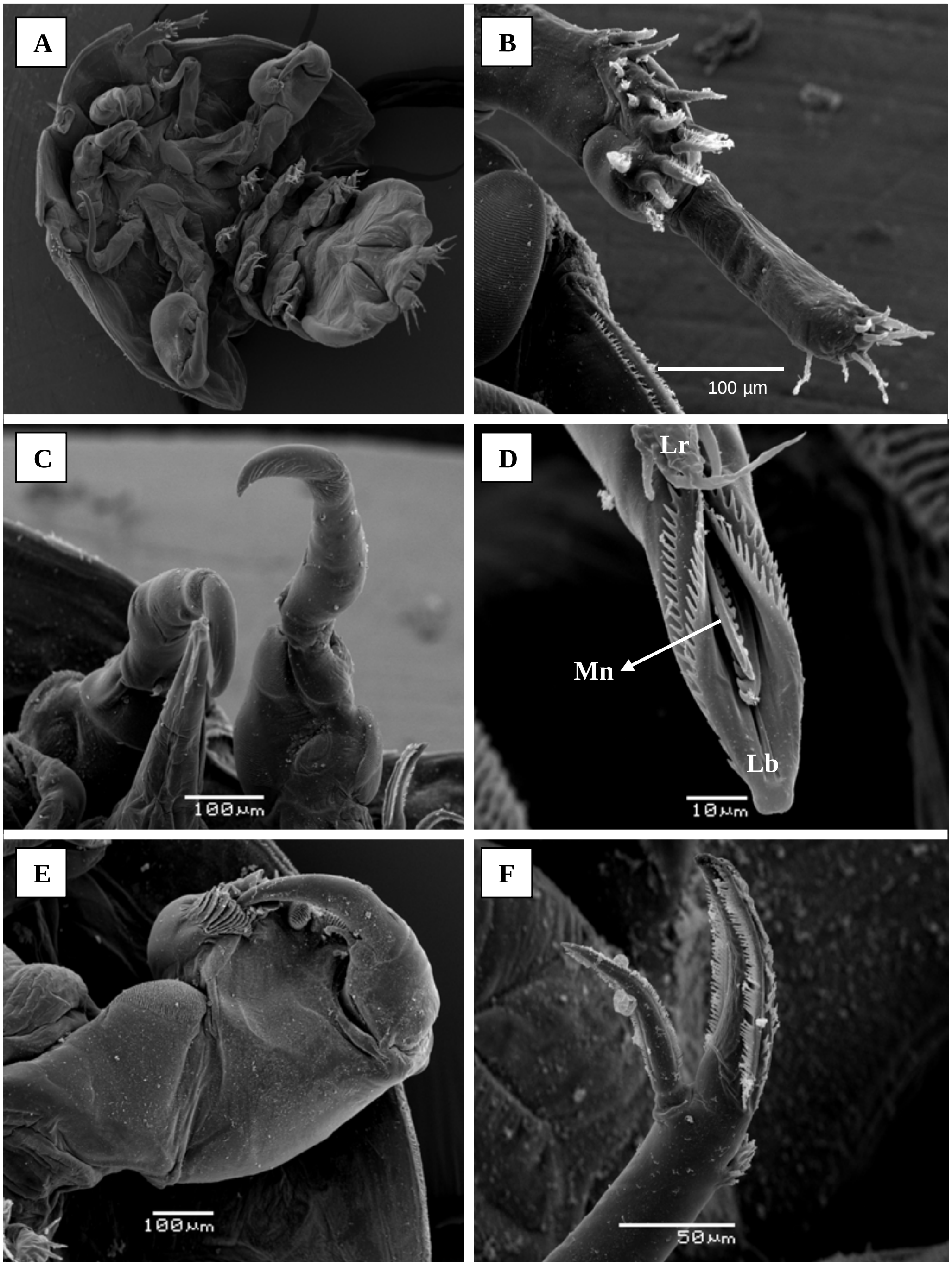

Figure 3.5 Scanning electron micrographs of ventral view of whole animal, and cephalic appendages of adult male copepod *Perissopus oblongus*, from scyliorhinid hosts, *Haploblepharus pictus* and *H. edwardsii*, from the west and south coasts of South Africa. **A.** Ventral view of whole animal. **B.** Antennule. **C.** Antenna. **D.** Detail of mouthtube showing labrum (Lr), labium (Lb) and mandibles (Mn). **E.** Maxilliped. **F.** Maxilla.

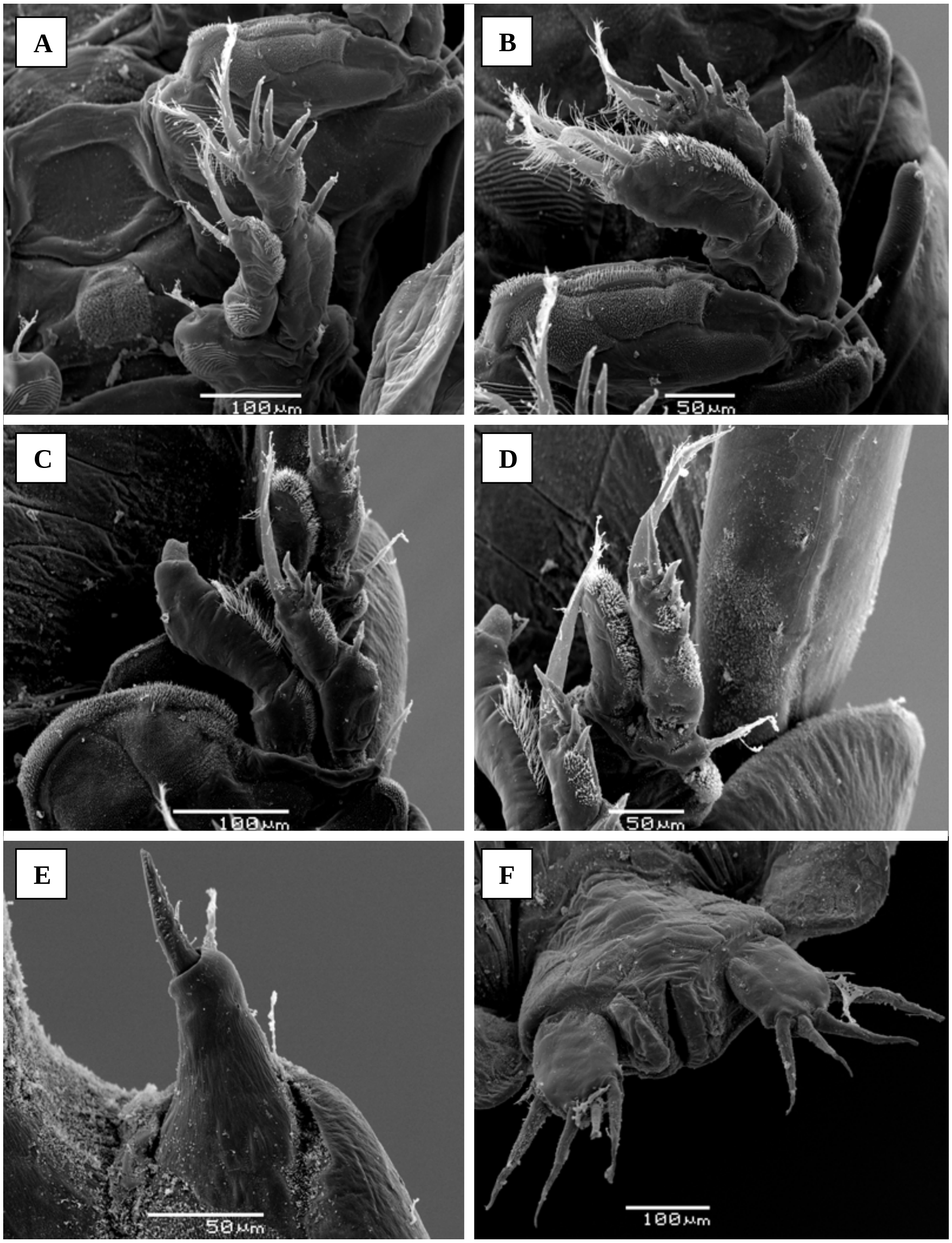

Figure 3.6 Scanning electron micrographs of swimming legs and furcal ramus of adult male copepod *Perissopus oblongus*, from scyliorhinid hosts, *Haploblepharus pictus* and *H. edwardsii*, from the west and south coasts of South Africa. **A.** First swimming leg. **B.** Second swimming leg. **C.** Third swimming leg. **D.** Fourth swimming leg. **E.** Fifth swimming leg. **F.** Furcal ramus and genital segment.

Both segments of endopodite bear patches of minute spines, and the terminal segment three pinnate setae (Figure 3.6B). Third swimming leg biramous. Basipodite bears single smooth seta adjacent to exopod. Exopodite bears single spine on first segment, and three spines and two pinnate setae on terminal segment. Endopodite bears patch of minute spines and single seta on first segment, terminal segment bears a row of setules and single pinnate seta (Figure 3.6C). Fourth swimming leg biramous, basipodite bears single smooth seta adjacent to exopodite. Exopodite bears four stout, short setae and one pinnate seta, lateral patches of minute spines present on endopodite (Figure 3.6D). Fifth swimming leg reduced and consists of single short, stout pinnate seta and a smooth spine (Figure 3.6E).

Furcal rami originate from posterior of abdomen and each bears four pinnate setae and a short spine on outer edge (Figure 3.6F).

Remarks

Although there are some minor differences between the above descriptions and previous descriptions of *P. oblongus* and its synonyms in terms of setation (both number and structure) of the swimming limbs, there is more than sufficient similarity in the diagnostic features to confirm the identification of the specimens.

Yamaguti’s (1936) description of *A. galeorhini* is referred to as the best description of appendages by Cressey (1967), and so has been the standard for comparison in terms of species diagnosis. However, this now poses problems, given the resurrection of *P. galeorhini*. The general body shape of the female seems to be the most constant factor among the various descriptions of synonyms of *P. oblongus*, with the main distinguishing feature being the posterior lobes of the genital segment, which are rounded rather than pointed pseudo-laterally, as in Oldewage's (1992) description of *P. dentatus*. The genital segment of the females found in this study do show a distinct posterior invagination in the genital segment, not noted by Oldewage (1992), but present in the figures of Wilson (1912).

There are a number of discrepancies in the setation of the appendages of both the female and male specimens described here, when compared with prior descriptions. This may be a result of several different causes. Handling of specimens can easily damage or remove setae, as can rough preparation techniques. Pandarids are also known to exhibit a broad range of intraspecific variability, which is extended by the marked morphological changes that occur with age differences in these copepods (Kabata, 1979). It has also been recommended that Oldewage's (1993) description of *N. orientalis* be discarded when studying that species (Dippenaar & Jordaan, 2006) and although this study is not conclusive enough to recommend the same in the case of *P. oblongus*, it should be borne in mind when comparing descriptions.

Functional morphology & attachment

Some adult female *P. oblongus* collected in this study were still grasping the placoid scales of the shark's skin. SEM studies on these specimens clearly showed that the maxillipeds are used for attachment to the host (Figure 3.7A and B). The maxilliped "claw" on the bifid tip clasps around the top of the placoid scale and holds it so that the dorsal surface of the scale fits onto the slightly concave myxal pad of the maxilliped. Ridges (lateral myxal projections) on the myxal pad fit around the edge of the scale and seem to prevent lateral movement, as suggested by Benz (1992) and Dippenaar & Jordaan (2006), therefore locking the copepod onto the shark's skin. Benz (1992) further suggested that when the scale is removed from the maxilliped, impressions left on the myxal pad indicate that the scale is actually slightly embedded in the myxa. This is a similar method of attachment to that described by Dippenaar & Jordaan (2006) for the related pandarid *Nesippus orientalis*, and seems to conform to standard Pandaridae attachment mechanisms (Benz, 1992). A notable difference in the attachment scheme of *P. oblongus* compared to those found by Benz (1981) and Dippenaar & Olivier (2006) is the lack of any clustering or packing. *P. oblongus* were found individually, with no association with any other individuals on the same host. The exception was males, found attached to females.

Males of *P. oblongus* in this study were exclusively found attached to the ventral side of females (Figure 3.7C and D). Studying these pairs with their attachment still intact, and using SEM photographs of the attached pair and then the same individuals after the attachment was broken, revealed the mechanism of attachment. In contrast to other pandarid copepods (Dippenaar & Jordaan, 2006), male *P. oblongus* attach to the ventral, rather than dorsal, surface of the female. Also, *P. oblongus* males use their antennae, rather than their maxillipeds, to hook onto the female between the cephalothorax and genital segment (Figure 3.7E and F). This attachment was found to be very strong, and when the male was forcibly removed from the female the antennae were broken off and left attached to the female. It is noteworthy that males in this position are not able to feed, given that their dorsal surface is resting on the host shark and their mouthtube is held against the ventral surface of the female. Since no males were found free of females, it would be of interest to do further investigation into the life cycle and behaviour patterns of this species, particularly the very poorly-known males.

3.3.3 Gnathia pantherina Smit & Basson, 2002

Systematics

Class CRUSTACEA

Order ISOPODA

Family GNATHIIDAE

Genus *Gnathia*

Gnathia pantherina Smit & Basson, 2002

(Figures 3.8 and 3.9)

Larval gnathiid isopods, *Gnathia pantherina* Smit & Basson, 2002, were found in the gills of nine pyjama sharks (100 %), *Poroderma africanum*, and 40 puffadder shysharks (97.6 %), *Haploblepharus edwardsii*, collected from De Hoop Nature Reserve. This isopod species was described by Smit & Basson (2002) from the related leopard catshark *Poroderma pantherinum* at Jeffrey's Bay, from *H. edwarsii* and from the blackspotted electric ray *Torpedo fuscomaculata* from De Hoop Nature Reserve, on the South Coast of South Africa. It has also been recorded from the lesser guitarfish *Rhinobatos annulatus* in the same area (Hayes *et al*., 2007). In this study it is also recorded from the pyjama catshark *P. africanum*, and from a far greater number of host specimens, providing more detailed information on host spectrum, prevalence and mean intensity of infection.

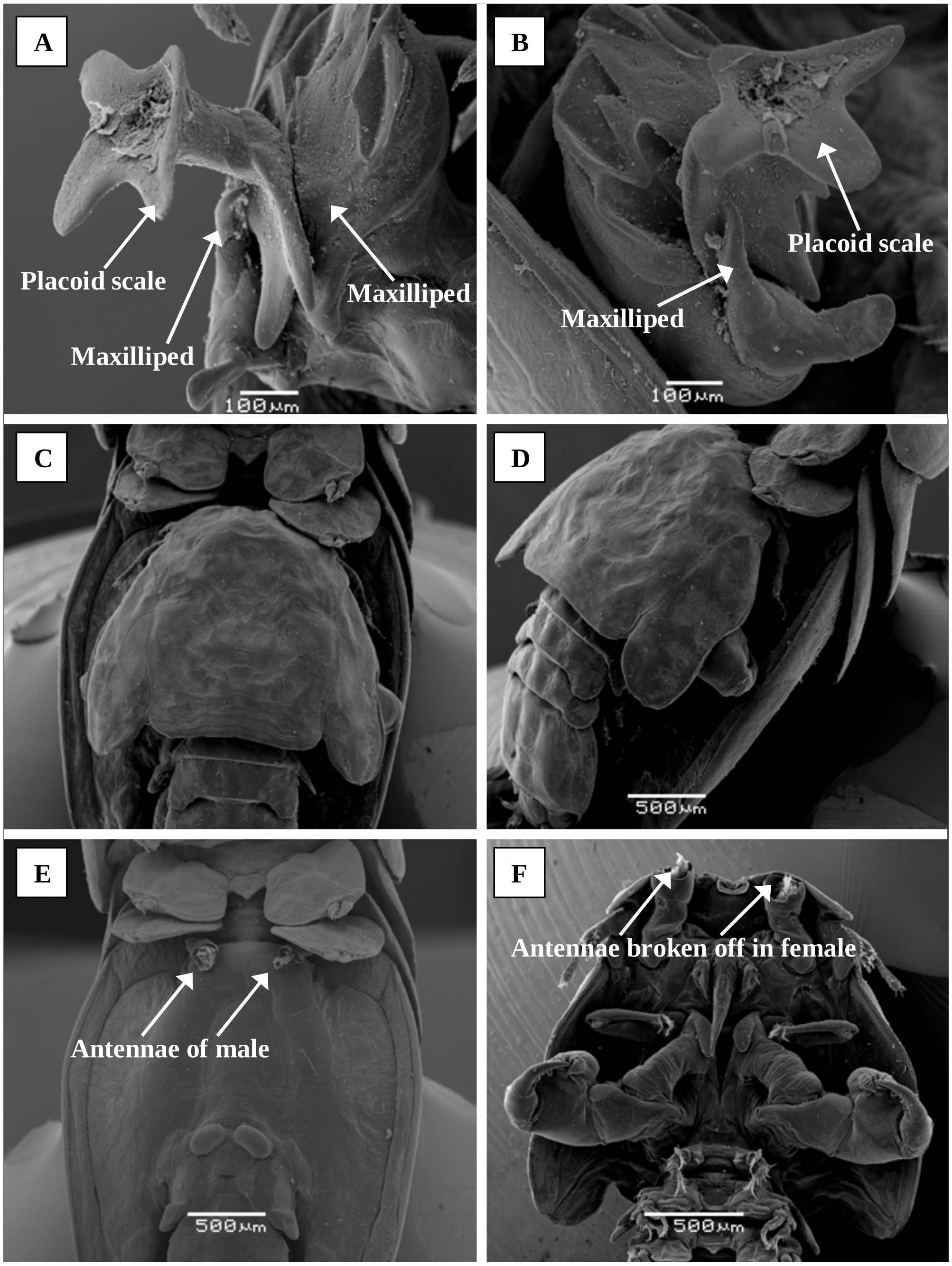

Figure 3.7 Scanning electron micrographs of copepod *Perissopus oblongus*, from scyliorhinid hosts, *Haploblepharus pictus* and *H. edwardsii*, from the west and south coasts of South Africa. **A.** and **B.** Maxilliped of female grasping host placoid scale. **C.** Ventral and **D.** Lateral view of male attached to female **E.** Ventral view of female after removal of attached male, showing position of male 2nd antennae used for attachment. **F.** Ventral view of male removed from female, showing broken ends of 2nd antennae used for attachment to female.

Figure 3.8 shows the larval isopods *in situ* on the gills of a dissected puffadder shyshark, *H. edwardsii*. Further scanning electron micrographs for this isopod species are provided (Figure 3.9) and additional information on prevalence, mean intensity of infection and host species is given below.

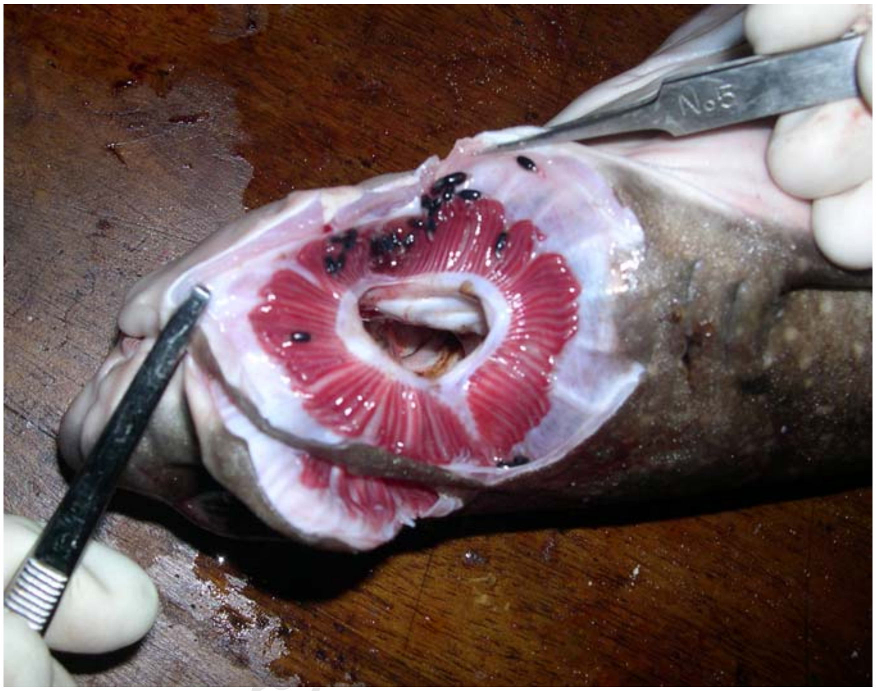

Figure 3.8 Larval isopods *Gnathia pantherina*: *in situ* in the gills of a puffadder shyshark, *Haploblepharus edwardsii*, from the De Hoop Nature Reserve, South Africa.

Members of the gnathiid isopod genus *Gnathia* are for the most part temporary ecto-parasites of marine fish, being parasitic in their young or larval stages (Sindermann, 1990). The South African species *G. pantherina* is one of very few to be described and named from elasmobranchs (Smit & Basson, 2002; Coetzee *et al*., 2008; Coetzee *et al*., 2009). Current descriptions of gnathiid isopod species are based on the morphological structure of the free-living adult males, although almost all current literature includes descriptions of females. Earlier descriptions created taxonomic confusion by describing gnathiid isopod species from larvae, rendering them virtually impossible to link to descriptions based on adults (Smit & Basson, 2002). The preferred method used of obtaining larval stages as parasites on their hosts, and allowing them to moult into the adult stages which have well-characterised and diagnostic feautres, allows male, female and larval morphological structure and characteristics to be unambiguously linked. The parasitic effect of the gnathiid infestation can also be determined, including any potential pathogenicity.

Although information on the life cycle and ecology of gnathiid isopods is even now rather sparse, enough has been elucidated to give a basic, general life cycle pattern for the Gnathiidae: Eggs develop inside the female into stage 1 zuphea larvae, which leave the maternal marsupium and find a suitable fish host. Feeding on blood or lymph occurs and consequently the zuphea develops into a praniza 1 phase. After feeding, praniza larvae detach and seek protection (e.g. in sponges or tunicates) until moulting into stage 2 zuphea larvae. These then feed again on a fish host, detaching once more as praniza 2 phase. These moult into stage 3 zuphea larvae, attach to the fish host, and detach at repletion as either male or female praniza 3 larvae (filled with eggs) that in turn moult into adults (Smit *et al*., 2003b). The feeding stages of gnathiid larvae on teleosts are known to be relatively short (Smit & Davies, 2004). The length and severity of praniza 3 larvae feeding on elasmobranchs is, however, unknown (Heupel & Bennett, 1999; Smit & Davies, 2004). However, an extended feeding stage is likely (McKiernan *et al*., 2005) and this may result in increased pathology, as noted by Hayes *et al*. (2007).

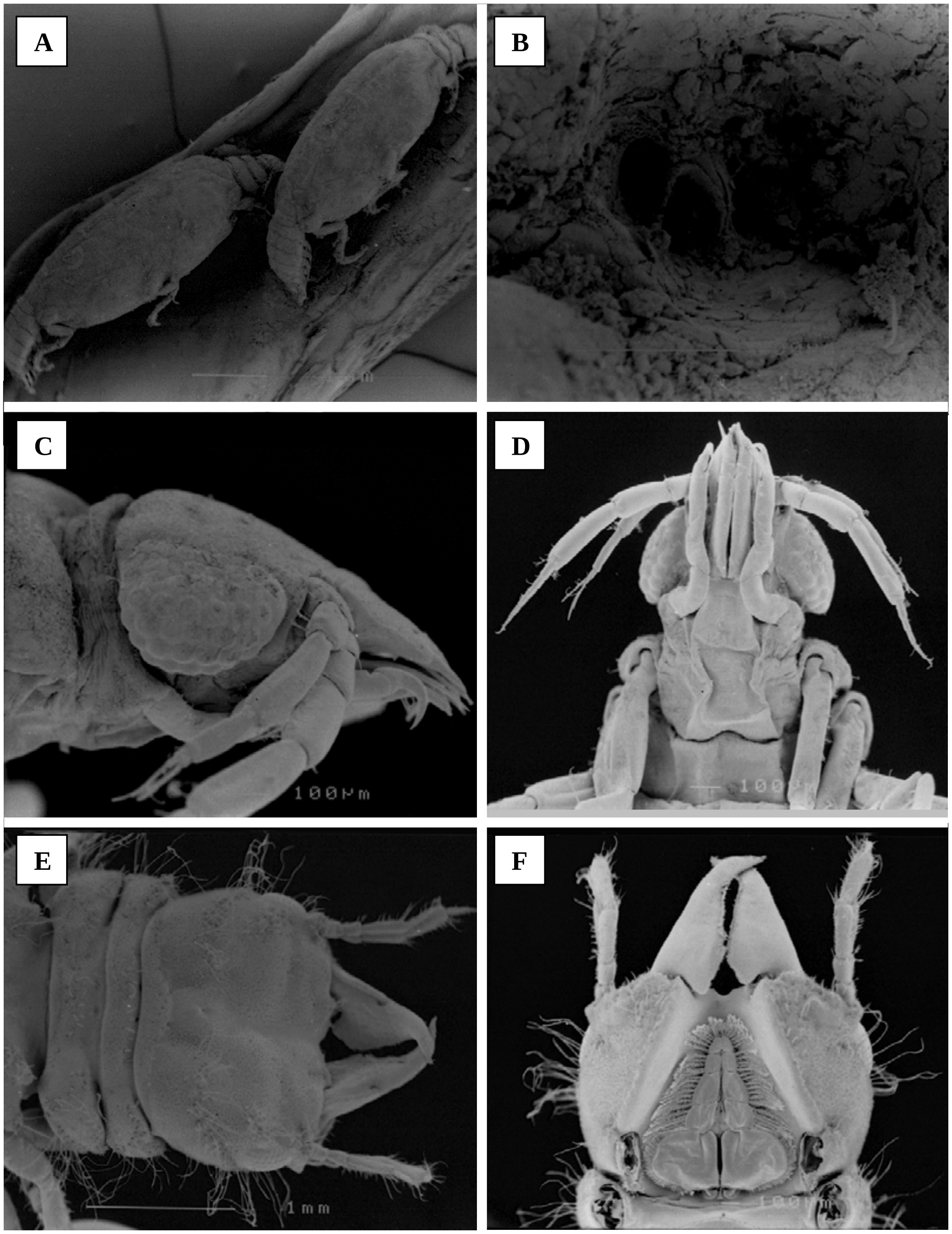

Figure 3.9 Scanning electron micrographs of characteristics of *Gnathia pantherinum* Smit & Basson, 2002 from scyliorhinid hosts, *Haploblepharus edwardsii* and *Poroderma africanum*, from De Hoop Nature Reserve on the south coast of South Africa. **A.** Praniza larvae attached to gill. **B.** Damage to gill resulting from larval feeding. **C.** Lateral and **D.** Ventral view of larval cephalosome. **E.** Dorsal and **F.** Ventral view of adult male cephalosome. Micrographs used with permission from NJ Smit.

Praniza larvae attached to the gill tissue of host sharks (Figure 3.9A) can cause visible damage to the gill tissue (Figure 3.9B). Other studies have also identified gnathiid isopod infections as causing serious pathological damage to elasmobranchs (Honma & Chiba, 1991; Honma *et al*., 1991; Heupel & Bennett, 1999). Hayes *et al*. (2007) first detailed the pathological effects of a gnathiid isopod infection on elasmobranch hosts, where the causative gnathiid species (*G. pantherina*) was known, and found that considerable pathology was seen. The largest infestation of *G. pantherina* seen by Hayes *et al*. (2007) was 27. In this study a maximum infestation of 272 individual gnathiids was recorded on the gills of a single host puffadder shyshark *H. edwardsii*. Taking into account the extremely large size of this gnathiid species, it is highly likely that such infestations are having a severely deleterious effect on the host.

Gnathiids have also been implicated as vectors in the transmission of fish diseases such as blood parasites (Davies & Smit 2001) and this area of research warrants further investigation. It is unlikely, however, that *G. pantherina* is implicated in the transmission of either the trypanosome or two haemogregarine species found in this study (Chapters 4 and 5). This is because of the widespread geographic occurrence of the blood parasites (encompassing the entire study area on both the west and south coasts of South Africa, as described in Chapter 2) and the extremely limited geographic occurrence of *G. pantherina*, which occurs only on the eastern edge of the south coast area sampled during this study. Furthermore, although transmission of apicomplexan blood parasites among teleost fish by juvenile gnathiids has been reported, this has not been the case with elasmobranchs (Davies & Smit, 2001; Davies *et al*., 2004; Smit & Davies, 2004; McKiernan *et al*., 2005; Hayes *et al*., 2007).

According to Smit & Basson (2002), the defining characteristics of *G. pantherina* include the large size of the final life cycle stages (as seen in the praniza 3 larvae found in this study, in Figure 3.8), the number of teeth on the mandibles (eight) and on the maxillules (seven) of the praniza larvae cephalosome (Figure 3.9C and D), the deeply divided mediofrontal process of the male cephalosome (Figure 3.9E and F), and the plyopod and maxilliped morphological stucture of the female (not shown). In this study larvae were only moulted into adult males. This proved sufficient for definite identification as *G. pantherina*.

3.4 Discussion

This chapter adds to the knowledge on three previously described species of parasite found from South African elasmobranchs: the nematode *Proleptus obtusus*, copepod *Perissopus oblongus* and isopod *Gnathia pantherina*.

Poroderma africanum and *Haploblepharus pictus* both represent new host records for *Proleptus obtusus*. The area north of Table Bay is a new distribution record for this nematode. Prevalence and mean intensity of infection from a large number of host sharks are provided, from which a much more accurate picture of the distribution and abundance of *P. obtusus* can be drawn. The first scanning electron micrographs of the general external morphological stucture of *P. obtusus* are shown (with the exception of those given by Moravec *et al.*, 2002, that only covered the oral aperture area). These clarify and confirm described details of the external morphological structure.

There is much scope for further work on *P. obtusus* and its infection of endemic South African catsharks. As with most of the parasite species in this study, the life cycle and intermediate hosts of the parasite, and the exact mechanism of infection need to be determined. The use of genetic analysis, combined with infection statistics and patterns, could potentially be a very useful tool in population studies on host species.

H. pictus, *H. edwardsii* and *P. africanum* are all new host records for the copepod *P. oblongus*. Prevalence and mean intensity of infection from a large number of host sharks give an accurate indication of the abundance and distribution of *P. oblongus*. Presence/absence were the only real data previously available on this species in South Africa.

The scanning electron micrographs presented here are the first for this species, and enable a detailed examination of the external morphological stucture of both male and female individuals. This is only the second documented description of the male since the initial description by Oldewage (1992). Also presented are the first evidence of the attachment mechanism of female *P. oblongus* to the host shark, and the attachment and positioning of the male to the female copepod whilst on the shark. These aspects of functional morphology are clearly documented by SEM of female specimens still clasping host placoid scales in the maxilliped, and by male specimens still attached to the ventral side of females using their second antennae. Benze *et al*. (2003) state that adult females of *Achtheinus* (including *A. oblongus* according to them) attach to the host b embedding their antennae into the host's skin as opposed to members of the genus *Perissopus* which cement the myxal region of the maxillipeds into the host placoid scales. This supports the identification of our specimens as *Perissopus*.

Research into the life cycle of *P. oblongus* is much needed, and this may explain the unexpected lack of specimens infecting sharks north of Table Bay and east of False Bay, given that the literature records its distribution in both these areas and that suitable host species occur there. *P. oblongus* is also known to infect a wide range of elasmobranchs (Barnard, 1955), and not only those found inshore in shallow temperate reef areas, which makes this apparent absence all the more intriguing. In order to try to disentangle the confused systematics surrounding the Pandaridae, particularly members of the genera *Perissopus* and *Achtheinus*, genetic analysis combined with detailed morphometric analyses is recommended.

P. africanum is a new host record for the gnathiid isopod *Gnathia pantherina*. This study represents by far the most intensive sampling of host sharks to date and provides more representative prevalence and mean intensity of infection for *H. edwardsii* and *P. africanum*.

Future research should focus on elucidating the full life cycle of *G. pantherina*, including the preferred habitat of the adults, and the detached praniza stage larvae whilst undergoing moult into zuphea stage. This should be combined with an exhaustive and fine spatial scale sampling programme both west and east (and offshore if possible) of the known habitat of *G. patherina*. Together these should go a long way towards elucidating the ecology of this isopod species. Genetic analysis is recommended to bolster the systematics of the Gnathiidae and the Isopoda in general. Although unlikely, the potential for *G. pantherina* to be a vector for blood parasites infecting catsharks should be thoroughly investigated. Its pathogenic potential, particularly given the extremely heavy infestation levels found in this study, should also be the subject of scientific study.

CHAPTER 4

A NEW SPECIES OF *TRYPANOSOMA* (KINETOPLASTIDA: TRYPANOSOMATIDAE) INFECTING CATSHARKS FROM SOUTH AFRICA

4.1 Introduction

Parasite community studies on three species of catshark (Elasmobranchii: Scyliorhinidae), the dark shyshark *Haploblepharus pictus* (Müller & Henle, 1838), the puffadder shyshark *H. edwardsii* (Voight, 1832) and the pyjama shark *Poroderma africanum* (Gmelin, 1789), revealed a trypanosome infecting the peripheral blood of all sharks examined.

At least ten species of trypanosomes have been described from elasmobranch species world-wide (Morillas *et al.*, 1987), with more species known from skates and rays than from sharks (Table 4.1). As far as can be determined, there has been no previous report of trypanosomes from elasmobranchs from the southern African coast. This chapter reports on the first trypanosome species from elasmobranchs in this region and provides information on its prevalence in *H. pictus*, *H. edwardsii* and *P. africanum*.

4.2 Materials and methods

As in Chapter 2 of this thesis, with the following additional methods specific to this chapter.

Table 4.1: Trypanosome species described from elasmobranchs, detailing host species, location, original description reference and morphometrics. All measurements given in micrometres (µm); mean ± standard deviation (minimum – maximum).

Trypanosome species	**Host species**	**Location**	**Reference**	**Body length**	**Flagellum length**	**Width**	**Nucleus length**	**Nucleus width**
Trypanosoma gargantua	*Raja nasuta*	South Pacific	Laird, 1951	66.7 – 131.1	absent	4.6 – 13.7	3.9 – 9.3	3.3 – 6.7
T. taeniurae	*Taeniura lymma*	Heron Island, Australia	Burreson, 1989	55	9	4	3.5	4.5
T. torpedinis	*Torpedo marmorata*	South-west France	Sabrazes & Muratet, 1908	n/a	n/a	n/a	n/a	n/a
T. rajae	*Raja* spp.	Northern Atlantic	Laveran & Mesnil, 1902	55 – 60	20	6	3	2.2
T. marplatensis	*Psammotics microps*	Atlantic, off Argentina	Bacigalupo & De la Plaza, 1948	60 – 65	Up to 15	10 - 12	7.2	4.2
T. giganteum	*Raja oxyrhynchus*	Mediterranean Sea	Neumann, 1909	100	25-30	8	3 – 5	2.5 – 4
T. carchariasi	*Odontaspis* sp.	South Pacific	Laveran, 1908 (in Wenyon, 1926)	35 – 40	25 – 30	n/a	n/a	n/a
T. scylli	*Scyliorhinus stellaris* & *S. canicula*	Roscoff, France	Laveran & Mesnil, 1902	54 – 61	14	5-6	n/a	n/a
T. mackerrasi	*Hemiscyllium ocellatum*	Heron Island, Australia	Burreson, 1989	125	absent	15	7	10
T. humboldti	*Schroederichthys chilensis*	Pacific coast, Chile	Morillas *et al.*, 1987	87.0 ± 3.8 (78 – 93)*		7.4 ± 1.5 (4 – 10)		5.3 ± 0.4 (5 – 6)
T. sp. A	*Haploblepharus pictus*	Atlantic, off South Africa	This paper	70.4 ± 9.4 (53.7 - 99.4)	n/a	17.4 ± 2.6 (12.6 - 24.3)	6.5 ± 0.8 (5.2 - 8.8)	6.5 ± 1.1 (4.7 - 8.8)

n/a, not available; *, includes free flagellum length.

Photomicrographs of trypanosomes on blood smear slides were obtained with a Zeiss photomicroscopic digital camera system and 55 trypanosomes were measured with the aid of Zeiss Axioplan Software, Version 4.1. Total body length was taken along the midline of the animal. Posterior end to kinetoplast centre (PK), kinetoplast centre to nucleus centre (KN), nucleus centre to start of free flagellum at anterior end (NA), cell width at nucleus (BWN) and nucleus dimensions (nuclear length = NuL and nuclear width = NuW) were also recorded following the methods of Karlsbakk *et al.* (2005). Measurements are presented as mean ± standard deviation (minimum – maximum). All sharks collected were also screened for leeches, which, when found, were removed and either preserved or kept alive in marine aquaria for transmission experiments.

4.3 Results

The identity, numbers and lengths of sharks captured, and the trypanosome prevalence in each species, are recorded in Table 4.2. A total of 145 sharks, of three species (*Haploblepharus pictus*, *H. edwardsii* and *Poroderma africanum*), were infected with trypanosomes, with infections ranging from 11–20 per blood smear to extremely heavy (50–72 per thin blood smear). All sharks examined were also infected with an unknown species of haemogregarine which will be described elsewhere. Some sharks were found to bear ectoparasitic haematophagous leeches that might act as vectors of these infections. Studies are underway in order to conclusively identify the leech species and to determine its role as possible vector.

Table 4.2: Identity, number and length of sharks captured from the west and south coasts of South Africa; and prevalence of the trypanosome blood parasite (*Trypanosoma* sp. A) in these sharks.

	Sharks		**Trypanosomes**
Species	N	ML ± SD (range) in mm	Prevalence (%)
Haploblepharus pictus	78	411.6 ± 89.5 (256-672)	78/78 (100)
Haploblepharus edwardsii	53	547.9 ± 105.9 (309-662)	53/53 (100)
Poroderma africanum	14	881.2 ± 106.4 (712-1068)	14/14 (100)

N, number; ML, mean length; SD, standard deviation

Systematics

Class MASTIGOPHORA Diesing, 1866

Order KINETOPLASTIDA Honigberg, 1963

Suborder TRYPANOSOMATINA Kent, 1880

Family TRYPANOSOMATIDAE Doflein, 1911

Genus *Trypanosoma* Gruby, 1843

Trypanosoma sp. A sp. nov.

(Figures 4.1 and 4.2)

4.3.1 Type material

Syntypes: One blood film with 72 trypanosomes (South African Museum, Cape Town; SAM A25081; Granger Bay, Western Cape, South Africa; coordinates: 33^{o}52’S, 18^{o}24’E). Collected by E.M. Yeld, 20 January 2005.

Twenty-five additional slides with trypanosomes in the collection of N.J. Smit in the Department of Zoology, University of Johannesburg.

4.3.2 Type host

Haploblepharus pictus (Müller and Henle, 1838).

Other hosts: *Haploblepharus edwardsii* (Voigt, 1832) and *Poroderma africanum* (Gmelin, 1789).

4.3.3 Vector

Unknown, but possibly the hirudinid (unconfirmed identity tentatively assigned as *Stibarobdella macrothela*) present on the sampled hosts.

4.3.4 Diagnosis

Very large deep blue staining trypanosome (53.7–99.4 µm body length) with short flagellum (6.9 µm). Body wide (12.6–24.3 µm), distinct undulating membrane with 8-16 undulations. Kinetoplast prominent, situated on average 16.8 µm from posterior. Nucleus circular with prominent karyosome and in anterior half of animal. Longitudinal striations visible on larger specimens (Figure 4.1).

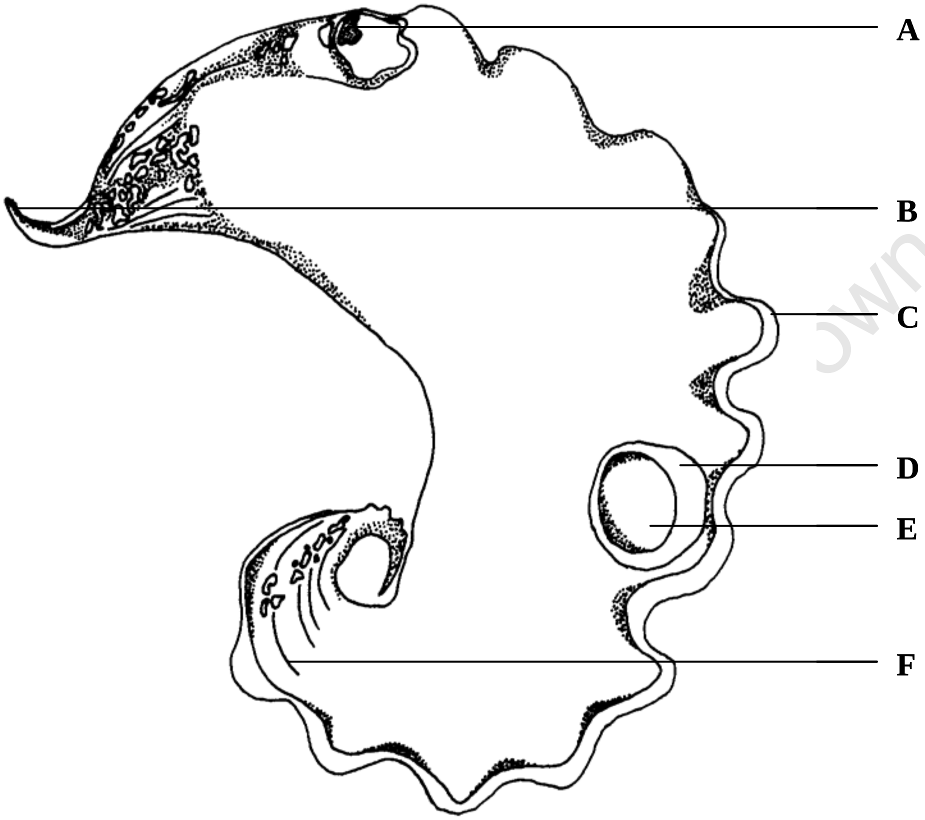

Figure 4.1. Line drawing of of *Trypanosoma* sp. A from the type host *Haploblepharus pictus* (Slide SAM A25081) from the west coast of South Africa. **A.** Kinetoplast. **B.** Site of free flagellum origination. **C.** Undulating membrane. **D.** Nucleus. **E.** Karyosome. **F.** Longitudinal striations.

4.3.5 Description

Trypanosomes found in the blood films taken from *Haploblepharus pictus* and *H. edwardsii* were morphometrically and morphologically indistinguishable and data provided here are of parasites from both hosts. Smaller (Figure 4.2A) and larger individuals (Figure 4.2B-E) occur. The cytoplasm, kinetoplast and nucleolus are basophilic and stain deep blue with Giemsa, with numerous chromatic granules visible in the cytoplasm. The karyosome is prominent within the nucleus (Figure 4.2B–E). The undulating membrane is richly spiralling, with numerous undulations (8–16), and is well developed and easily stained (Figure 4.2B). The width of the undulating membrane fluctuates between 1–4 µm. A distinct, very short free flagellum was observed, but was not easily stained or measured in trypanosomes greater than 68 µm. In animals less than 68 µm it was easier to observe, measuring 6.9 µm (Figure 4.2A), although sometimes just visible in larger individuals (Figure 4.2E). A distinct kinetoplast (Figure 4.2B), approximately 2 µm in diameter, is found near the posterior end of the body, PK = 16.8 µm or 23.9% of body length. The posterior end in smaller stages tends to be slender, elongated and pointed (Figure 4.2A), whereas the larger stages have a more variable posterior end shape, often tending to be blunt and rounded (Figure 4.2C).

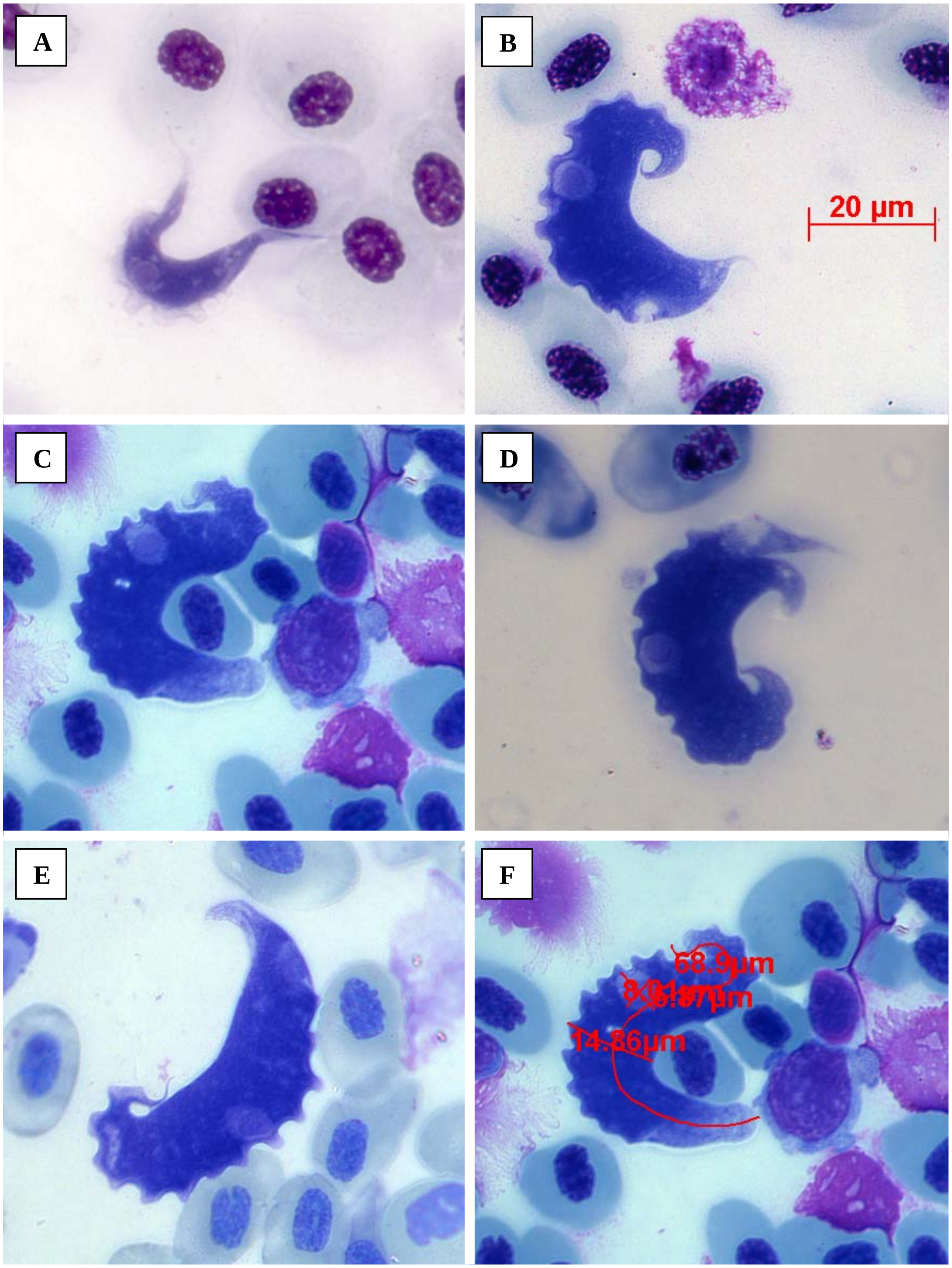

Figure 4.2. Light micrograph pictures of *Trypanosoma* sp. A from the type host *Haploblepharus pictus* (Slide SAM A25081) from the west coast of South Africa. **A.** Small stage with short free flagellum. **B.** Large stage with distinct kinetoplast and undulating membrane. **C.** Large stage with rounded, blunt posterior end. **D.** Possible division in peripheral blood. **E.** Large stage with distinct kinetoplast and short free flagellum. **F.** Example of measurements being taken.

Body length 70.4 µm ±9.4 (range 53.7–99.4 µm) (N = 55); body width (including the undulating membrane) BWN = 17.4 µm ± 2.6 (12.6-24.3 µm) (n = 55). Nucleus approximately circular, NuL = 6.5 µm ± 0.8 (5.2-8.8 µm) and NuW = 6.5 µm ± 1.1 (4.7-8.8 µm) (n = 55). The location of the nucleus NA = 34.7 µm from the anterior end, or 49.3% of the body length, thus lies just anterior to the midpoint of the body for the majority of specimens examined. Kinetoplast to nucleus KN = 18.9 µm or 26.9% body length. Longitudinal striations sometimes visible on the more deeply stained, larger specimens, particularly over the nuclear area (Figure 4.2C).

Figure 4.2F shows the measurements generated by the intrinsic measuring tool.

4.3.6 Remarks

Given the reported morphometrics of this trypanosome, the only possible overlap would be with the largest reported trypanosome species, such as *Trypanosoma giganteum* Neumann, 1909 and *T. gargantua* Laird, 1951. *Trypanosoma* sp. A is markedly different from both these trypanosomes, since *T. giganteum* has a length of up to 95-100 µm, a width of only 8 µm, a free flagellum of up to 30 µm, and a wider undulating membrane of up to 6 µm; whereas *T. gargantua* measures 66.7-131.1 x 4.6-13.7 µm. Since *T.* sp. A measures 70.4 µm ± 9.4 (53.7-99.4 µm) in length with a body width (including the undulating membrane) of 17.4 µm ± 2.6 (12.6-24.3 µm), it clearly differs from the aforementioned two, most strikingly in body width. The shape and size of the nucleus also separates *T.* sp. A from the other trypanosome species, being very large and circular.

Trypanosoma mackerrasi Burreson, 1989, although overlapping in some measurements, differs clearly in that it has a much longer body, a larger and distinctly oval nucleus, and no free flagellum (Burreson, 1989). Although measurements for the free flagellum of the new species were difficult to obtain, it is present and visible (Figure 4.2A).

Trypanosoma sp. A, unusually, does not display any notable pleomorphism in the specimens examined. Interestingly, dividing forms have been found in the peripheral blood in this study (Figure 4.2D), whereas other studies of trypanosomes infecting Scyliorhinidae (although not the same host species) found no dividing forms in peripheral blood, kidney or spleen (Pulsford, 1984). Other reported species of trypanosome do not overlap with the morphometrics of *T.* sp. A (Table 4.1), and given the features described, its geographical location and the endemicity of the host shark species, we propose it as a new species.

4.4 Discussion

More than 40 trypanosomes are known from marine teleosts, but only 10 have been reported from elasmobranchs and of these, more have been reported from rays and skates (6) than sharks (4). This is the first report of a trypanosome from elasmobranchs from the Southern African coast.

Taxonomic confusion exists in the blood parasites of fishes. Species are usually described using morphological criteria, and this results in a lack of knowledge about host specificity

(James & Woo, 1990). It is difficult to do a thorough comparison of morphometrics from elasmobranch trypanosomes due to the paucity of valid data available, and, since there are only minor morphological differences among many of the trypanosome species, they may still prove to be synonymous with biochemical characterization (Pulsford, 1984). When considering factors influencing similarity between species this is further complicated, since geographical proximity, which is usually given priority, does not have any significance in this case, where the type location is so far removed from any prior elasmobranch descriptions. Trypanosomes have, however, been shown to be unequivocally monophyletic (Stevens & Gibson, 1999).

The most marked pleomorphism examples can be drawn from marine species, for example *Trypanosoma gargantua*, *T. giganteum*, *T. rajae* and *T. murmanense*. *T. rajae* is the best-known example of extreme polymorphism, which prompted Minchin and Woodcock (1910) to refer the species *T. variabile* as a synonym (Laird & Bullock, 1969). There are a few fish trypanosomes which seem to be virtually monomorphic with variability displayed only in size and/or width to length ratio. No indication of sexuality has been discerned in fish trypanosomes, unlike mammal trypanosomes e.g. *T. brucei*. Unlike in other studies (Pulsford, 1984), dividing forms were found in the peripheral blood in this study (Fig. 4.2D.

Given the observed morphometrics of this trypanosome, the only possible overlap would be with the two largest reported trypanosome species, such as *T. giganteum* and *T. gargantua*. *T.* sp. A is markedly different from both these trypanosomes, most strikingly in the body width. The nucleus shape and size also separates *T*. sp. A from the other trypanosome species, being very large and circular. *T. mackerrasi*, although overlapping in some measurements, differs clearly in that it has a much longer body, a larger and distinctly oval nucleus, and no free flagellum (Burreson, 1989). Although measurements for the free flagellum of *T*. sp. A are not presented here, it is present and visible. *T*. sp. A, unusually, does not display any notable pleomorphism in the specimens examined. Other reported species of trypanosome do not even nearly overlap with the morphometrics of *T*. sp. A, and given the features described, its geographical location and the endemicity of the host shark species, there is sufficient evidence to consider it a new species.

The impact of infection on host populations is difficult to assess – partly because there is no agreement in the literature on the criteria used to evaluate parasite-induced cost to the host (Davies & Johnston, 2000). Few blood infections of fishes, amphibian and reptiles have proven pathogenicity, contrary to many intraerythrocytic parasites of mammals and birds (Davies & Johnston, 2000). Trypanosomes of marine fish seem to very rarely cause acute disease (Pulsford, 1984), although there is relatively little known about the diseases of elasmobranchs, or the haematological effects of their blood parasites (Aragort *et al.*, 2005).

Trypanosome infections are known to kill juvenile fish, especially in concurrent infections with gill parasitic copepods such as *Lernaeocera branchialis* (Khan & Lacey, 1986). Although parasitic gill copepods are not present in this case, there is a gnathiid isopod (*Gnathia pantherina* Smit & Basson, 2002) parasitic on the gills of those specimens from De Hoop Nature Reserve in infestations of up to 272 per shark, which may increase the stress load posed by the trypanosome infection, particularly on juvenile sharks. Heavy trypanosome infections have also been suggested to have debilitating sublethal effects on adult cod and other fish hosts (Hemmingsen *et al.*, 2005). Close scrutiny of the effect of trypanosomiasis on juvenile hosts is likely to reveal remarkable cases of morbidity and mortality, while the pathogenic potential of fish trypanosomes has not been appreciated until relatively recently (Lom & Dykova, 1992), although Neumann (1909) noted brain inflammation, anaemia, eosinophilia and some fatty degeneration in organs of skates (*Raja* sp.) possibly related to heavy infections with *T. variabile* (syn. *T. rajae*).

This infection of *T.* sp. A is unique in that sharks from all size and age categories were infected, unlike previous studies where only rays (family Rajidae) less than 500 g were infected with *T. giganteum* (Aragort *et al.*, 2005), or only the larger classes of dogfish *Scyliorhinus canicula* were infected with *T. scylli* (Pulsford, 1984). Also unlike previous studies, the infection of sharks with *T.* sp. A is relatively high, with a mean of 11 trypanosomes per blood smear, as opposed to infection rates of 0-2 (Aragort *et al.*, 2005) or 1-4 (Pulsford, 1984).

Marine fish trypanosomes are transmitted by piscicolid leeches, which are the only known vectors, although the vectors of most described trypanosome species have not been identified (Molyneux, 1977; Jones & Woo, 1990). However, not all leeches will transmit trypanosome infections (Khan *et al.*, 1980) and thorough transmission experiments need to be carried out before the vector can be conclusively known. Benthic lifestyle (such as the shark hosts *Haploblepharus pictus* and *H. edwardsii*) increases exposure to leeches, and therefore to the transmission of trypanosomes and other blood parasites. Khan, Barrett and Murphy (1980) showed that there was a higher prevalence of trypanosome infection observed in cold-water, benthic, sedentary fishes. In other words, both ecological and behavioural factors of the vertebrate and invertebrate hosts will have an effect on marine fish blood infections. Trypanosomes may be specific to their leech vector, but infect several fish hosts (Karlsbakk *et al*., 2004). Leeches known to be vectors of elasmobranch trypanosomes include *Pontobdella muricata*, a vector of *Trypanosoma scylli*, and *Branchellion ravenelli,* a suspected vector of *T. humboldti* (Morillas *et al.*, 1987). Early stages of marine fish trypanosomes (amastigotes and sphaeromastigotes) develop and divide in the crop and intestine of the leech vector, followed by movement of the epimastigotes and metatrypanosomes to the proboscis sheath (Molyneux, 1977). However this migration to the proboscis sheath is not noted in all marine species, which indicates that the pattern of trypanosome development in the leech may have some taxonomic value (Jones & Woo, 1990).

A leech species, tentatively identified as *Stibarobdella macrothela* (Prof. E. Burreson, Virginia Institute of Marine Science, USA; this has yet to be confirmed) has been noted on sharks infected with *T.* sp. A. Although this has not been conclusively shown to be a vector, possibly it can transmit *T.* sp. A, since leeches are known to transmit haematozoans. A study is currently underway to test this hypothesis. There is no apparent correlation between the prevalence of the trypanosome infection (100%) and the leech infestation levels in the sharks examined, but this can possibly be attributed to the behaviour of leeches, which detach from their hosts after feeding (Morillas *et al.*, 1987).

According to Lom & Dykova (1992) sharks and rays are known to host some of the largest trypanosomes known, possibly influenced by the large size of elasmobranch blood cells. Our discovery of *T.* sp. A in the blood of South African catsharks further emphasises this phenomenon.

CHAPTER 5

TWO NEW SPECIES OF HAEMOGREGARINE BLOOD PARASITE (APICOMPLEXA: HAEMOGREGARINIDAE) FROM SOUTH AFRICAN SCYLIORHINIDAE

5.1 Introduction

Haemogregarines belong to the protozoan phylum Apicomplexa Levine, 1970, which is represented by parasites with an apical complex, believed to be specialised to aid in the invasion into host cells (Lom & Dykova, 1992). Within this phylum, parasites of fishes are found in the class Coccidea Leuckhart, 1879. Members of the coccidian family Haemogregarinidae Leger, 1911 use both vertebrate and invertebrate hosts during their life cycle: the vertebrates host merogony and gamogony in a variety of cells; and sexual development, sporogony and further merogony is hosted by invertebrates (Davies, 1995; Smit, 2000). A non-motile zygote that secretes a flexible membrane, stretched during the sporogonous stage in the invertebrate host, is characteristic of the haemogregarines (Lom & Dykova, 1992).

In order to give some background information on elasmobranch blood haemogregarines, and to put the current study into context, a review of the research in this field is provided. Transmission of blood parasites, although an integral part of this field of research, will be only briefly touched on, as this is the subject of another ongoing study.

Five species of haemogregarine worldwide are known to infect elasmobranch fishes. These belong to two genera: *Desseria* Siddall, 1995 and *Haemogregarina* Danilewsky, 1885. The known species are *D. (Haemogregarina) dasyatis* (Saunders, 1958) Siddall, 1995; *D. (Haemogregarina) heterodonti* (von Prowazek, 1910) Siddall, 1995; *D. (Haemogregarina) torpedinis* (Neumann, 1909) Siddall, 1995 (synonym *H. lobianci* Yakimov and Kohl-Yakimov, 1912); *H. carchariasi* Laveran, 1908 (synonym *H. hemiscylli* Mackerras and Mackerras, 1961); and *H. delagei* Laveran & Mesnil, 1902 (Smit, 2000). Their morphometrics, host and distribution records are given in Table 5.1.

Knowledge of marine fish haematozoans from South Africa is very limited, and that of haemogregarines even more so. To date, only five species of haemogregarine have been identified from the region: *H. bigemina* Laveran and Mesnil, 1901, a very widespread and cosmopolitan intraerythrocytic parasite of marine fish worldwide (Smit, 2000; Davies & Smit, 2001; Davies *et al*., 2004); *H. koppiensis* Smit and Davies, 2001 described from specimens of the evileye pufferfish *Amblyrhynchotes honckenii* from De Hoop Nature Reserve and Jeffrey's Bay, both on the south coast of South Africa (Smit & Davies, 2001, 2005); *H. curvata* Hayes, Smit, Seddon, Wertheim and Davies, 2006, also from the south coast of South Africa (Hayes *et al*., 2006); *Desseria zei* Smit and Davies, 2006 in *Zeus capensis* from deep water trawls on the south and west coasts (Smit & Davies, 2006); and *D. mugili* (Carini, 1932) from several mullet species (Smit *et al*., 2002).

During the past four years, blood smears of 145 sharks of three different species (*Haploblepharus pictus* Muller & Henle, 1838 [78], *H. edwardsii* Voight, 1832 [53] and *Poroderma africanum* Gmelin, 1789 [14]) were collected from six sites on the west and south coasts of South Africa, spanning the area between Lambert's Bay on the west coast and De Hoop Nature Reserve on the south coast. Of these, 100 % showed haemogregarine infections. Two different forms of haemogregarine were found, both undescribed species, and referred to here as *Haemogregarina* sp. A and *H.* sp. B. Detailed descriptions of both species follow, including the use of light microscopy, morphometrics, and transmission electron microscopy.

Table 5.1. Haemogregarine species described from elasmobranchs, detailing host species, location, original description reference and morphometrics. All measurements given in micrometres (µm) unless otherwise stated; mean ± standard deviation (minimum – maximum) where available. N/A: not available.

Haemogregarine species	Host species	Location	Reference	Mature gamonts (length x width)	Nucleus (length x width)	vector
Desseria dasyatis	*Dasyatis americana*	Bahamas, British West Indies	(Saunders, 1958)	N/A	N/A	Unknown
D. heterodonti	*Heterodontus japonicus*		(von Prowazek, 1910)	N/A	N/A	Unknown
D. torpedinis	*Topedo ocellata*, *T. marmoratus*		(Neumann, 1909)	18 x 4.5	N/A	Unknown
Haemogregarina carchariasi (syn. *H. hemiscylli*)	*Carcharias* sp., *Hemiscyllium ocellatum*	Australia	Laveran, 1908; Mackerras & Mackerras 1961	(20 – 27) x (7 – 10); [(16 – 19) x (5 – 8)]	N/A	Unknown
H. delagei	*Raja punctata*, *R. mosaica*, *R. erinacea*, *R. radiate*, *R. senta*, *Squalus acanthias*	North America	Laveran & Mesnil, 1902	(6.3 – 13.7) x (1.6 – 3.6);		*Oxytonostoma typica*
			Becker & Overstreet, 1979	(10.8 – 15.2) x (4.3 – 6.6)	(3.0 – 4.3) x (3.2 – 5.3)	
Haemogregarina sp A	*Haploblepharus pictus*, *H. edwardsii*, *Poroderma africanum*	South western coast of South Africa	This paper	13.94 ± 0.61 (12.72 – 15.18) x 7.47 ± 0.58 (6.57 – 8.63)	3.75 ± 0.48 (2.72 – 4.90) x 5.99 ± 0.50 (5.08 – 7.42)	Unknown
Haemogregarina sp. B	*Haploblepharus pictus*, *H. edwardsii*, *Poroderma africanum*	South western coast of South Africa	This paper	14.15 ± 0.79 (12.24 – 15.62) x 8.00 ± 0.69 (4.39 – 6.84)	5.67 ± 0.69 (4.39 – 6.84) x 5.57 ± 0.62 (4.55 – 7.38)	Unknown

5.2 Materials and methods

As in Chapter 2 of this thesis, with the following additional methods specific to this chapter.

Photomicrographs of haemogregarines found on blood smear slides were obtained with a Zeiss photomicroscopic digital camera system and intraerythrocytic haemogregarines were measured with the aid of Zeiss Axioplan Software, Version 4.1. Where possible, 30 individuals of each stage (trophozoite, meront, immature gamont and gamont) were measured, and the two forms found measured separately, resulting in the measurement of over 200 individual haemogregarines. Total body length (TL) was taken along the midline of the animal, and total body width (TW) at the widest part of the cell. Nucleus dimensions (nuclear length NL and nuclear width NW) were measured, as was anterior end to mid-nucleus (AN) and mid-nucleus to posterior end (NP). The total area (TA) of the erythrocyte was also recorded (30 each where possible): uninfected erythrocytes, and those infected with one, two and three of Form A and Form B respectively, as well as those infected with one Form A and two Forn B, and one Form B and two Form A. Measurements are presented as mean ± standard deviation (minimum – maximum). Specimen line drawings were made with the aid of a *camera lucida*.

Statistical analyses were done using the Statistica Package software. Gamonts of *Haemogregarina* sp. A and *H.* sp. B were compared, with respect to TL, TW, NL, NW, AN and NP. Levene's Test for Homogeneity was performed in order to test whether the data were homoscedastic, and data were plotted on a probability plot in order to ascertain whether they were normally distributed. When data were homoscedastic and normally distributed, a t-test was performed. When data were either heteroscedastic or not normally distributed, the non-parametric tests of Kolmogorov-Smirnov and the Mann-Whitney U Test were performed. Cell areas of uninfected erythrocytes and erythrocytes infected with one and two of *Haemogregarina* sp. A, and one and two of *Haemogregarina* sp. B, as well as erythrocytes infected with one of each (i.e. two) of the aforementioned, were compared. After testing for heteroscedasticity and normal distribution, the non-parametric Kruskal-Wallis Test was performed on the data.

Transmission electron microscopy (TEM) was done on *Haemogregarina* sp. A and *H.* sp. B in order to elucidate the cell ultrastructure and be able to give an accurate, detailed description of the two new species.

All sharks collected were also screened for leeches, which, when found, were removed and held for up to 32 days post feeding. In order to examine for transmission of blood parasites, squash preparations of two leeches were made. They were crushed and smeared between two clean glass slides (Davies & Smit, 2001; Hayes *et al.*, 2006). Squashes were then fixed in absolute methanol, stained and screened as for blood films. Histological sections of four individual leeches were also made and screened for stages of haemogregarine blood parasites.

5.3 Results

Unknown intraerythrocytic apicomplexan haemogregarines were found in the blood of all three shark species under investigation (*Haploblepharus pictus, H. edwardsii* and *Poroderma africanum*). Furthermore, they were found in all the sampled areas of coastline.

Two distinct species of haemogregarine were found: *Haemogregarina* sp. A and *H.* sp. B, differing in morphometrics and staining properties (Figure 5.1). Developmental stages (trophozoites, meronts and gamonts) conforming to the defining characteristics of both these species were noted in the blood (Figure 5.2–5.4).

Systematics

Phylum APICOMPLEXA (SPOROZOA) Levine, 1970

Class COCCIDEA Leuckhart, 1879

Order ADELEINA Leger, 1911

Suborder ADELEIORINA Levine, 1988

Family HAEMOGREGARINIDAE Leger, 1911

Genus *Haemogregarina* Danilewsky, 1885

Haemogregarina sp. A sp. nov. and *Haemogregarina* sp. B sp. nov.

(Figure 5.1)

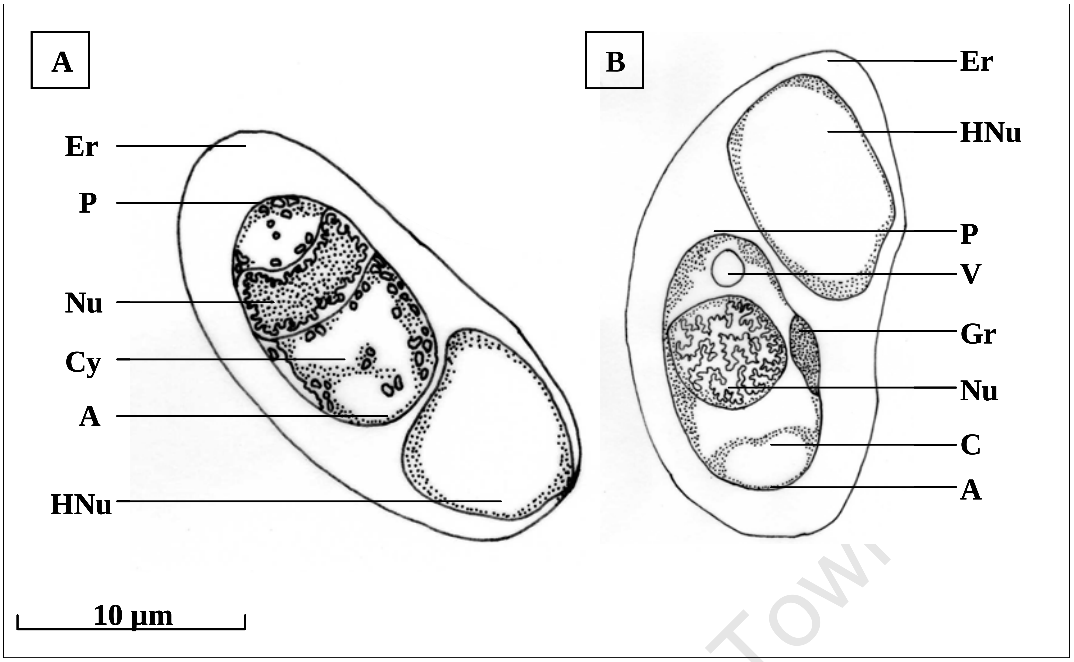

Figure 5.1. Line drawing of intraerythrocytic gamonts of *Haemogregarina* sp. A sp. nov. and *H*. sp. B. sp. nov. from a scyliorhinid host, *Haploblepharus pictus*, from the west coast of South Africa. Host erythrocyte (Er), host cell nucleus (HNu); parasite anterior (A), posterior (P), nucleus (Nu). **A.** *H*. sp. A showing typical speckled, "foamy" cell cytoplasm appearance (Cy) and densely compact nucleus. **B.** *H*. sp. B showing characteristic vacuole (V), anterior "cap" (C), deep-staining protruding granule (Gr) and diffuse nucleus.

5.3.1 Haemogregarina **sp. A**

The smallest stages of *Haemogregarina* sp. A were identified as trophozoites (Figure 5.2A and B). These were mostly found in the erythrocytes, between the nucleus and the cell end, with the red blood cell nuclei often found much further towards the other end of the cell. The trophozoites had a very distinctive and predictable shape, with a broad and rounded anterior end, and a narrow and pointed posterior end, resembling a bivalve mussel in outline. They measured 9.24 ± 1.11 (7.06 – 10.86) µm long by 5.17 ± 0.49 (4.34 – 6) µm wide (n = 13). The cytoplasm stained very lightly, with a number of minute deeply stained granules. The nucleus was compact, stained dark purple with Giemsa, and was generally centrally situated with a slight bias towards the anterior end of the cell (anterior to mid-nucleus 3.95 ± 0.75 (3.09 – 5.22) µm and mid-nucleus to posterior 5.29 ± 1.02 (3.42 – 7.1) µm (n = 13)). It was rounded in shape although very slightly longer than broad, with the dimensions 3.91 ± 0.74 (2.8 – 5.43) µm wide by 4.118 ± 0.97 (3.05 – 5.78) µm long (n = 30).

Large, rounded meronts of *Haemogregarina* sp. A were found (Figure 5.2C and D), also in the erythrocytes of infected sharks, and were situated right adjacent to the host blood cell nucleus, in some cases causing a distortion of the nucleus. Their shape was distinctly round, measuring 9.83 ± 1.87 (7.53 – 11.40) µm long by 8.71 ± 0.74 (7.48 – 9.66) µm wide (n = 11). Their cytoplasm was very lightly stained, with the darkly stained "speckled" granules which appear characteristic of this species. The defined, darkly purple stained nuclei were sometimes centrally placed, but usually found at one end of the cell, and frequently much rounder in shape than the nuclei of the other developmental stages of *Haemogregarina* sp. A, measuring 5.15 ± 0.73 (4.14 – 6.34) µm in length and 6.13 ± 1.10 (4.61 – 8.25) µm in width (n = 11).

Dividing forms of *Haemogregarina* sp. A were found in host shark erythrocytes – usually with the development of two daughter nuclei visible in the haemogregarine (Figure 5.2E and F).

Gamonts of *Haemogregarina* sp. A were generally oval shaped cells tending to be tapered towards one end. They had light-coloured, practically non-staining cytoplasm speckled with miniscule deeply purple stained granules, giving it a foamy or "speckled" appearance (Figure 5.2G–I). The densely compact nucleus stained very dark purple, and was laterally elongated with an almost linear aspect, stretching the width of the cell, sometimes associated with a slight indentation. Gamonts measured 13.94 ± 0.61 (12.72 – 15.18) µm in length and 7.47 ± 0.58 (6.57 – 8.63) µm in width (n = 33). The nuclei, measuring 3.75 ± 0.48 (2.72 – 4.90) µm in length and 5.99 ± 0.50 (5.08 – 7.42) µm in width (n = 33), were generally situated across the posterior end of the parasite, but occasionally towards the middle of the cell. Anterior to mid-nucleus measured 9.42 ± 0.88 (7.33 – 11.18) µm and mid-nucleus to posterior measured 4.52 ± 0.89 (3.25 – 6.52) µm (n = 33).

5.3.2 Haemogregarina sp. B

Trophozoites corresponding to *Haemogregarina* sp. B were found in erythrocytes and erythroblasts of host shark blood (Figure 5.3A and B). There was no preference shown for position within the cell, but 75 % were found in the cytoplasm of erythroblasts, with the remaining 25 % in erythrocytes. These stages displayed a very characteristic sickle or crescent shape, and measured 9.72 ± 1.31 (7.13 – 12.71) µm long and 5.37 ± 0.64 (4.26 – 6.81) µm wide (n = 28). The posterior end of the cell was somewhat tapered, and the anterior end rather more blunt. Their cytoplasm stained a light blue purple, with a diffuse, darker purple nucleus situated centrally (anterior to mid-nucleus measured 5.56 ± 0.93 (3.61 – 7.41) µm and mid-nucleus to posterior measured 4.17 ± 1.05 (2.57 – 6.02) µm (n = 28)). The nuclei were generally circular in shape, 4.59 ± 0.83 (2.91 – 6.10) µm long by 4.06 ± 0.59 (2.92 – 5.14) µm wide (n = 28).

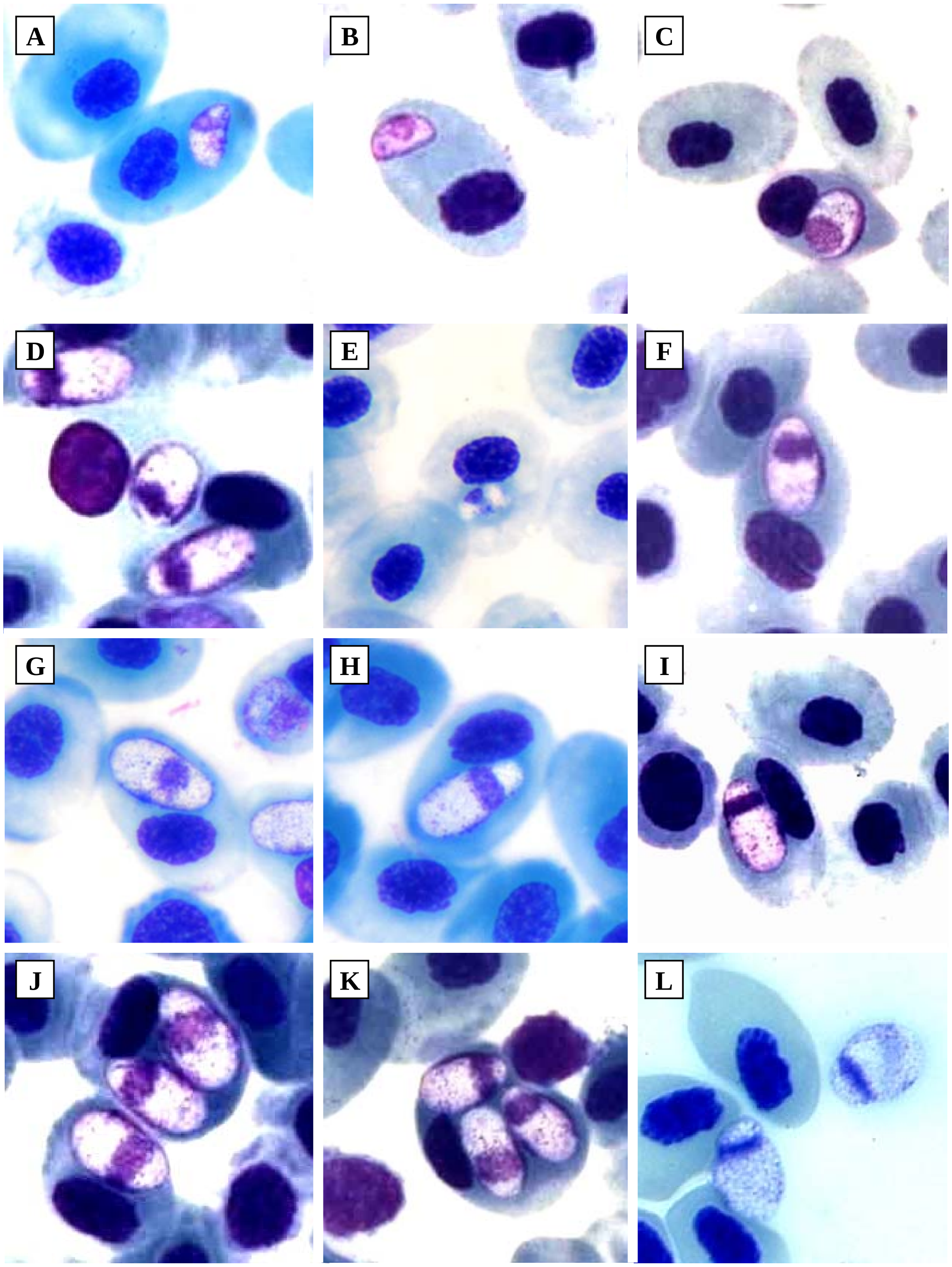

Figure 5.2. Light micrographs of the different life cycle stages of *Haemogregarina* sp. A in scyliorhinid hosts, *Haploblepharus pictus*, *H. edwardsii* and *Poroderma africanum*, from the west and south coasts of South Africa (X630). **A-B.** Small trophozoite. **C-D.** Meronts. **E-F.** Dividing stages. **G.** Immature gamont. **H-I.** Gamonts. **J.** Paired gamonts in host erythrocyte. **K.** Three gamonts in host erythrocyte. **L.** Extra-erythrocytic gamonts.

Meronts of *Haemogregarina* sp. B were very characteristic, unmistakeable stages in host erythrocytes (Figure 5.3C and D). They were large, very round cells, pressed up tightly adjacent to the erythrocyte nucleus, sometimes causing minor distortion of the nucleus. Meront dimensions were 9.90 ± 1.25 (7.12 – 12.40) µm long by 8.90 ± 1.02 (7.20 – 11.66) µm wide (n = 33). The meront cytoplasm stained light indigo, with a slightly granular appearance. The nucleus appeared roughly circular, measuring 5.80 ± 0.88 (3.99 – 7.63) µm in length and 6.26 ± 0.78 (4.28 – 7.60) µm in width (n = 33). It was most often located right against one end of the cell, and was made up of loosely aggregated, pinkish purple stained granules. A distinctive vacuole occured in the cytoplasm. A large very dark red purple granule could be found (either paired on two sides of the cell or singly) along the periphery of the meront, sometimes protruding from the cell.

Dividing forms of *Haemogregarina* sp. B were found in host shark erythrocytes – usually with the development of two daughter nuclei visible in the haemogregarine (Figure 5.3E and F).

Gamonts of *Haemogregarina* sp. B (Figure 5.3G–L)) were also oval shaped cells, but had no defined taper towards either end. The cytoplasm stained a light indigo blue colour. The diffuse nuclei, which were situated approximately in the middle of the cell lengthwise, tending towards the cell edge, stained a slightly darker, pinker shade of purple, with a very granulated appearance and were much rounder in shape than that of *H.* sp. A. Anterior to mid-nucleus measured 8.10 ± 1.36 (5.74 – 10.37) µm and mid-nucleus to posterior measured 6.05 ± 1.43 (3.99 – 9.13) µm (n = 34). Gamonts measured 14.15 ± 0.79 (12.24 – 15.62) µm in length and 8.00 ± 0.69 (4.39 – 6.84) µm in width (n = 34). The nuclei were roughly oval in shape and measured 5.67 ± 0.69 (4.39 – 6.84) µm in length and 5.57 ± 0.62 (4.55 – 7.38) µm in width. There was a distinct darker purple anterior cap present in mature gamonts, a conspicuous vacuole between the posterior end and the nucleus, and a large, very dark reddish purple granule present lateral to the nucleus and frequently protruding from the normal boundary/outline of the cell.

Although the two species usually occurred individually in erythrocytes, they did occasionally occur in pairs (Figure 5.2J and Figure 5.3J), and, fairly rarely, both forms were present in one erythrocyte (Figure 5.4A–C). In particularly heavy infections, up to three individual parasites were found in one erythrocyte, both single form infections (e.g. three *Haemogregarina* sp. A gamonts) (Figure 5.2K and Figure 5.3K) or in any combination of the two forms (Figure 5.4B) and the three developmental stages (Figure 5. 4A–C). Extracellular gamonts of both species (Figure 5.2L and Figure 5.3L) were also observed.

5.3.3 Transmission Electron Microscopy

Figure 5.5 shows toluidene blue-stained semi-thin slides, from which haemogregarines of both *H*. sp. A and *H*. sp. B were identified (circled in Figure 5.5A-D). Ultra-thin sections were then cut from these areas of interest, and these micrographs are shown in Figure 5.6 and Figure 5.7.

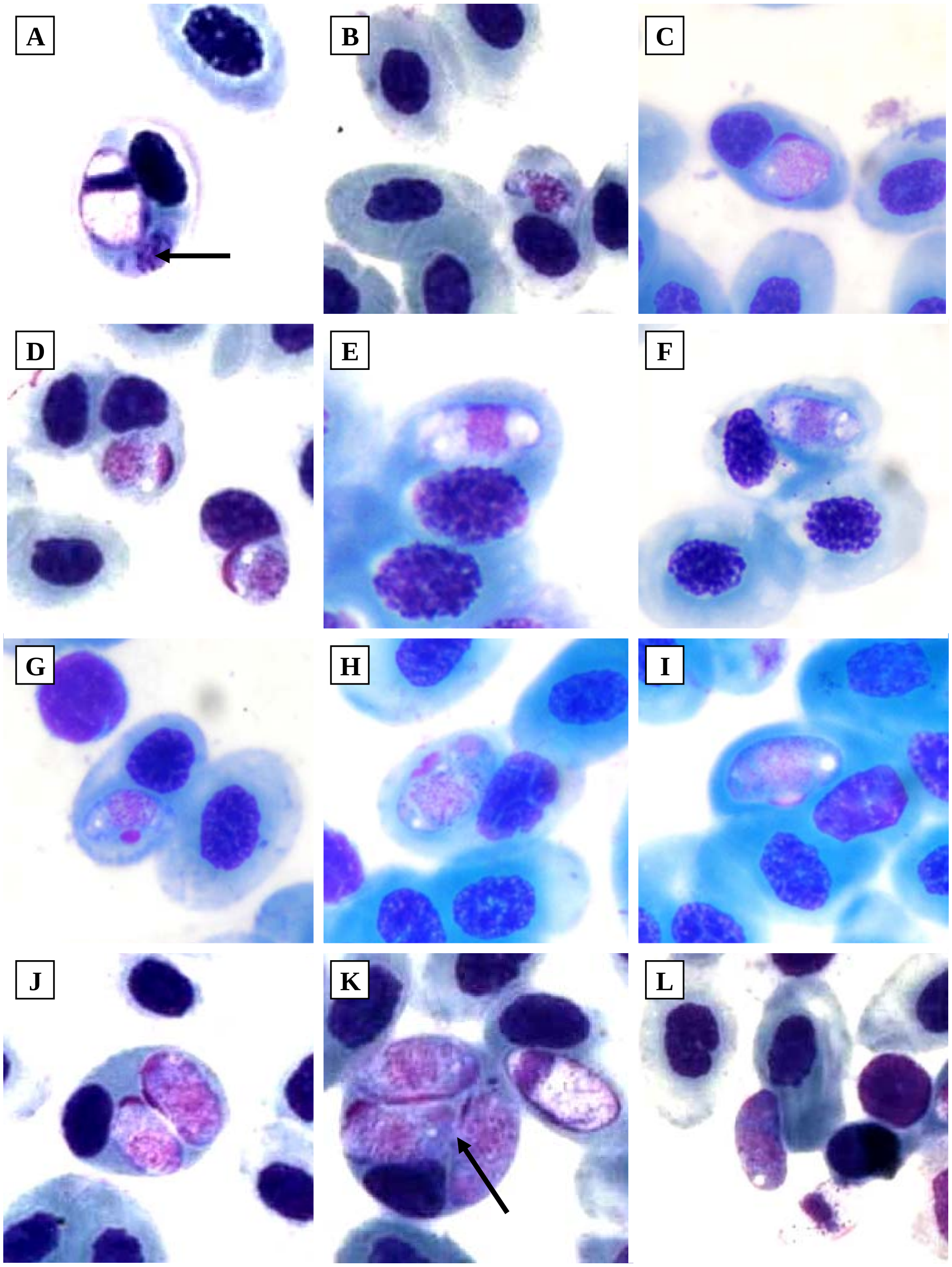

Figure 5.3. Light micrographs of the different life cycle stages of *Haemogregarina* sp. B in scyliorhinid hosts, *Haploblepharus pictus*, *H. edwardsii* and *Poroderma africanum*, from the west and south coasts of South Africa (X630). **A.** Small trophozoite (indicated by arrow) sharing cell with gamont of *H.* sp. A. **B.** Small trophozoite. **C-D.** Meronts. **E-F.** Dividing stages. **G.** Immature gamont. **H-I.** Gamonts. **J.** Paired gamonts in host erythrocyte. **K.** Three gamonts in host erythrocyte (indicated by arrow). **L.** Extra-erythrocytic gamont.

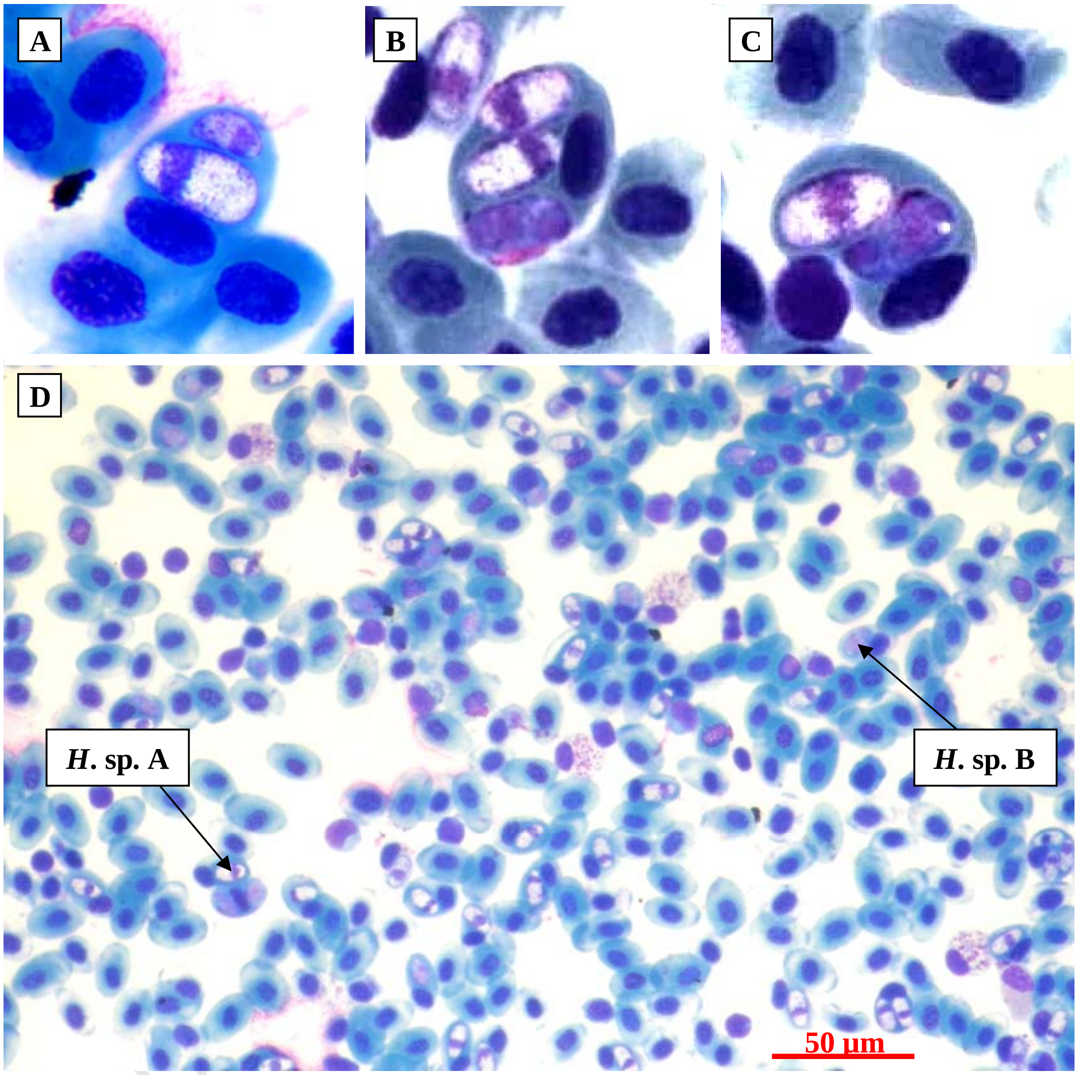

Figure 5.4. Light micrographs of multiple infections and stages of *Haemogregarina* sp. A. and *Haemogregarina* sp. B in a scyliorhinid host, *Haploblepharus pictus*, from the west coast of South Africa. **A.** Small trophozoite and mature gamont of *H.* sp. A in one host erythrocyte (X630). **B.** Two *H.* sp. A and one *H.* sp. B gamonts in one host erythrocyte (X630). **C.** One gamont of each haemogregarine species in one erythrocyte (X630). **D.** Heavy haemogregarine infection of both species in host peripheral blood (scale bar = 50 µm).

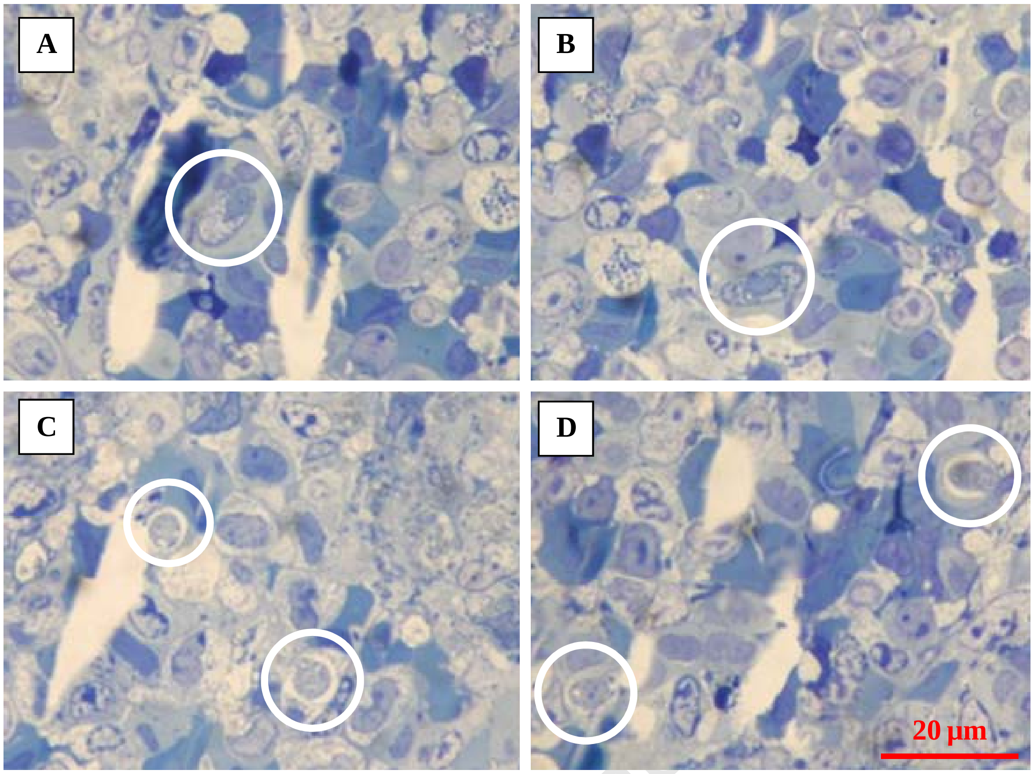

Figure 5.5. Toluidine Blue stained light micrographs of semi-thin section of spleen showing specimens of *Haemogregarina* sp. A and *Haemogregarina* sp. B (circled) in a scyliorhinid host, *Haploblepharus pictus*, from the west coast of South Africa. **A.** and **B.** *H.* sp. A. **C.** and **D.** *H.* sp. B.

Both new species of haemogregarine showed the ususal organelles typical of the phylum Apicomplexa (Diniz *et al*., 2002; Abdel-Nasser, 2006) (Figure 5.6 and Figure 5.7). The anterior of each parasite was characterized by the presence of an apical complex, consisting of a conoid, a few dense rhoptries and a large mass of micronemes. The nucleus was situated more or less centrally, with visible chromatin masses and small dense particles. Dense bodies were visible, mostly towards the posterior of the parasite. Mitochondria were often visible, usually (but not exclusively) at the periphery of the cell.

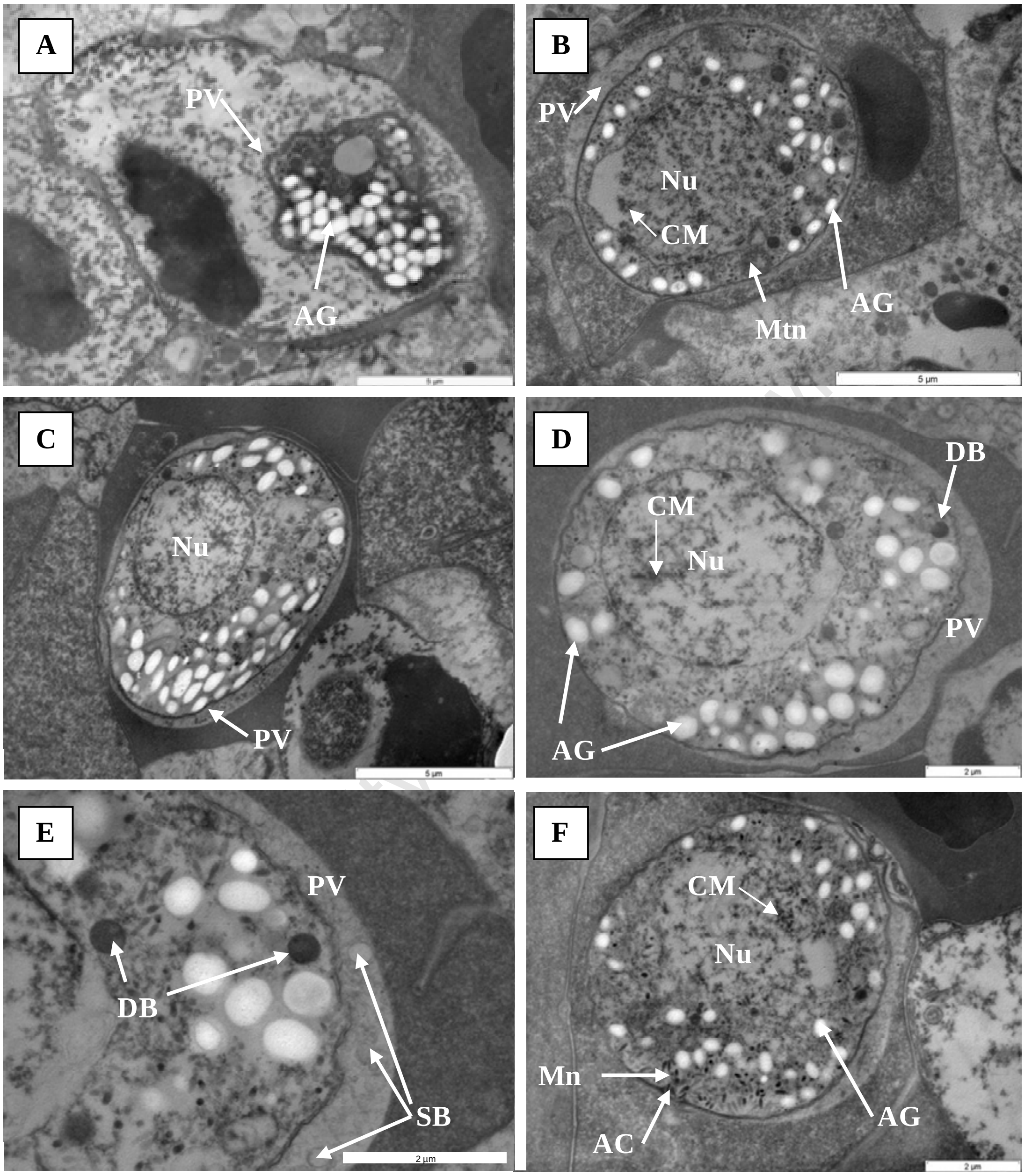

Figure 5.6. A.–F. Transmission electron micrography showing ultrastucture of *Haemogregarina* sp. A in a scyliorhinid host, *Haploblepharus pictus*, from the west coast of South Africa, including apical complex (AC), amylopectin granules (AG), chromatin mass (CM), dense bodies (DB), micronemes (Mn), mitochondrion (Mtn), nucleus (Nu), parasitophorous vacuole (PV) and spherical bodies (SB).

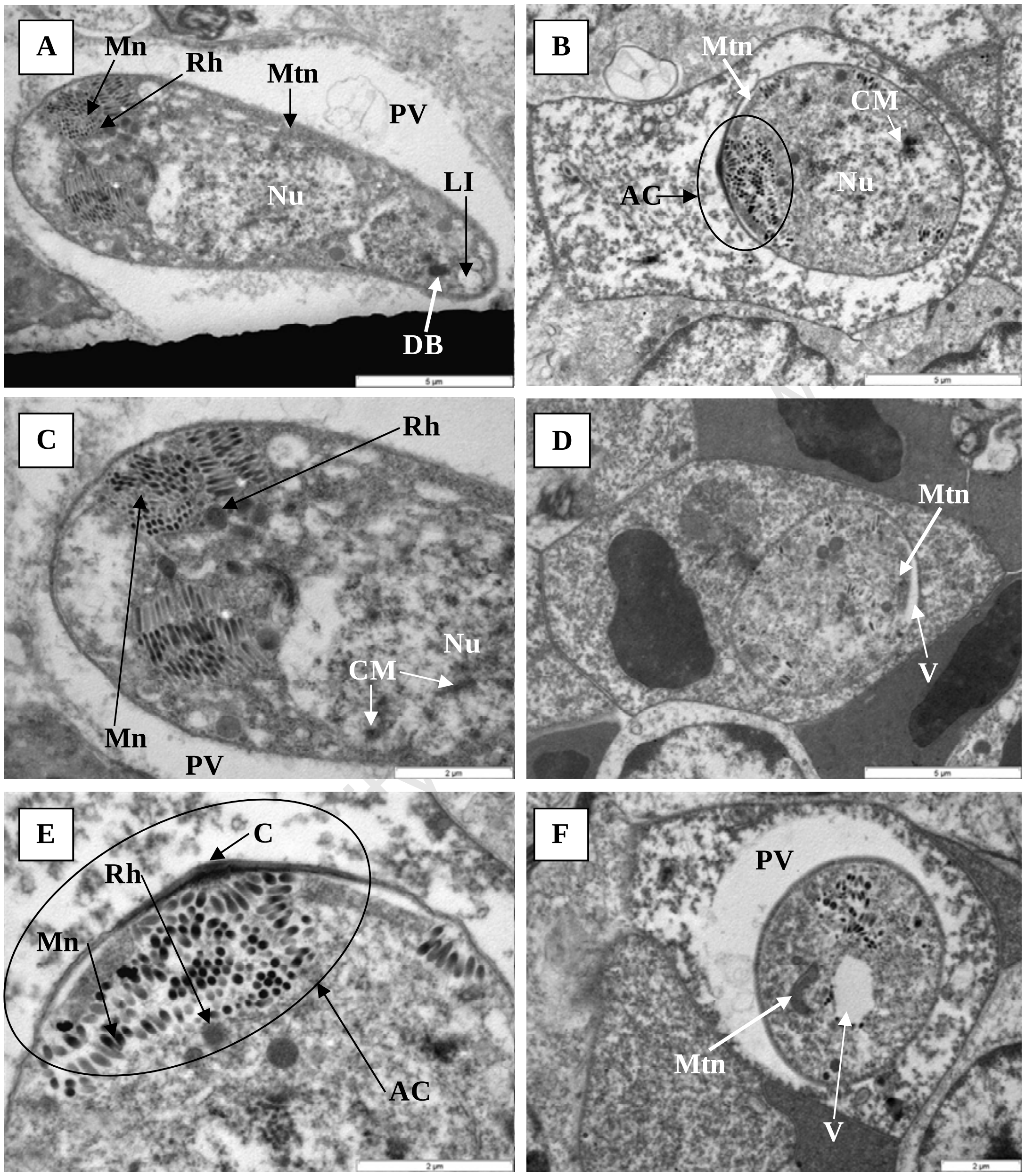

Figure 5.7. A.–F. Transmission electron micrography showing ultrastucture of *Haemogregarina* sp. B in a scyliorhinid host, *Haploblepharus pictus*, from the west coast of South Africa, including apical complex (AC), conoid (C), chromatin mass (CM), dense bodies (DB), lipid inclusions (LI), micronemes (Mn), mitochondrion (Mtn), nucleus (Nu), parasitophorous vacuole (PV) and rhoptries (Rh).

There were, however, striking differences in the ultrastructure of *H*. sp. A and *H*. sp. B. First and most obvious is the presence of a very large number of amylopectin granules in all stages of *H*. sp. A (Figure 5.6A-F). These were completely absent in all stages of *H*. sp. B (Figure 5.7A-F). This would account for the very light, almost non-staining appearance of *H*. sp. A under light microscopy, and also for the “foamy” or “speckled” appearance of the cytoplasm. Another noticeable difference in the ultrastucture of the two species is the parasitophorous vacuole (PV) surrounding the protozoan. In *H*. sp. A, the membrane lining of the PV is reasonably close to the parasite membrane, and there is a narrow but defined vacuole, always visible, surrounding the parasite inside the host cell (sometime containing faint spherical bodies) (Figure 5.6A-E). In *H*. sp. B, the PV seems to contain a material less dense than the host cytoplasm, and forms a large area around the parasite, giving the appearance of a “clearing” with no visible membrane (Figure 5.7A, C and F), and is not always visible. There is a vacuole (V) present in *H*. sp B (Figure 5.7D and F), also visible under light microscopy, which (although not seen in all the TEM, given its ultrathin sectioning) is almost always present (see Section 5.3.2). This is not seen in *H*. sp. A.

5.4 Remarks

Haemogregarina sp. A and *Haemogregarina* sp. B are differentiated for a number of reasons. Firstly, the obvious differences in appearance caused by staining properties and morphological structures. This is supported by TEM, which shows very distinct differences in cell ultrastucture of the two species, whilst confirming both as apicomplexan haemogregarines.

Table 5.2 shows the results of statistical analyses between the dimensions of *Haemogregarina* sp. A and *H.* sp. B. A t-test showed no significant difference in the gamont cell lengths of *Haemogregarina* sp. A and sp. B. The gamont widths of *H.* sp. B, however, proved to be significantly greater than those of *H.* sp. A. Gamont nucleus lengths of the two species were shown to be significantly different (t-test), however the data were heteroscedastic and therefore the non-parametric Mann-Whitney U test was used to confirm this highly significant difference (Table 5.2). T-test results showed a significant difference in the width of the nuclei of the two haemogregarine species, *H.* sp. A having a significantly wider nucleus. The Mann-Whitney U test showed that anterior to mid-nucleus length of *H.* sp. A was significantly greater than *H*. sp. B, and that mid-nucleus to posterior length of *H*. sp. B was significantly greater than *H*. sp. A. Therefore the nucleus of *H*. sp. B is more centrally situated than that of *H*. sp. A, which is towards the posterior end of the cell. Thus although no distinction can be made between the gamonts of the two species in terms of cell length, they differ significantly in terms of width, nucleus length, nucleus width, and nucleus position.

Table 5.2.: Results of statistical analyses of comparisons between the morphometrics of gamonts of *Haemogregarina* sp. A and *Haemogregarina* sp. B, from the peripheral blood of scyliorhinid catsharks from South Africa. Statistical significance is indicated by *

Gamont morphometrics	**t-test**			**Mann-Whitney U-test**	
	df	**t**	**p**	**U**	**p**
Cell length	65	-1.21	>0.05		
Cell width	65	-3.36	<0.01*		
Nucleus length				15.00	<0.01*
Nucleus width	65	3.05	<0.01*		
Anterior to mid-nucleus				221.50	<0.001*
Mid-nucleus to posterior				210.00	<0.001*

Table 5.3 shows the results of statistical analyses among the erythrocyte cell areas of blood cells infected with haemogregarines. Only erythrocytes infected with one each of *H.* sp. A and *H.* sp. B have an area significantly different to that of uninfected erythrocytes, as well as having a statistically significantly different area to erythrocytes infected with one of either haemogregarine species. Blood cells infected with one *H.* sp. A had a statistically indistinguishable area from those of uninfected erythrocytes, and those infected with one *H.* sp. B, but had a significantly different area from those infected with two haemogregarines of any combination. Cells infected with two *H.* sp. A were only found to have a different area to cells infected with one *H.* sp. A, which was also the case with erythrocytes infected with two *H.* sp. B.

Despite this statistical justification that *H.* sp. A and *H.* sp. B are two species, the most valid justification for separating the two is the fact that different developmental stages (trophozoite, meront, merogony, immature gamonts and gamonts) can be clearly seen for *H.* sp. A and *H.* sp. B, separately, and obviously differentiated. In addition, trophozoites of *H.* sp. B show a marked preference for erythroblasts, with 75 % occurring in these cells. Trophozoites (and all other intracellular stages) of *H.* sp. A on the other hand are found solely in erythrocytes. This is not merely a case of dimorphic gamonts mistaken for different species of parasite.

Table 5.3.: Results of statistical analysis using the Pairwise Unequal N HSD test comparing cell area among uninfected erythrocytes and erythrocytes infected with a range of gamonts of *Haemogregarina* sp. A and *Haemogregarina* sp. B, from the peripheral blood of scyliorhinid catsharks from South Africa. Statistical significance is indicated by *

Infection	{1}	{2}	{3}	{4}	{5}	{6}
Uninfected erythrocyte {1}	-	>0.05	>0.05	>0.05	>0.05	<0.05*
1 *Haemogregarina sp*. A {2}	>0.05	-	<0.01*	>0.05	<0.01*	<0.01*
2 *H. sp*. A {3}	>0.05	<0.01*	-	>0.05	>0.05	>0.05
1 *H. sp*. B {4}	>0.05	>0.05	>0.05	-	>0.05	<0.01*
2 *H. sp*. B {5}	>0.05	<0.01*	>0.05	>0.05	-	>0.05
1 each *H. sp* A & *H. sp*. B {6}	<0.05*	<0.01*	>0.05	<0.01*	>0.05	-

The two species of haemogregarine found in the blood of *Haploblepharus pictus*, *H. edwardsii* and *Poroderma africanum* differ in morphometrics to the other known species from elasmobranchs, being much shorter than *H. carchariasi* (20-27 x 7-10 µm) although cell width measurements overlap. The gamonts are also different from those of *H. delagei*, which has elongated gamont structure (11.3 x 3.7 µm) and is close to half the width of the parasites in the current study, and more than one gamont per erythrocyte occurs frequently. The shape of the nucleus, particularly of *H.* sp. A, and the staining properties of the cell, do not agree with known descriptions of haemogregarines, particularly those described from sharks. It is thus recommended that both be treated as species new to science. The two haemogregarine species found differ significantly from each other in both staining properties and morphometrics, and it is therefore further recommended that they be maintained as two separate species.

Leeches

Neither the squash preparations nor the histological sections of any of the leeches examined, showed any stages of haemogregarine blood parasite, either *H*. sp. A or *H*. sp. B. Histological results did, however, show mature gametes which was unexpected given the tentative identification of these leeches as juvenile *Stibarobdella macrothela* (Prof. Eugene Burresson, VIMS, USA, *pers. comm*). It is, however, still possible that the species identification is correct, and that the unprecedented finding of individuals of such extremely small size is an example of pronounced intraspecific variability. More research is needed into this question and it is the subject of a new study.

5.5 Discussion

Hameogregarines are apicomplexan parasites of the suborder Adeleina, with vertebrate intermediate and invertebrate definitive hosts (Barber *et al*., 1987). They seem to be especially common in marine fishes, found mostly in circulating erythrocytes, but also in erythroblasts and leukocytes (Kirmse, 1979; Davies *et al*., 2004). Studies on infections in chelonians, reptiles and fishes show that single or mixed infections occur with some frequency. These mixed infections can be dual infections of two species of haemogregarine, infections of haemogregarine and trypanosome species, or, as in this study, infections of two species of haemogregarine and a trypanosome species (Hayes *et al*., 2006; Cook, 2008).

Generally accepted haemogregarine biology dictates that merogony and gametogony occur in the blood cells of the vertebrate (in this case fish) host (Barber *et al*., 1987), however, in his 1995 systematic revision of the Adeleorina Siddall (1995) attempted to resolve some of the existing taxonomic confusion. The genus *Haemogregarina (sensu lato)* is characterized by intraerythrocytic merogony in the fish host, and the genus *Desseria* by the lack of this process (Davies *et al*., 2004). Siddall (1995) erected the genus *Desseria* and moved a large number of existing species, mainly from the genus *Haemogregarina*, into the new genus on the basis that they were devoid of intraerythrocytic merogony, although secondary merogony can take place in the internal organs of the fish host (Smit, 2000). However, the author agrees with the opinion of Becker and Overstreet (1979) that the classification of piscine haemogregarines on the presence (or more pertinently the absence) of intraerythrocytic schizogony must be viewed with some caution. Since this is seen in so few infected fish (with the exception of *H. bigemina*, in which it is a relatively constant feature), it seems that having the systematic placement of a species depend on the lack of observation of a developmental process, rather than definitive proof that it does not occur, is neither logical nor taxonomically accurate. Particularly given the nature of fish haemogregarine taxonomy, where it is well-nigh impossible to view all the blood cells and parasitic individuals present in an host, the placement of a species into a genus based on whether or not intraerythrocytic merogony was observed in a few blood smears cannot be robustly supported. This said, however, Siddall and Desser (1990) are justified in criticizing the generic placement of adeleid parasites depending on the appearance of bloodstream gamonts (as indeed has been questioned since the earliest descriptions of haemogregarines). Siddall's 1995 review attempted to clear this up, however, it is felt that this revision, whilst adding clarity, was not necessarily adding taxonomic worth.

Other apicomplexan diseases, e.g. malaria, toxoplasmosis and coccidiosis, have been studied in much greater detail than haemogregarines, particularly *Haemogregarina*. Consequently, the development of haemogregarines has escaped the scrutiny given to these other groups. This is due both to the perception that haemogregarines are non-pathogenic, and the fact that they infect hosts of little economic or social interest (Siddall & Desser, 1993). Haemogregarine pathology has, however, been noted in various studies, and it has been shown that haemogregarines, both individual and dividing stages, can adversely affect their hosts (definitive and intermediate). Additionally, and in fish of economic importance e.g. turbot and Atlantic mackerel, haemogregarine parasites are thought to be associated with a much broader pathology (Davies, 1995). Pathology can be broken down into two areas: pathological effects on the intermediate host, and pathological effects on the definitive host. Since this study focused on the stages found in the shark species under investigation, only the effects on the intermediate host will be explored.

The most notable effects of haemogregarine infections on the intermediate host are on the erythrocytes and erythroblasts, and can incorporate changes to their number, cytoplasm structure, position and/or appearance of the nuclei, or a change in their size (Davies, 1995). In this study, no parasites were found in the leucocytes and so no comment can be made on the pathological effects of the haemogregarine infection on this cell type. In addition, no shark was found without infection, so quantifying a change in the number of red cells is not feasible, given that it is impossible to determine the pre-infection numbers.

The impact of usual intraerythrocytic haemogregarine parasites is to cause hypertrophy of the host cells and/or nucleus of the host cell, and to cause displacement of the erythrocytic nucleus (Khan, 1972, Siddall & Desser, 1993). Siddall and Desser (1993) found that erythrocytes of fish infected with haemogregarine gamonts were smaller, with a less regular outline, than uninfected erythrocytes. They exhibited shrinkage, and their overall shape was altered in a fashion consistent with loss of cytoskeletal integrity. Khan (1972), in a study on the elasmobranch haemogregarine *Haemogregarina delagei*, found that neither hypertrophy nor displacement of the nucleus was found – although doubly infected cells were not uncommon. In some facets this is similar to the findings of the current study. Erythrocytes infected singly, with either *H.* sp. A or *H.* sp. B, showed no significant difference in cell area from uninfected erythrocytes, although cells infected with a single gamont of *H.* sp. A had a lower mean area (perhaps similar to Siddall & Desser, 1993) this was not statistically justified. However, what no studies seem to have dealt with previously is the pathogenicity associated with multiple infections that have been found in this case to characterize heavy infections. Hypertrophy of erythrocytes is displayed, along with very distorted and displaced host cell nuclei, and a frequent blurring of host cell outline.

Transmission electron microscopy (TEM) has been used to elucidate the cell ultrastructure of haemogregarine parasites. This process enables internal apicomplexan features to be determined, and increases the robustness of species descriptions from the morphometrics and staining properties of gamonts. Typical apicomplexan features seen by TEM include the conoid, subpellicular microtubules, micronemes and rhoptries (Siddall & Desser, 1992). The presence of these and other diagnostic apicomplexan and haemogregarine feature in the TEM of *H*. sp. A and *H*. sp. B confirm and support their identification as haemogregarines.

The commonly held assumption that, like species of haemogregarine found from chelonians, most (if not all) haemogregarines of marine fish exhibit sexual dimorphism of the gamont (Kirmse, 1979), can also be explored. TEM has shown, for example (supporting light microscope evidence), that sexual dimorphism is not apparent in *H. myxocephali* (Siddall & Desser, 1992). Yet another difference between both *H*. sp. A and *H*. sp. B and other elasmobranch haemogregarines is the lack of any demonstrated sexual dimorphism. The best-known elasmobranch haemogregarine, *H. delagei*, is thought to display sexual dimorphism: gamonts can be characterized by either the presence or absence of a (poorly defined) chromophilic "cap" (Laird & Bullock, 1969). The grouping of fish haemogregarines into the "rovignensis group" having a prominent polar cap (often free forms rather than intraerythrocytic) by Laird and Bullock (1969) is therefore to be viewed with caution, since either the staining procedure affects the appearance of the cap, or the cap stains differently depending on the developmental stage of the gamont, or in fact sexual dimorphism is present (Davies, 1995). Any of these possibilities would cause the categorization into the "rovignensis group" to be systematically irrelevant.

Whilst the subject of vectors and transmission of marine fish haemogregarines is not going to be dealt with in great detail, it is worthwhile mentioning briefly. It is thought likely that the association between haemogregarine blood parasites and leeches is an old and conserved one, and may be marked by a strong pattern of cospeciation (Siddall & Desser, 2001). Although leeches have traditionally been identified as the vectors of piscine haemogregarines (Davies & Smit, 2001), haematophagous isopods have also been identified as vectors of certain species (Hayes *et al*., 2006). Developmental stages of haemogregarines reported from leeches, thus identifying leeches as vectors of transmission, include *Cyrilia*, *Desseria* and *Haemogregarina*. In terms of elasmobranch haemogregarines, the leech *Oxytonostoma typica* has been shown to transmit *H. delagei* in little skates *Raja erinacea* (Siddall & Desser, 2001). Definitive hosts of most presumed *Haemogregarina* species are unknown – and those which have been identified (e.g. see above) have yet to demonstrate unequivocal biological transmission to a vertebrate intermediate host (Siddall & Desser, 1990).

This chapter presents the first description of haemogregarine species from elasmobranchs in Southern Africa. Furthermore, it documents an exceptionally heavy and widespread joint infection of three host chatshark species, *Haploblepharus pictus*, *H. edwardsii* and *Poroderma africanum*, with 100 % of sampled sharks from all sampling areas on the west and south coasts of South Africa infected by both *H*. sp. A and *H*. sp. B.

CHAPTER 6

PROBOLITREMA RICHIARDII (LÓPEZ, 1888) (PLATYHELMINTHES: GORGODERIDAE) INFECTING CATSHARKS (SCYLIORHINIDAE) FROM THE WEST AND SOUTH COASTS OF SOUTH AFRICA

6.1 Introduction

During an investigation of the parasite community found on a number of species of catshark (family Scyliorhinidae) endemic to the South African coastline, a digenean endoparasite, *Probolitrema richiardii* (López, 1888), was found in the body cavity of some of the sharks examined.

Eight generally accepted species of *Probolitrema* are known so far, all parasites of the body cavity of elasmobranchs, both sharks and rays (Table 6.1)**.** At least four genera of digeneans of the family Gorgoderidae, including *Probolitrema*, have been reported from the peritoneal body cavity of elasmobranch species (both sharks and rays), which suggests that this may be a common site for these trematodes (Caira & Healy, 2004). As far as can be determined, there have been only two previous reports of trematodes from elasmobranchs from the southern African coast. The first of these was Ofenheim's identification of *Anaporrhutum richiardii* (previously *Distomum richiardii* Lopez 1888) from *Scyllium* sp. in the Atlantic Ocean off Cape Town (Table 6.1). However, Looss (1902) moved *D. richiardii* from *Anaporrhutum* into a new genus, *Probolitrema*. Ofenheim's *Anaporrhutum richiardii* was also designated as a new species, *Probolitrema capense* (Looss, 1902) by Stunkard (1935). Secondly, Gibson (1976) makes mention of a specimen of *P. capense* (now synonymous with *P. richiardii*) from the ray *Raja marginata* caught off Houtjes Bay (assumed to be what is currently known as Hoedjiesbaai in Saldanha Bay, although this cannot be confirmed) north of Cape Town, South Africa in 1926.

In the same paper, Gibson (1976) synonymised all known species of *Probolitrema* with *P. richiardii*, which still stands as the last valid taxonomic revision. Accordingly, we provisionally refer to the specimens collected in this study as *P. richiardii*, but later in this chapter (Discussion, Section 6.4) dispute this synonymy.

The identification of *Scyllium* as the host of *P. richiardii* itself adds to the general confusion surrounding the systematics of *Probolitrema*. Members of the now invalid and defunct genus *Scyllium* have been reallocated to a number of genera including (amongst others) *Poroderma*, *Haploblepharus* and *Scyliorhinus*, all three of which contain species found off the Atlantic South African coast around Cape Town (Heemstra & Heemstra, 2004). This makes the designation of historic host records somewhat challenging, and for the purpose of clarity, every shark species found to host *P. richiardii* here is designated as a new host record.

This paper reports on the only trematode species known from elasmobranchs in this region and provides a detailed redescription of the species, including scanning electron microscopy (SEM). As recommended by Gibson (1976) as “well worthy of study” – the first detailed structural assessment of the tegument of this species by SEM is included. Also included are observations on the detailed morphological structure of *P. richiardii*, new host records and information on its prevalence and intensity in endemic catsharks on the west and south coasts of South Africa.

Table 6.1: The eight described species of *Probolitrema* (Trematoda: Gorgoderidae) from the body cavity of sharks and rays worldwide, detailing host species, location, morphometrics and reference. N/A: not available. †Described from a single specimen. *Described from specimens collected in this study.

Trematode species	Host species	Location	Reference	body length x width (mm)	Testis number	oral:acetabulum	Ovary	Vitellaria	Eggs (µm)
P. richiardii	*Squalus acanthias, Mustelus vulgaris, Myliobatis aquila*	Mediterranean	Lopez, 1888	N/A	35-36	1:3	Crescentic to lobed	Loose; long digitiform branches	N/A
P. capense	*Scyllium sp.*	Cape Town, South Africa	Looss, 1902	N/A	28-38	1:1	2-3 lobed	Loose; moderately long slender branches	50 x N/A
P. rotandatum	*Trygonorrhina fasciata*	Australia	Johnston, 1934	6.0 x 5.0	25-34	2:3	Slightly trilobed	Compact; few branches with short lobes	50-54 x 36-39
P. clelandi	*Mustelus antarcticus*	Australia	Johnston, 1934	10.5 x 7.7	31-37	1:1	Compact	Compact; short rounded lobes	42-74 x 37-57
P. antarcticum	*Mustelus antarcticus*	Australia	Woolcock, 1935	N/A	24-30	2:3	3-5 lobed	Loose; long digitiform branches	36-45 x 35-40
P. philippi†	*Heterodontus portusjacksoni*	Australia	Woolcock, 1935	N/A	24	1:2	Crescentic; indistinctly trilobed	Small "scattered follicles"	48-50 x 36-42
P. californiense	*Myliobatis californica, Platyrhinoides triseriatus, Rhinobatos productus*	California	Stunkard, 1935	7-13 x 5.7-10.2	16-26	1:2	2-5 lobed	Compact to moderately loose; long digitiform branches	43-60 x 40-45
P. mexicana	*Urobatus maculates, Dasyatis brevis*	Baja California, Mexico	Markell, 1956	13.7 x 7.5	17-39	1:1.3	3-5 lobed	Compact or loose; tubular	50-66 x 34-40
*P. capense**	*Haploblepharus pictus*	Cape Town, South Africa	This chapter	9.5 ± 1.39 x 6.4 ± 0.88	24-36	1:1.5	Round to oval	Clustered branched tubules	42- 54 x 29-31

6.2 Materials and methods

As in Chapter 2 of this thesis, with the following additional methods specific to this chapter.

Trematodes were relaxed using warm water, then fixed in 70 % ethanol between two microscope slides with slight pressure. Specimens were stained using the standard Mayer Carmalum protocol for staining helminths, and mounted whole. Alternatively, specimens were stained using Van Cleave’s haematoxylin, carmine and counter-stained with fast green, and dehydrated through several concentrations of ethanol, for one hour in each concentration. Mounting was done using DPX as mounting medium. They were then photographed, drawn with a *camera lucida*, measured, and described. Thirty specimens were measured.

Measurements were made of total body length along the longitudinal axis of the body, total body width, length and width of the oral and acetabulums, and positions of the suckers relative to the body of the animal: anterior to oral sucker, anterior to acetabulum, oral sucker to posterior, acetabulum to posterior; and position of the suckers relative to each other. Measurements were also made of pharynx length and width; oesophagus length and width; testicular follicle length and width; vas deferens length; ovary length and width; seminal receptacle length and width; vitelline glands length and width; and egg length and width. Shape, position and relative proportion morphometrics were noted for all relevant organs and body structures. Measurements are presented as minimum – maximum (arithmetic mean ± standard deviation), and given in millimetres (mm) unless otherwise indicated.

Scanning electron microscopy (SEM) for the trematode specimens was done at the University of Johannesburg (SPECTRAU), as in Chapter 2, but additional SEM was performed at the Electron Microscope Unit at the University of Limpopo (Medunsa campus). For this SEM, specimens were fixed overnight in 2.5 % glutaraldehyde, washed in Millonig's phosphate buffer (pH = 7.2) and dehydrated through a graded ethanol series for 30 seconds to one minute in each concentration. Thereafter, they were critical point dried (Polaron, U.K.) overnight, mounted on stubs, sputter-coated with gold (Emscope, U.K.) and examined using a Leica Stereoscan 420 scanning electron microscope (Leica Electron Optics, U.K.) at 10 – 20 kV.

6.3 Results

The identity, numbers and lengths of sharks captured, and the trematode prevalence in each species, are recorded in Table 6.2. A total of 145 sharks, of three species, *Haploblepharus pictus*, *H. edwardsii* and *Poroderma africanum*, were examined and 15 *H. pictus* from the west (Atlantic) coast of South Africa were found to be infected with trematodes in the body cavity (Figure 6.1), with infections ranging from 1–22 per shark and a mean infection rate of seven per shark.

Table 6.2: Identity, number and length of sharks captured from the west and south coasts of South Africa; and infection levels and prevalence of trematode parasites (*Probolitrema* sp.) in these sharks.

N, number; ML, mean length; SD, standard deviation

Scyliorhinidae (hosts)			**Trematodes (parasites)**	
Species	N	ML ± SD (range) in mm	Infection levels	Prevalence (%)
Haploblepharus pictus	78	411.6 ± 89.5 (256-672)	7 per shark	15/90 (16.7%)
Haploblepharus edwardsii	53	547.9 ± 105.9 (309-662)	0	0
Poroderma africanum	14	881.2 ± 106.4 (712-1068)	0	0

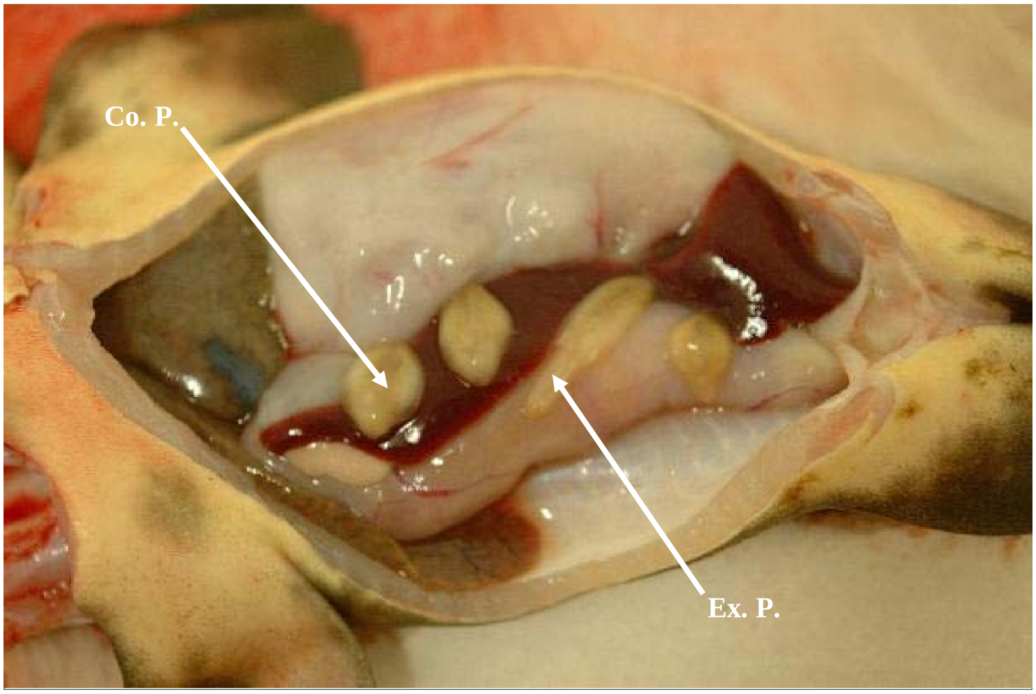

Figure 6.1. Trematodes *Probolitrema richiardii* (Lopez, 1888) Looss, 1902; *in situ* free in the dissected body cavity of the dark shyshark, *Haploblepharus pictus*, from the west coast of South Africa. Note normal, compact posture (Co.P.) and the extended posture (Ex. P.) indicating the method of locomotion.

Systematics

Class TREMATODA Rudolphi, 1808

Subclass DIGENEA Carus, 1863

Family GORGODERIDAE Looss, 1901

Genus *Probolitrema* Ofenheim, 1900

Probolitrema richiardii (Lopez, 1888) Looss, 1902

(Figure 6.2)

Synonyms *Probolitrema capense* Looss, 1902; *P. rotundatum* Johnston, 1934; *P. clelandi* Johnston, 1934; *P. antarcticum* Woolcock, 1935; *P. philippi* Woolcock, 1935; *P. californiense* Stunkard, 1935; *P. mexicana* Markell, 1956.

6.3.1 Description

This is the first description of this species based on a representative sample (30 specimens) rather than the usual low number (often just a single specimen). Due to the large size differences found within the collected and stained material, measurements will be presented as three separate groups. The main group of measurements is used in Table 6.1.

Measurements of the main group constitute 24 worms with more or less the same body form and size. The worms measure 6.4 – 12.3 (9.5 ± 1.39) in length and 4.6 – 7.8 (6.4 ± 0.88) in width (Figure 6.2). The slightly oval-shaped oral sucker (Figure 6.3A) is positioned at the terminal end of the body and measures 0.6 – 1.1 (0.8 ± 0.13) long and 0.8 – 1.9 (1.2 ± 0.24) wide (Figure 6.2). The edge of the sucker is rough (Figure 6.3B and C) and displays numerous papilla-like (micro-sucker) structures (Figure 6.3C). The pre-pharynx is absent in stained specimens. The muscular pharynx is large and posterior to the oral sucker (although sometimes overlaying the posterior end of the oral sucker), measuring 0.3 – 0.8 (0.5 ± 0.12) x 0.5 – 1.1 (0.7 ± 0.13). It is followed by a short oesophagus, measuring 0.04 – 0.4 (0.1 ± 0.07) x 1.12 – 0.28 (0.20 ± 0.03), leading to two long intestinal caeca that extend to near the posterior end of the body (Figure 6.2). Bifurcation of these caeca takes place in the region midway between the oral sucker and the acetabulum. The caeca measure 5.9 – 11.1 (8.3 ± 1.4) long and 0.1 – 1.1 (0.7 ± 0.2) wide. The caeca wind slightly and display many small diverticulate sacculations, particularly towards the terminal ends of the caeca (Figure 6.2). The acetabulum (Figure 6.3E) is situated 1.1 – 2.8 (1.9 ± 0.46) from the posterior end of the oral sucker. It is larger than the oral sucker, round, and measures 0.9 – 1.7

(1.2 ± 0.18) in diameter (Figure 6.2). The edge of the acetabulum is rougher, compared to the oral sucker (Figure 6.3B), displaying tegumental ridges (Figure 6.3F). The oral sucker to acetabulum ratio is approximately 1:1.5. The body tegument is spineless, displaying an uneven surface (Figure 6.3D) that is able to extend and retract considerably.

The testes are situated alongside the body, outside of the intestinal caeca in the posterior half of the body. The testes are grape-like in appearance and display 27 – 36 (32) oval to lobed testicular follicles on the left and 24 – 33 (28) on the right side of the body, measuring 0.2 – 0.5 (0.4 ± 0.07) x 0.15 – 0.4 (0.3 ± 0.05) (Figure 6.2). The vasa efferentia lead laterally from the testes to posterior of the acetabulum to unite into a narrow vas deferens, leading to the seminal vesicle, measuring 0.1 – 1.1 (0.7 ± 0.2) x 0.2 – 0.6 (0.3 ± 0.11). The seminal vesicle includes a narrow ejaculatory duct and a wider pars prostatica. The cirrus is not visible. The genital pore opens medially and close to the ventral side of the bifurcation of the intestinal caeca.

A large seminal receptacle is situated posterior to the acetabulum, sometimes overlapping the posterior part of the acetabulum (Figure 6.2). It measures 0.5 – 1.3 (0.9 ± 0.20) x 0.5 – 1.7 (1.0 ± 0.25). Medially and posterior to the seminal receptacle is a small round to oval ovary, measuring 0.4 – 0.8 (0.7 ± 0.13) x 0.4 – 1.0 (0.7 ± 0.13) (Figure 6.2). Mehlis's organ is situated medially between the ovary and the seminal receptacle. The uterus loops posteriorly and is filled with many thin-shelled eggs, measuring 42 – 54 µm (49 µm ± 4.8) x 29 – 31 µm (30 µm ± 0.7) (Figure 6.2). The uterus occupies the entire inter-caecal space posterior of the ovary before winding anteriorly until it opens at the female genital pore, just posterior to the intestinal bifurcation. The vitellaria form two cluster groups of branched tubules, extra-caecally and anterior to the testicular follicles (Figure 6.2).

Receptors on the body are limited and most receptors are without flagella. Most receptors are concentrated on the oral sucker (Figures 6.3B, C and G), and a few on the edge of the acetabulum (Figure 6.3F). Similar receptors are also present on the tegument (Figures 6.3C and H), particularly a few large ones anteriorly around the acetabulum (Figure 6.3E), and even towards the posterior end of the trematode (Figure 6.3I). The excretory pore opens terminally (Figure 6.3I) and is visible at the posterior end of the body as an invagination, leading into the excretory vesicle between the caeca and posterior loops of the uterus (Figure 6.2).

The smallest worm measures only 4.5 mm in length and 3.4 mm in width. Although this specimen was very small and originally thought to be an immature worm, the body was filled with eggs. The oral sucker measures 0.6 x 0.7 mm, followed by a small pharynx, 0.3 x 0.4 mm and a very short oesophagus, 0.1 x 0.1 mm, leading to the intestinal caeca that stretched until the posterior end of the worm, measuring 3.4 mm long x 0.2 mm wide. The acetabulum measures 0.9 x 0.8 mm and is situated 1.1 mm posterior to the oral sucker and 1.8 mm from the posterior end of the body. Just posterior to the acetabulum lies a large seminal receptacle measuring 0.5 mm in diameter, followed by a small ovary measuring 0.4 mm in diameter. The vitellaria measure only 0.08 mm in diameter, followed by the testes measuring 0.1 mm in diameter. The seminal vesicle measures 0.22 mm in length and 0.12 mm wide.

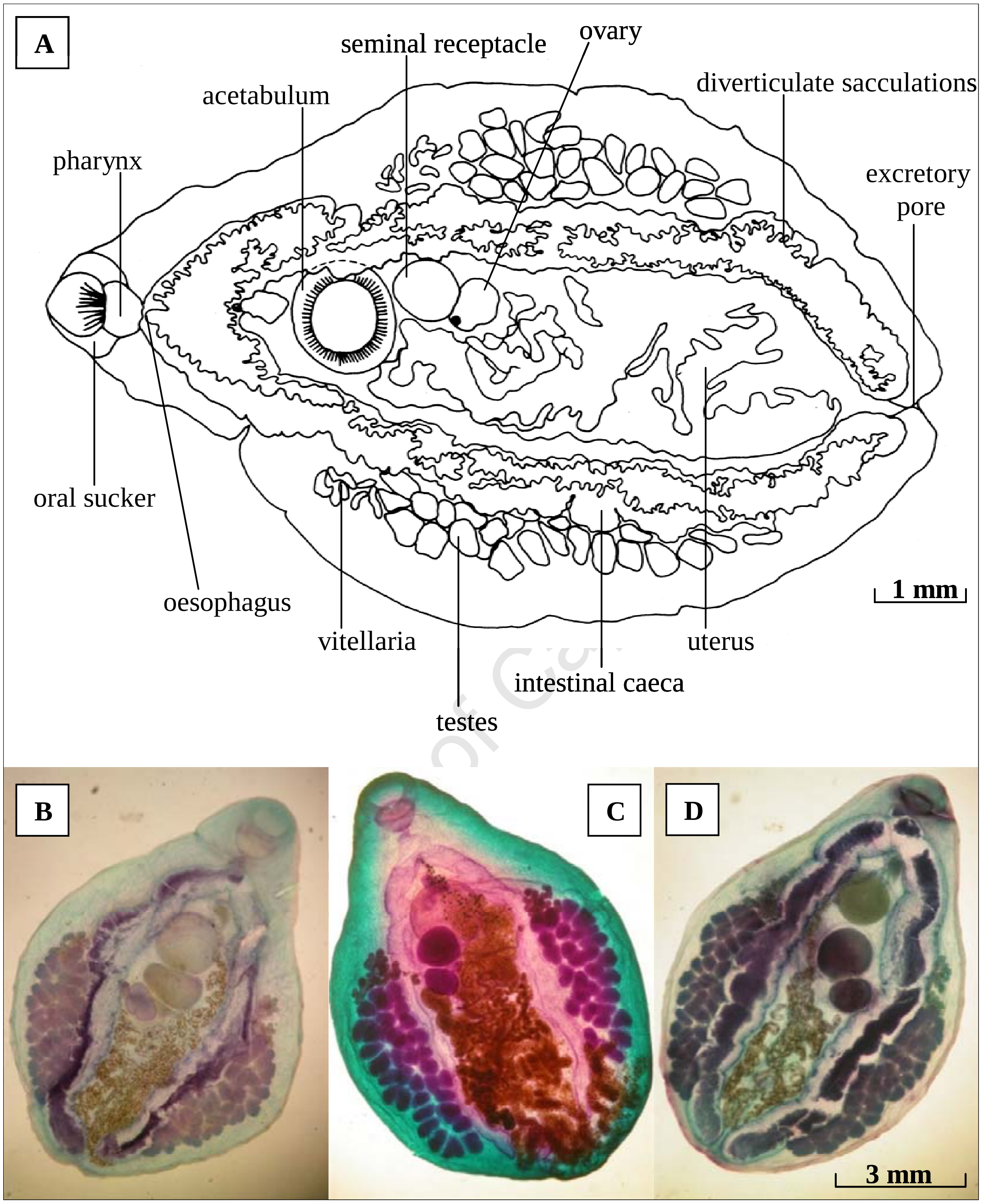

Figure 6.2. The digenean trematode *Probolitrema richiardii* (Lopez, 1888) Looss, 1902 infecting the dark shyshark *Haploblepharus pictus* from the west coast of South Africa. **A.** Drawing of whole mount. **B. C.** and **D.** Whole mounts stained with Van Cleave's haematoxylin.

Five specimens were found to be much larger, compared to all of the other worms sampled. Their body length measures 15.7 – 21.6 (18.0 ± 2.3) long and 9.8 – 11.4 (10.9 ± 0.67) wide. The oral sucker is 1.1 – 1.2 (1.15 ± 0.05) long and 1.5 – 2.1 (1.9 ± 0.22) broad. It is followed by a broad pharynx, measuring 0.6 – 0.8 (0.7 ± 0.08) x 0.8 – 1.1 (1.0 ± 0.12). The short oesophagus, 0.1 – 0.2 (0.17 ± 0.04) x 0.2 – 0.3 (0.28 ± 0.04), leads to two long and broad intestinal caeca measuring 13.3 – 18.8 (16.2 ± 2.16) x 1.1 – 2.2 (1.6 ± 0.41). The acetabulum is 3.3 – 4.7 (3.9 ± 0.59) posterior of the oral sucker and measures 1.6 – 2.1 (1.9 ± 0.19) x 1.7- 2.4 (2.1 ± 0.26). The distance between the acetabulum and the posterior end of the body measures 9.5 – 13.5 (11.1 ± 1.59). A large seminal receptacle, 1.3 – 3.3 (1.9 ± 0.77) x 1.1 – 3.2 (1.7 ± 0.89) lies posterior to the acetabulum, and anterior to the smaller ovary measuring 0.6 – 1.1 (0.8 ± 0.17) x 0.9 – 1.4 (1.1 ± 0.19). The small testes measure 0.6 – 0.7 (0.67 ± 0.07) x 0.3 – 0.4 (0.39 ± 0.05) and the smaller vitellarian follicles measure 0.15 – 0.18 (0.17 ± 0.01) in diameter. The seminal vesicle measures 0.8 – 1.4 (1.2 ± 0.24) in length and 0.4 – 0.8 (0.6 ± 0.13) wide.

6.3.2.Remarks

Figure 6.2A shows a detailed drawing of the dorsal view, and Figure 6.2B–D three stained whole mounts. Notably, the trematodes were found free in the body cavity, as depicted by Figure 6.1, and not internal to any organs, or the digestive tract. Also notable in Figure 6.1 is the extended posture of the trematode, indicating the method of locomotion.

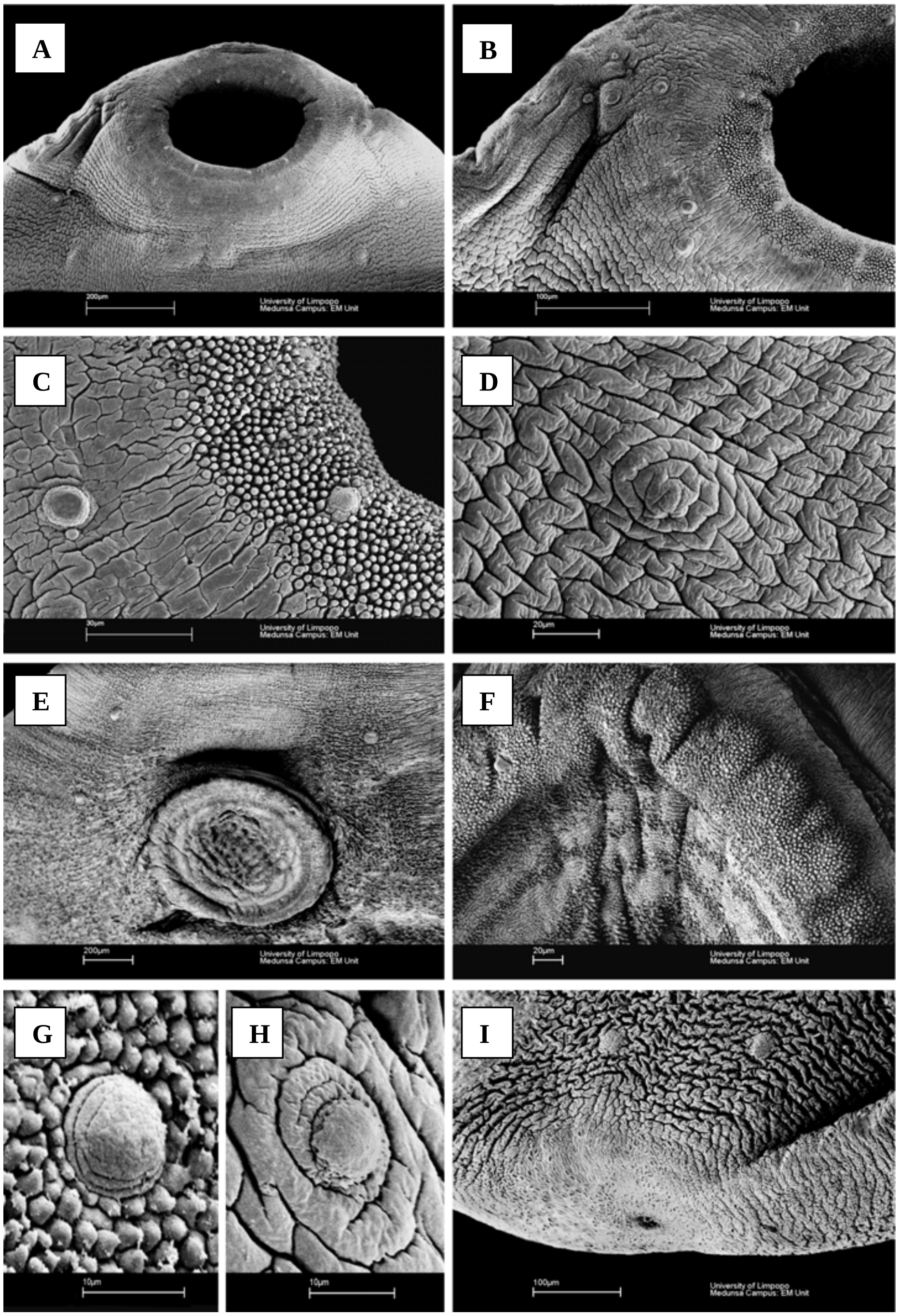

Figure 6.3. Scanning electron micrographs of the digenean trematode *Probolitrema richiardii* (Lopez, 1888) Looss, 1902 infecting the dark shyshark *Haploblepharus pictus* from the west coast of South Africa. **A.** Oral sucker. **B**. Enlargement of oral sucker. **C**. Edge of oral sucker and anterior tegument, showing non-ciliated receptors. **D**. Tegument. **E**. Acetabulum. **F**. Enlargement of acetabulum. **G**. Non-ciliated receptor on edge of oral sucker. **H**. Non-ciliated receptor on body tegument. **I**. Posterior end of body showing excretory opening and non-ciliated receptors.

Tegument

The tegument is rough and ridged, with folds and crevices in its surface (Figure 6.3D). These are irregular and orientated to enable movement in all directions. This doubtless accommodates the extreme degree of stretching involved in the locomotion of this trematode. It does not display any spines or papillae on the general body surface. The tegument of the oral sucker, in contrast, is covered in micro-papillae, although also not ciliated or bearing spines (Figure 6.3C). The acetabulum also displays micro-papillae, although the surface is rougher, and still bearing ridges (unlike the oral sucker) (Figure 6.3F). Non-ciliated receptors on the body surface are also ridged, once more presumably to accommodate stretch (Figure 6.3H), whereas those found on the oral sucker are not (Figure 6.3G).

6.4 Discussion

The platyhelminths found in the body cavity of several of the sharks examined belong the class Trematoda Rudolphi, 1808. They are members of the subclass Digenea Carus, 1863, of which there are more than 2500 nominal genera. The vast majority of Digenea use molluscs as their primary hosts and vertebrates as their secondary hosts (Gibson *et al.*, 2003). Within the Digenea, these trematodes belong to the order Opecoelatea Brooks, Baudoni, Macdonald & O’Grady, 1989, which are parasites of fish, amphibians and turtles, with cercaria in prosobranchs or bivalves and metacercaria in aquatic arthropods or fish. The specimens belong to the Superfamily Gorgoderoidea Looss, 1901, which parasitise vertebrates, with their cercaria in bivalves as first intermediate host, and to the family Gorgoderidae Looss, 1901, and genus *Probolitrema* Looss, 1902 (Gibson *et al.*, 2003). Taking into account the feeding habits of *Haploblepharus pictus*, which is often an opportunistic scavenger that feeds on benthic macroinvertebrates (Lechanteur & Griffiths, 2002), transmission of a *Probolitrema* species seems credible and in fact quite likely.

Anaporrhutine species are known to be parasites of elasmobranch fishes (Curran *et al.,* 2003), to be very large and conspicuous, and to be parasitic in the body cavity, rather than associated with a particular organ or tract. Anaporrhutine gorgoderids are thought to have a long history of association with chondrichthyans (Cribb *et al.,* 2001). These specimens are also typical of anaporrhutine morphological structure (Gibson, Parasitic Helminths Division, Natural History Museum, London, pers. comm.).

The digenean genus *Distomum* underwent a thorough revision by Looss (1902), leading to the establishment of the family Gorgoderidae, and within this several subfamilies were set up (Markell, 1953a). The family Gorgoderidae includes two subfamilies, the Gorgoderinae (without the presence of either a pharynx or a seminal receptacle, but with Laurer’s canal) and the Anaporrhutinae (showing a pharynx and seminal vesicle, but lacking Laurer’s canal). In 1900, Ofenheim placed *Distomum albidum* Brandes (from the coelom of the ray *Aetobatis narinari*) and *D. richiardii* Lopez (from the coelom of the shark *Squalus acanthias*) into a new genus, *Anaporrhutum*, after identifying *A. richiardii* from a shark, *Scyllium* sp., in the Atlantic Ocean off Cape Town, South Africa (Stunkard, 1935). These two species, with the addition of *Plesiochorus cymbiforme* (previously *Phyllodistomum*) were placed in the new subfamily Anaporrhutinae. Looss (1902) also broke down the genus

Anaporrhutum into *Anaporrhutum* (type species *A. albidum*) and *Probolitrema* (type species *P. richiardii*) with two main diagnostic characteristics. *Anaporrhutum* was distinguished by: the presence of a cross-connecting tubule, known as an anastomose (which was absent in *Probolitrema*), uniting the testes from the vasa efferentia and running below the ovary; and the position of the testicular follicles – traversing the caeca in *Anaporrhutum* and entirely extracaecal in *Probolitrema* (Markell, 1953a). During this process differences were noted by Looss (1902) and *Distomum richiardii* of Lopez was moved from *Anaporrhutum* and put into *Probolitrema*, whereas Ofenheim's identification of *A. richiardii* from *Scyllium* was erroneous, and designated as a new species named *Probolitrema capense*.

Of the six genera originally designated within the Anaporrhutinae, only five were considered valid by Curran *et al.* (2003): *Anaporrhutum*, *Probolitrema*, *Nagmia*, *Petalodistomum* and *Staphylorchis*. Major generic-level diagnostic characteristics (see Table 6.3) of *Probolitrema* (illustrated by Markell, 1953b) compared to the other four genera include: Position of the testicular follicles, which are exclusively extracaecal, traversing neither the lateral and/or medial sides of the caeca, in all genera except *Anaporrhutum* where they traverse the caeca; smooth-sided, non-sinous or undulating caeca (unlike *Staphylorchis*) with no major diverticulations in caecal margins (unlike *Nagmia* and *Petalodistomum*); opposing fields of vitelline follicles entirely extracaecal (unlike all four other genera); and a Y (or I) shaped excretory vesicle caused by the only most distal ends of the lateral collecting arms being observable, directed in an upward angle from the midline of the body (unlike *Anaporrhutum* and *Staphylorchis* which have H-shaped vesicles) (Curran *et al.*, 2003) (Figure 6.4, redrawn from Markell, 1953b).The presence of an anastomose joining the testes below the ovary was one of the original diagnostic characteristics of *Anaporrhutum*, however, although the presence of an anastomose in *A. albidum* is obvious from the drawings of Ofenheim and was used by Looss (1902) as part of his rationale for the formation of *Probolitrema* and the rearrangement of the existing *Distomum* and *Anaporrhutum* species, it has ceased to be used as a genus-level diagnosis and, in fact, is frequently not mentioned in more recent literature. Campbell (2008) has further reduced the number of recognized anaporrhutine genera to three: *Anaporrhutum*, *Probolitrema*, and *Petalodistomum*, with *Nagmia* and *Staphylorchis* reclassified as subgenera.

Table 6.3: The three genera and two subgenera (as classified by Campbell, 2008) belonging to the Gorgoderid sub-family Anaporrhutinae (parasitic in the body cavity of elasmobranchs), showing the main diagnostic morphological features of each genus.

Genus	**Diagnostic morphological characteristic**					
	Anastomose joining testes below ovary	Excretory vesicle shape	Testicular follicle position	Vitelline follicle position	Diverticulate caeca	Non-sinuous/non-undulating smooth-sided caeca
Anaporrhutum	Yes	H	Traversing caeca	Not extracaecal	No	Yes
Probolitrema	No	Y (I)	Extracaecal	Entirely extracaecal	No	Yes
Nagmia (Syn. *Petalodistomum*)	No	Y (I)	Extracaecal	Not extracaecal	Yes	Yes
Petalodistomum	No	Y (I)	Extracaecal	Not extracaecal	yes	Yes
Staphylorchis (Syn. *Anaporrhutum*)	no	H	extracaecal	Not extracaecal	No	Sinuous/ undulating

Notable in many species of anaporrhutine trematodes, and all previously described species of *Probolitrema*, is the fact that the trematodes are not permanently attached in the body cavity, and lack any permanent association with organs or viscera. They do not attach themselves firmly, but rather move around freely (Figure 6.1), and there is no evidence of irritation, no spots on the viscera or body wall or any other indications of their presence in the host body, as noted by other studies on *Probolitrema* (Markell, 1953b; Stunkard, 1935). Although life cycle information is not available for many (if any) species of anaporrhutine Gorgoderidae, and in fact little information is available in this area on any digenean species (Cribb *et al.*, 2001), enough is known of the typical life-cycle of monogeneans and digeneans to speculate on how adult life in the peritoneal cavity of their host elasmobranchs is possible, and the implications for entry and exit (Caira & Healy, 2004). According to Gibson & Bray (1977) one such appropriate portal is the abdominal pore which opens directly into the cloaca. This, however, needs further investigation and completing life-cycle studies of some of these trematodes should be a priority area of future study.

Gibson (1976) reviewed the gorgoderid sub-family Anaporrhutinae, in which the genus *Probolitrema* came under particular scrutiny. In his opinion, the incomplete nature of the earlier species descriptions of new *Probolitrema* species, and the small number of individuals examined by the majority of authors, often from a very limited (frequently only one or two) number of host elasmobranchs, results in there being only one valid species of *Probolitrema* so far described. He therefore synonymized all species of *Probolitrema* with *P. richiardii* (Lopez, 1888) Looss, 1902. The geographic distribution of the various *Probolitrema* species, however, and the implications therein implied for transmission, pose a problem with this synonymy.

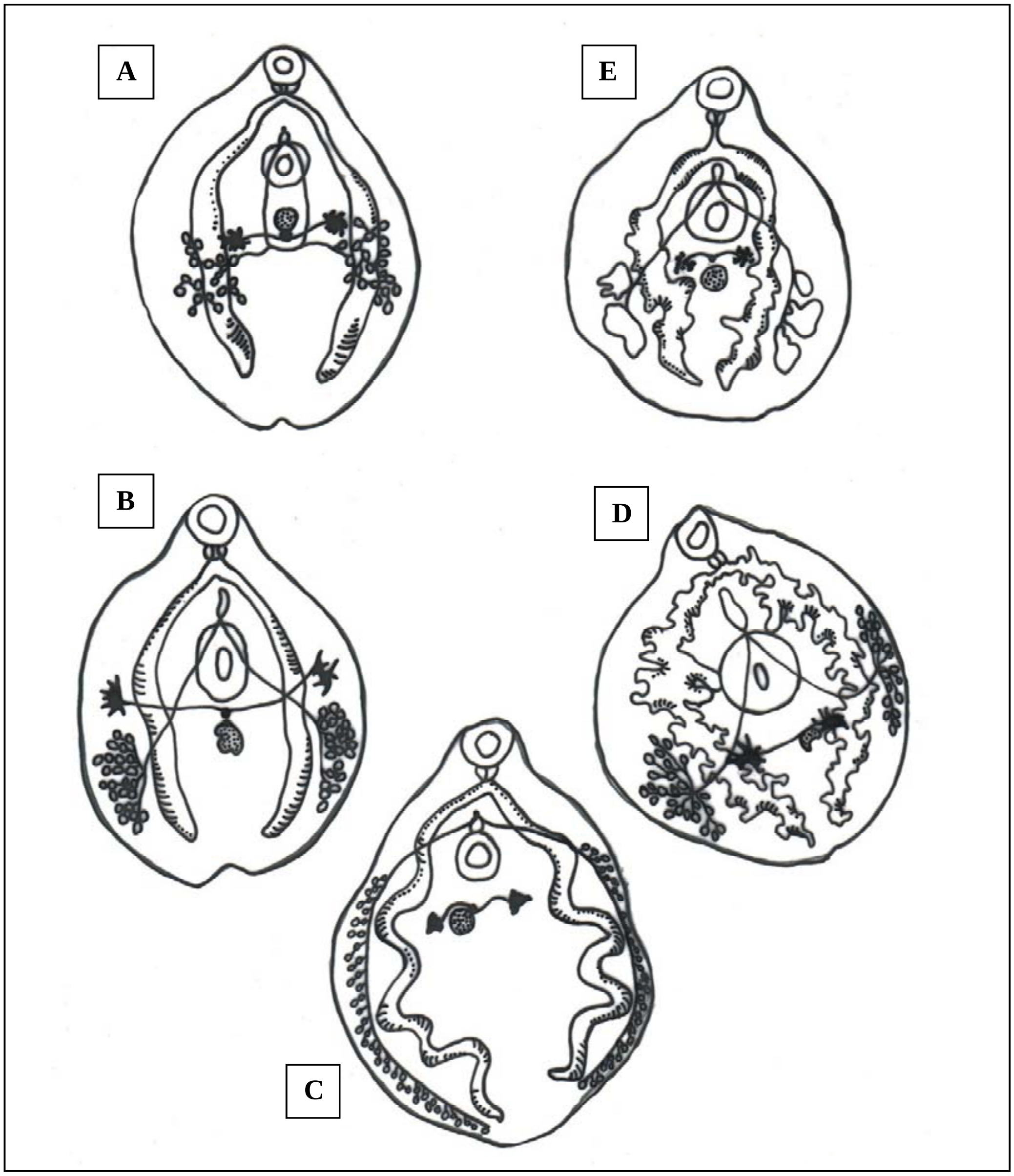

Figure 6.4. Anatomical characteristics (described in text) of valid genera and subgenera of the Digenean Subfamily Anaporrhuntinae, family Gorgoderidae: found in the body cavity of elasmobranchs (redrawn from Markell, 1953). **A.** Genus *Anaporrhutum*. **B.** Genus *Probolitrema*. **C.** Subgenus *Staphylorchis* (of *Anaporrhutum*). **D.** Subgenus *Nagmia* (of *Petalodistomum*). **E.** Genus *Petalodistomum*.

The life history and ecology of the host catsharks in South Africa is a good example of this problem. *Haploblepharus pictus*, found to host *P. capense*, is an endemic, highly resident shark, displaying very little range movement, and is found exclusively in shallow coastal rocky seas, usually associated with kelp forests. In addition, although sharks were sampled extensively throughout their range, only a limited number (15/90 or 16.7%) were infected. Furthermore, only sharks sampled from Granger Bay and Langebaan (which together encompass Gibson's 1926 Houtjes Bay [Hoedjiesbaai?] and possibly Ofenheim's "Atlantic coast off Cape Town" samples) were found to be infected. Given that the range of these sharks extends both north and south of these sites, and that sharks were sampled from these areas, but lacked specimens of *Probolitrema*, it seems very likely that *Probolitrema* is limited in its distribution, either by availability of intermediate host, or by some other physical or biological barrier. Thus the worldwide distribution of one species of internal trematode, infecting a large range of diverse elasmobranchs (Table 6.1), seems unlikely in the extreme, especially when the distribution of suitable intermediate hosts and environmental conditions also needs to be taken into account. The above, plus the extreme rarity of a true monotypic genus, leads us to recommend that the majority of the species described (Table 6.1) remain as valid, until such time as genetic or molecular studies, or more thorough descriptions, prove otherwise. And thus, the species under study in this thesis shall hereafter be referred to *Probolitrema capense* Looss, 1902.

This paper reports on the first documented occurrence of *P. capense* Looss, 1902 since its original description by Ofenheim (1900), and gives prevalence and infection intensity details, with a new confirmed host record in *H. pictus* in the southwestern cape of South Africa. Measurements and observations from 30 individual specimens are presented. Further characteristics and morphometrics of the species are elucidated, with a particular focus on the tegument. In addition, a thorough comparison of all known species of the genus *Probolitrema* is undertaken. The first scanning electron micrographs are made available for this species (and genus).

CHAPTER 7

THE PARASITE ASSEMBLAGES OF SOUTH AFRICAN CATSHARKS: THEIR ECTO-, ENDO- AND BLOOD PROTOZOAN PARASITES AND SOME ECOLOGICAL APPLICATIONS

7.1 The parasite assemblages of South African catsharks

This section of the chapter provides an overview of the parasite assemblages infecting three species of catshark sampled at each of the four sample sites (Saldanha Bay, Granger Bay, False Bay and De Hoop). The parasites found were identified as far as possible, with reasons given for identifications detailed in Chapters 3 – 6. Infection sites, prevalence and infestation rates of the parasites are detailed for each of the three host shark species, giving an overview of the parasitic fauna on the three host catshark species, incorporating the standard indicative parasitic indices.

7.1.1 Catsharks sampled

The identity and number of catsharks captured at the different sampling locations are shown in Table 7.1. Along the West Coast only one species, *Haploblepharus pictus*, was captured at Saldanha Bay, while two species from two genera, *H. pictus* and *Poroderma africanum*, were captured at Granger Bay. Three species belonging to two genera *H. pictus*; *H. edwardsii* and *P. africanum*, were captured at sites along the Western shore of False Bay (Simonstown, Miller's Point and Buffels Bay). At the most southern sampling locations, Koppie Alleen and Lekkerwater in De Hoop Nature Reserve, two species from two genera, *H. edwardsii* and *P. africanum*, were captured. All sampling took place over the period February 2004 – November 2006.

Table 7.1. Species and number of catshark (Scyliorhinidae) collected from four sites on the West and South coasts of South Africa. Dashes indicate where collection site is outside of species distribution and/or no samples were collected.

Scyliorhinid species	Common name(s)	Saldanha Bay	Granger Bay	False Bay	De Hoop Nature Reserve
Haploblepharus edwardsii	Puffadder shyshark; Happy Eddie	–	–	12	41
Haploblepharus pictus	Dark shyshark; skaam-oog	44	18	29	–
Poroderma africanum	Pyjama shark	–	3	2	9

7.1.2 Ectoparasites

Three types of haematophagous ectoparasites were observed on collected sharks. These included leeches, pandarid copepods (both males and females, as well as juvenile potential lfemales), and juvenile gnathiid isopods. The specimens observed and collected are reported below.

Leeches

Leeches were found on all three shark species examined, and at all four collection sites on the West and South coasts. They were located on the skin surface, with a slight preference shown for the anal and pelvic fins, but were also found from different sites on the skin. The total number of individuals collected was 18, with a maximum infestation of two found on several *H. edwardsii* from De Hoop Nature Reserve.

The leeches were tentatively identified as juvenile *Stibarobdella macrothela* (Schmarda, 1861). This is a cosmopolitan leech on oceanic sharks and has a relatively large caudal sucker and two large brown ocular spots on the oral sucker (Prof. E. Burresson, VIMS, USA, pers. comm.). Should this identification be confirmed, all three host species will be new host records and the entire collection area will represent a new distribution record for this species of leech.

Scanning electron microscopy was done on several of the leech specimens in order to elucidate cuticle detail and fine scale morphological structure, but this forms part of a further study and is not applicable to this thesis. Leeches were also examined both as squash preparations, and by histology, as potential vectors for the haemogregarine and trypanosome blood parasites.

Copepods

Adult female, adult male and juvenile (possibly) female copepods, *Perissopus (Achtheinus) oblongus* (Copepoda: Pandaridae), were found on catsharks sampled from Granger Bay on the West coast and False Bay on the South coast. Although the taxonomic and systematic placement of *P. oblongus* is not entirely resolved, this species identification was readily made (Chapter 3). *H. edwardsii* and *H. pictus* are new host records for this species.

Perissopus oblongus was observed on the skin – both body surface and fins – of all three scyliorhinid species. Preference for attachment site seemed to be the dorsal surface of the body, just anterior to the midpoint of the dorsal fin. Males were never found alone, but always attached to the underside of adult females, which were themselves firmly attached to the shark. The maximum infestation of seven individuals was found on *H. pictus* from False Bay.

Of the 37 copepods collected, a number were cleared, stained, dissected, drawn and photographed for identification purposes and detailed descriptions. Adult female, adult male, and a female with male still attached *in situ* were examined by scanning electron microscopy (SEM). This allowed the first detailed morphological structure of this species to be observed through the use of SEM, including: appendage details, host attachment clarification and mechanism, and male/female attachment clarification and mechanism (Chapter 3).

Gnathiid isopods

Juvenile gnathiids isopods (Isopoda: Gnathiidae), zuphea and praniza larval stages, were found on *H. edwardsii* and *P. africanum* sampled from De Hoop Nature Reserve on the South coast throughout the study period, but were not present at any of the other sites. These juvenile gnathiids were readily identified as *Gnathia pantherina* Smit & Basson, 2002, from features of adult males after captive juveniles moulted. *P. africanum* is a new host record for *G. pantherina*. The total number of gnathiids collected was 2292, with a maximum infection of 272 from a puffadder shyshark *H. edwardsii* from De Hoop Nature Reserve (Chapter 3).

Praniza larvae from instar stages P3 and Z3 were found on the gills of both host species, with little (or no) site preference being shown for any particular gill, or for either side of the fish. Unfed, small, clear zuphea larvae were found on the skin surface of both shark species.

Larvae were examined through scanning electron microscopy, and infestation data collected. Although gnathiids isopods have been implicated in the transmission of blood parasites, in particular haemogregarines, this is not thought to be the case for these species, as the exceedingly limited distribution of the gnathiids, coupled with the extremely widespread, ubiquitous and heavy infections of blood parasites, makes their probability as the vector very low.

7.1.3 Metazoan endoparasites

Three species of endoparasites were observed on collected sharks – tapeworms (Cestoda), a digenean trematode and a physalopteroid nematode. The tapeworms were found in the spiral intestine, but in such small numbers and in so few hosts that they were preserved for future study and identification and not considered further in this thesis. The trematode and nematode were as follows:

Digenean trematodes

Specimens of the digenean gorgoderid trematode, *Probolitrema capense* , were found from specimens of *H. pictus* collected from Saldanha Bay and Granger Bay, both on the West coast of South Africa. Taxonomy and systematic placement of *P. capense* has undergone a number of changes, but species identification was relatively straightforward (Chapter 6). In light of the confusion surrounding first descriptions and host records for this species, *H. pictus* is designated as a new host record for *P. capense*.

When present, *P. capense* was observed in the body cavity of *H. pictus*. There was no preference for any particular site, and, typically for anaporrhutine trematodes, the worms were mobile and moving unattached around the coelomic cavity. Trematodes of a large range of sizes were found, often together in the same host. The maximum infection encountered (22), was found in the body cavity of an *H. pictus* from Granger Bay.

Of the 105 trematodes collected, a number were cleared, stained, dissected, drawn and photographed for identification purposes and used for detailed descriptions. Additional worms were examined by SEM, which allowed detailed morphological structure of the species to be observed for the first time, particularly focusing on the hitherto unexplored finer structure of the tegument. This was the most thorough examination, and by far the largest sample size, of these trematodes yet examined.

Nematodes

Adult male and female physalopteroid nematodes, *Proleptus obtusus*, were found in the stomachs (and occasionally the intestines) of 100 % of all species of host sharks from all four collection sites. *P. obtusus* has a number of easily identifiable characteristics which enabled identification, despite a paucity of research on this species.

P. obtusus is found either loose in the stomach or attached to the stomach wall, where removal of the worm leaves a visible scar. The greatest infection of 162 individual nematodes was found in a puffadder shyshark, *H. edwardsii,* from False Bay. Of the total of 5458 worms collected, a number were drawn and photographed for identification purposes and for detailed descriptions. Other worms were used for SEM, which allowed for confirmation of the species identification and for additional elucidation of fine morphological details (Chapter 3). *H. pictus* and *P. africanum* are new host records for *P. obtusus*.

7.1.4 Blood protozoans

All sharks collected were examined for blood protozoans – and 100 % of sharks at all four sites were infected with both one trypanosome and two haemogregarine species, all three of which are new to science.

Trypanosomes

A kinetoplastid trypanosome was found in the blood plasma of 100 % of sharks examined from all species and at all sampling sites. This trypanosome species was determined to be new to science and subsequently described as *Trypanosoma* sp. A (Chapter 4). *H. pictus*, *H. edwardsii* and *P. africanum* are therefore original host records (type host *H. pictus*) for *Trypanosoma* sp. A and the west and south coasts of South Africa are the initial geographical range (type locality Granger Bay).

Blood smears of one cell thick were fixed and stained with Giemsa, and subsequently examined under a compound microscope. Photomicrographs and measurements were taken from 55 individuals for calculation of standard morphological indices.

These are very large, deep-blue staining trypanosomes with a short free flagellum. The body is wide with a distinct undulating membrane. The kinetoplast is prominent, and is on average an quarter of the body length from the posterior. The nucleus is circular with a prominent karyosome and situated in the anterior half of the animal. There are longitudinal striations visible on larger specimens. Infection levels ranged from 11–20 per blood smear to an extremely heavy 50–72 per thin blood smear.

Haemogregarines

Two species of intraerythrocytic apicomplexan haemogregarine, differing in morphometrics and staining properties, were found in the blood of 100% of sharks examined and collected, from all four sampling sites and of all species. Developmental stages (trophozooites, meronts and gamonts) which conformed to the defining characteristics of both these species were noted in the blood. Both haemogregarines were determined to be new to science, and were subsequently described as *Haemogregarina* sp. A and *Haemogregarina* sp. B (Chapter 5). *H. pictus*, *H. edwardsii* and *P. africanum* are therefore original host records for *Haemogregarina* sp. A and *Haemogregarina* sp. B and the west and south coasts of South Africa are the initial geographical range.

Photomicrographs were obtained with a photomicroscopic digital camera system and intraerythrocytic haemogregarines were measured with the aid of intrinsic software (Zeiss Axioplan). Where possible, 30 individuals of each stage (trophozooite, meront, immature gamont and gamont) were measured from each species, resulting in the measurement of over 200 individuals. Transmission electron microscopy (TEM) was done on *Haemogregarina* sp. A and *H.* sp. B in order to elucidate cell ultrastructure, and enable accurate, detailed descriptions of the two new species. Infection levels for both species ranged from an already heavy average of 20–40 individuals per 1000 erythrocytes to an extremely heavy 90–100 per 1000 erythrocytes, particularly of *H.* sp. A.

7.1.5 Infection sites, prevalence and infestation rates

Tables 7.2 and 7.3 summarise the sites of infection and total number of parasite collected; and the prevalence and infestation rates of the above listed parasites on the catshark species *Haploblepharus pictus*, *H. edwardsii* and *Poroderma africanum*.

Table 7.2. Ecto-, endo- and blood protozoan parasites of three scyliorhinid catshark species, *Haploblepharus pictus*, *H. edwardsii* and *Poroderma africanum*, from the west and south coasts of South Africa, showing sites of infection and total number collected (n) (N/A: not applicable)

Category	Parasite Taxon	Species	Site of infection	n
Ectoparasites	Leech	*Stibarobdella macrothela* (?)	skin	18
	Copepod	*Perissopus (Achtheinus) oblongus*	skin	37
	Isopod	*Gnathia pantherinum*	Gills and skin	2292
Metazoan endoparasites	Trematode	*Probolitrema capense*	Body cavity	105
	Nematode	*Proleptus obtusus*	Stomach	5458
Blood protozoan parasites	Trypanosome	*Trypanosoma* sp. A	Blood plasma	N/A
	Haemogregarine	*Haemogregarina* sp. A	Intraerythrocytic and blood plasma	N/A
		Haemogregarina sp. B	Intraerythrocytic and blood plasma	N/A

Table 7.3. Ecto-, metazoan endo- and blood protozoan parasites of three scyliorhinid catshark species, *Haploblepharus pictus*, *H. edwardsii* and *Poroderma africanum*, from four sites on the west and south coasts of South Africa, showing prevalences (%) and infection/infestation rates where applicable (average per shark). Site 1: Saldanha Bay; Site 2: Granger Bay; Site 3: False Bay; Site 4: De Hoop Nature Reserve. Note that only sites within host sharks' range are included.

Parasite species		***Haploblepharus pictus***			***Haploblepharus edwardsii***		***Poroderma africanum***		
		Site 1	**Site 2**	**Site 3**	**Site 3**	**Site 4**	**Site 2**	**Site 3**	**Site 4**
***Stibarobdella macrothela* (?)**	**Prevalence (%)**	0	17.07	6.90	9.09	17.07	0	0	22.22
	Infestation rate (per shark)	0	0.22	0.07	0.09	0.22	0	0	0.22
Perissopus (Achtheinus) oblongus	**Prevalence (%)**	0	37.93	0	18.18	0	0	0	0
	Infestation rate (per shark)	0	1.14	0	0.36	0	0	0	0
Gnathia pantherina	**Prevalence (%)**	0	0	0	0	97.56	0	0	100
	Infestation rate (per shark)	0	0	0	0	43.22	0	0	78.33
Probolitrema capense	**Prevalence (%)**	13.64	50.00	0	0	0	0	0	0
	Infectation rate (per shark)	1.11	3.11	0	0	0	0	0	0
Proleptus obtusus	**Prevalence (%)**	100	100	100	100	100	100	100	100
	Infectation rate (per shark)	14.91	13.67	27.24	38.09	64.44	18.33	20.00	47.22
***Trypanosoma* sp. A**	**Prevalence (%)**	100	100	100	100	100	100	100	100
***Haemogregarina* sp. A**	**Prevalence (%)**	100	100	100	100	100	100	100	100
***Haemogregarina* sp. B**	**Prevalence (%)**	100	100	100	100	100	100	100	100

7.2 Ecology and community study applications of South African scyliorhinid parasite assemblages

Parasites represent a large proportion of global biodiversity. In the marine environment, the vast majority of parasite species have a planktonic first intermediate host and a benthic secondary intermediate hosts (Sanmartin *et al*., 2000). Marine fish are generally vagile and gregarious and many have generalized and/or opportunistic feeding habits, which can result in prey switching and dietary overlap (Gonzalez *et al*., 2006), with implications for parasite infestation and the transmission of parasite species. Parasites tend to be specific for elasmobranchs (with very few, if any, known exceptions), both because elasmobranchs have high concentrations of urea, which inhibits successful maturation of other parasite species, and because of the highly specialised adaptations needed for ectoparasites to attach to and feed through the denticles and placoid scales characteristics of this group. In fact, many species of elasmobranch parasites are specific to a single host species, particularly such taxa as cestodes (Sanmartin *et al*., 2000). Elasmobranchs tend to be either at, or towards, the top of the food chain, therefore typically would act as the definitive host, meaning that autogenous parasite species are fairly frequent (Sanmartin *et al*., 2000).

Studying community ecology of parasite fauna has many advantages. With free-living organisms, defining the borders of a community is often problematic. From an ecological viewpoint a community would include all organisms living in the same area. However, the boundaries of that area are frequently subject to the whims of the investigator, or to geographic factors, logistic limitations, and so on. This is sometimes solved by use of discrete units of habitats e.g. islands or ponds. However, in parasite community ecology the borders are relatively well-defined, for example at the host individual level, host population level or internally at organ level. This means that hosts can be considered individual patches of habitat for parasites, with the added advantage that history (depicted by phylogeny) can be taken into account (Morand *et al*., 1999). Few species are independent of other species, and in parasites this is definitional. Studies on parasite communities can sometimes expose much about the hosts, for example food resources, and presence/absence data can be used to identify different populations of one host species, or to extrapolate host dietary information etc.

One of the central themes in biogeography is longscale geographic patterns in species distributions and abundances (Gonzalez *et al*., 2006). Parasites are frequently used as indicators of ecological relationships between hosts, such as feeding and migration patterns (Chambers & Dick, 2005). There is abundant evidence that parasites can be used as biological indicators of the movement of fish populations, because variability in the parasites of marine fish hosts has been shown both across geographic areas, as well as among fish species. Parasite communities have been demonstrated to differ substantially among different fish hosts, even within the same geographic area. Parasitic fauna, particularly endoparasites, of any fish species with an extensive geographic distribution may vary geographically (both qualitatively and quantitatively) as a consequence of zoogeographical breaks that affect distribution of the parasites' intermediate hosts (Gonzalez *et al*., 2006). In addition, environmental variability can cause species richness to show differences along a latitudinal gradient. Studies relating host variables and parasite community composition have allowed identification of ecological stocks and additional clarification and/or support for the phylogenetic relationships of host species. These studies have also contributed to understanding biogeographical patterns of hosts, habitat use, food and feeding mechanisms, and integration with other members of the host community (Sepúlveda *et al*., 2006).

In order to study parasite community ecology it is necessary to define the various levels at which the community is structured. The infracommunity is the most frequently referred to when studying hosts at an individual level. This is defined by Bush & Holmes (1986) as all parasite infrapopulations in a single host individual and the most fundamental level of parasite community structure, or the level at which all data on parasite communities begin. The component community includes all infrapopulations of parasite associated with a host species (or more realistically with a subset of a host species) (Homes & Price, 1986). This definition has been expanded upon by Bush *et al*. (2001) to include all free-living stages of parasite associated with a subset of the abiotic environment. When the focus is on the identity of the hosts, the guild community can be used, which is the parasite community found in a guild of hosts. Parasite communities can also be viewed on spatio-temporal scales that go beyond a hierarchical nature, which means that issues of, amongst others, host gender or size can be addressed. Component community concepts seldom encompass studies of the same host species that occurs in different habitats according to the season, or that is stage-structured. However, it must be noted that these host individuals still represent infracommunities, and they are all still part of the supercommunity. Communities are further classified as either inter- or non-interactive, where interactive communities occur among residents of local habitat (Morand *et al*., 1999). Given the problems of scale, in both a geographic and a taxonomic sense, almost all community level studies are conducted on a subset of the infracommunity, and, with reference to the general methods for this thesis, for the purposes of this study when the term “community” is used it is defined as the subset of the infracommunity collected during dissection and blood collection.

Clearly, protozoan blood parasites are part of the parasite community. However, the scale of both parasite individual size and parasite infestation rate between protozoan blood parasites and other macroparasites in this study differ by orders of magnitude. Additionally there was a 100 % prevalence of all three species of protozoan blood parasite, making them a constant in the parasite community. Cestodes are also part of the parasite assemblage and it is acknowledged that they make up a critical part of the assemblage and are excellent indicator species. Furthemore it is admitted that their inclusion could potentially change the conclusions reached in this study. However the extremely small, insignificant number found, and the inability at this time to identify them (see Chapter 2) led to their exclusion in this study Therefore, only the macroparasite species (with the exception of the cestodes) found in this study were used in the following analyses and are hereafter referred to as the parasite assemblage.

In this study, three hypotheses were tested.

> H01: There is no relationship between either parasite load or assemblage and host catshark size.
>
> H02: There is no difference in parasite assemblage on one host species between its zone of exclusive occurrence and the overlap zone**.**

> H03: The host catshark species does not affect the species composition of the parasite assemblage.*****

*****Should H02 be rejected, the host catshark species will only be compared in the overlap zone, to avoid any potential confusion in the interpretation of the results.

7.2.1 Materials and methods

As in Chapter 2 of this thesis, with the following additional methods specific to this chapter.

The four sampling locations were chosen to encompass geographic zones where only one of the two congeneric (*Haploblepharus*) host shark species occurred, and also to include zones where the two species co-occurred. Only *H. pictus* is found west of Cape Point (designated "*H. pictus* exclusive zone"), both species occur in False Bay (designated "overlap zone") and only *H. edwardsii* occurs in De Hoop Nature Reserve (designated "*H. edwardsii* exclusive zone"). These designations were used throughout the following analyses.

Frequency distributions of host shark total length (TL) were plotted for both species from their exclusive and overlap zones. A 2-sample Kolmogorov-Smirnov test was used to test for significant differences in the size frequencies of: *H. pictus* in the exclusive versus ovelap zone, *H. edwardsii* in the exclusive versus the overlap zone, and *H. pictus* versus *H. edwardsii* in the overlap zone. Additionally, a subsample of similar sized sharks (350 – 510 mm TL) of both species from the overlap zone, were selected in order to control for any possible effects of size on interspecific and inter-regional comparisons. The size frequency distributions of *H. pictus* and *H. edwardsii* in the resulting subsample were then also tested using a 2-sample Kolmogorov-Smirnov test to confirm that they were not significantly different.

In order to test for the effect of host size on parasite load, the relationship between host shark length and parasite load (expressed as total number) was plotted. This was done separately for each host species and each occurrence zone, and each macroparasite species (infecting that host species in that zone). A linear trend line was fitted to the relationships and the correlation coefficient tested for significance. When testing the significance of a correlation coefficient, it is assumed that both variables are normally distributed (Zar, 1996). For some parasite species encountered in this study, parasite load was not normally distributed. Nevertheless, this method was still deemed useful in examining the relationship between parasite load and host size. This relationship was further examined using nonparametric multivariate analyses, which do not assume normality.

In order to test the interactive effect of host size on the whole assemblage of macroparasites, nonparametric multivariate analyses were performed. Host sharks were *a priori* assigned into 100 mm size classes as follows: size class 0 = 0 – 99 mm; size class 1 = 100 – 199 mm; 2 = 200 – 299 mm; 3 = 300 – 399 mm; etc. These tests take into account a subset of the entire assemblages, both presence/absence and abundance data. Each host shark species in each zone was tested.

Within each host shark species, the difference in parasite assemblage between the exclusive and overlap zones (assigned *a priori*) was tested for using nonparametric multivariate analyses.

Interspecific differences between *H. pictus* and *H. edwardsii* were tested. This was done in the overlap zone to control for any possible effects of geographic zone differences mentioned above. The aforementioned subsample of sharks sampled from this overlap region, of between 350 – 510 mm TL, was subsequently tested in the same way to ensure that size effects were not confounding the results.

All multivariate analyses were performed as follows. Similarities between host catsharks were calculated using the Bray-Curtis similarity index, were displayed on dendrograms (Cluster analysis) and two-dimensional ordination plots, using Multi-Dimensional Scaling (MDS). Statistical differences in parasite composition were tested for by Analysis of Similarities (ANOSIM); in the case of statistical significance being shown, the parasite species primarily responsible for similarities within and dissimilarities among the groups were identified using Simper analysis. All data were transformed prior to analysis: square root for MDS, Cluster and ANOSIM, and fourth root for Simper.

Results of statistical tests were considered significant at $p<0.05$. All univariate statistical analyses were performed using STATISTICA, version 7 (StatSoft, 2004). All multivariate analyses were performed using the Cluster, MDS, ANOSIM, and Simper modules of the PRIMER computer package, version 6.0 (Plymouth Marine Laboratory, Plymouth, U.K.).

7.2.2 Results

Length frequency distributions of *Haploblepharus pictus* and *H. edwardsii* from their exclusive and overlap occurrence zones are shown in Figure 7.1. Kolmogorov-Smirnov 2-sample tests on these distributions showed a significant difference between *H. pictus* exclusive and overlap zones, between *H. edwardsii* exclusive and overlap zones, and between *H. pictus* and *H. edwardsii* in the overlap zone (Table 7.4). When a sub–sample of sharks (of both species) from the overlap zone between 350 – 510 mm was selected, and the resulting length frequency distributions were tested, no significant difference was found (Table 7.4). This sub–sample allowed comparisons of the parasite assemblage between the shark species without any confounding effect of host size on the result.

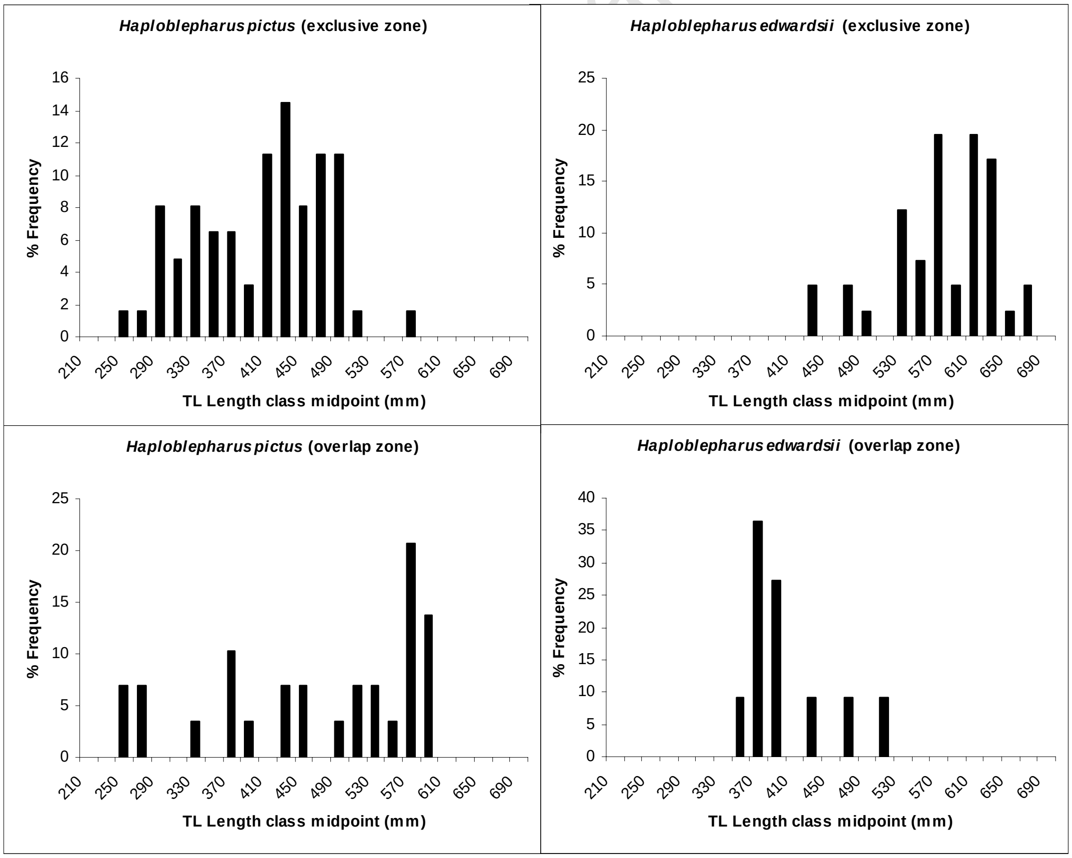

Figure 7.1. Total length (TL) frequency distributions of scyliorhinid catsharks *Haploblepharus pictus* and *H. edwardsii*, sampled west of Cape Point (*H. pictus* exclusive zone), in False Bay (overlap zone) and at De Hoop Nature Reserve (*H. edwardsii* exclusive zone)**.**

Table 7.4. Results of Kolmogorov-Smirnov 2-sample tests of length frequency distributions of scyliorhinid catsharks *Haploblepharus pictus* and *H. edwardsii*, sampled west of Cape Point (*H. pictus* exclusive zone), in False Bay (overlap zone) and at De Hoop Nature Reserve (*H. edwardsii* exclusive zone)**.** A subsample of 350-510 mm total length was also tested for both shark species in the overlap zone * indicates a statistically significant difference

Length frequency distributions tested between	Max Neg.	Max Pos.	p value
H. pictus exclusive vs. overlap	-0.487	0.122	$< 0.001^{*}$
H. edwardsii exclusive vs. overlap	0.000	0.878	$< 0.001^{*}$
H. pictus overlap vs. *H. edwardsii* overlap	-0.483	0.172	$< 0.05^{*}$
Subsample *H. pictus* overlap vs. *H. edwardsii* overlap	-0.364	0.000	> 0.1

Since the length frequency distributions of the sharks sampled from the separate areas were significantly different, the effect of host shark size on parasite load was tested individually, by each shark species from each zone, and each macroparasite species infecting those sharks. For *H. pictus* from the exclusive occurrence zone (Figure 7.2) the correlation coefficients of the relationships were not significant for any of the parasite species, indicating no relationship between parasite load and host shark size. For *H. pictus* from the overlap zone a similar result is shown (Figure 7.3), with the exception of the nematode parasite, *Proleptus obtusus*, which shows a significant increase in number with increasing shark size (r^2 = 0.3363, F (1,27) = 13.68, p<0.001). *H. edwardsii* from the overlap zone (Figure 7.4) and *H. edwardsii* from the exclusive zone (Figure 7.5) both have non-significant correlation coefficients, indicating no relationship between parasite load and host shark size.

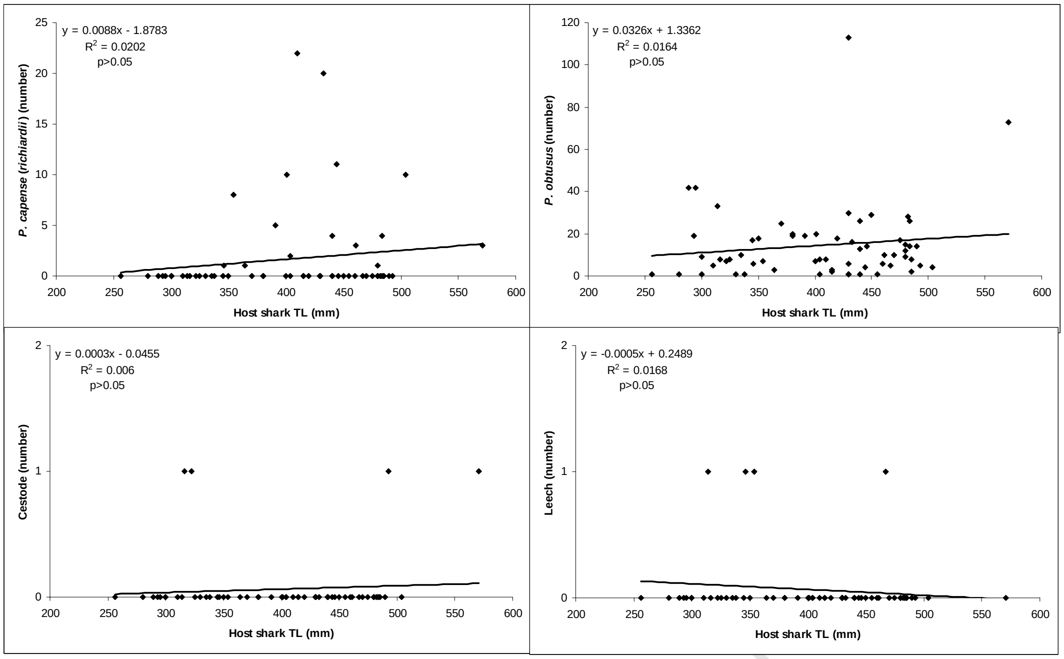

Figure 7.2. Relationship between parasite load and host shark total length (TL) of four species of macroparasite: *Probolitrema capense* (*richiardii*), *Proleptus obtusus*, a cestode (unidentified) and a leech (*Stibarobdella macrothela*?), infecting *Haploblepharus pictus* sampled from the zone of exclusive occurrence west of Cape Point. None of the correlations are statistically significant.

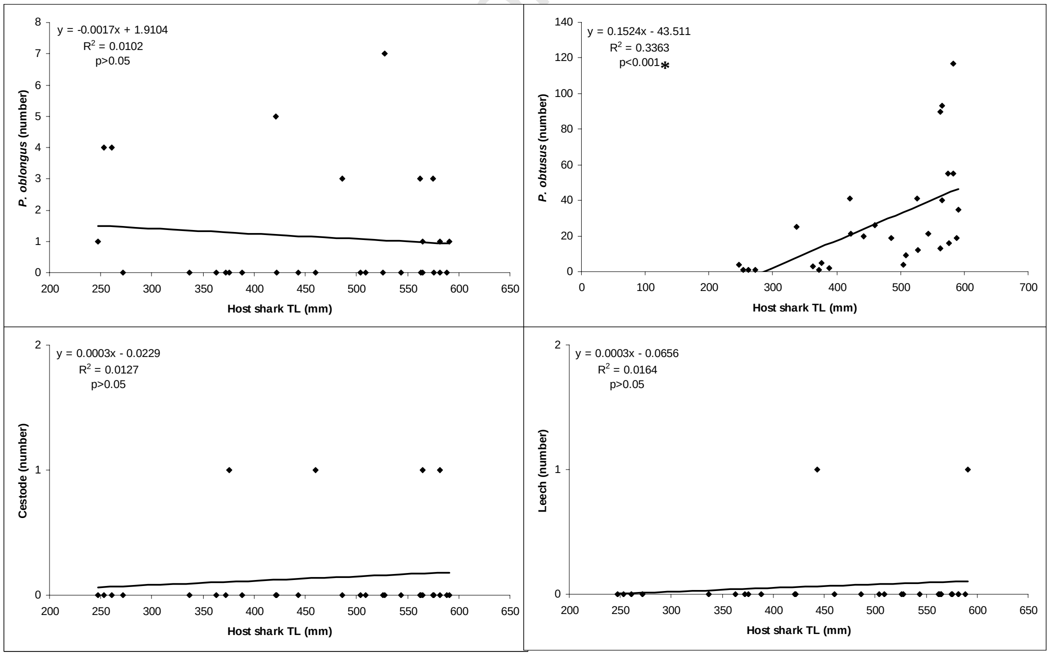

Figure 7.3. Relationship between parasite load and host shark total length (TL) of four species of macroparasite: *Perissopus oblongus*, *Proleptus obtusus* (statistically significant), a cestode (unidentified) and a leech (*Stibarobdella macrothela*?), infecting *Haploblepharus pictus* sampled from the zone of overlap occurrence (with *H. edwardsii*) in False Bay. Parasite load of *P. obtusus* shows a significant relationship with host shark size.

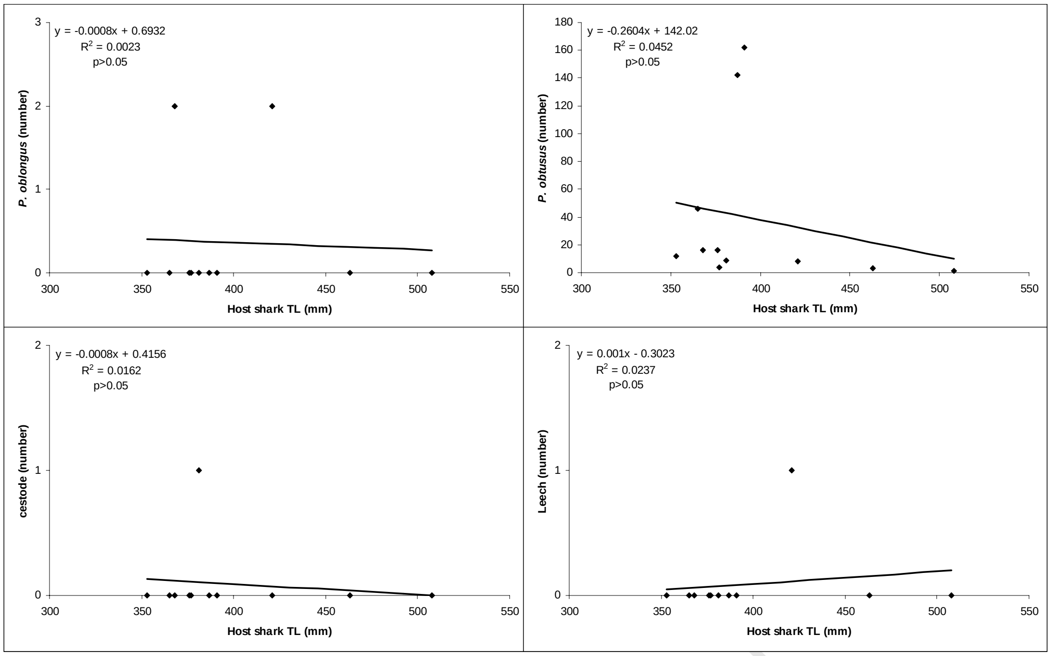

Figure 7.4. Relationship between parasite load and host shark total length (TL) of four species of macroparasite: *Perissopus oblongus*, *Proleptus obtusus*, a cestode (unidentified) and a leech (*Stibarobdella macrothela*?), infecting *Haploblepharus edwardsii* sampled from the zone of overlap occurrence (with *H. pictus*) in False Bay. None of the correlations are statistically significant.

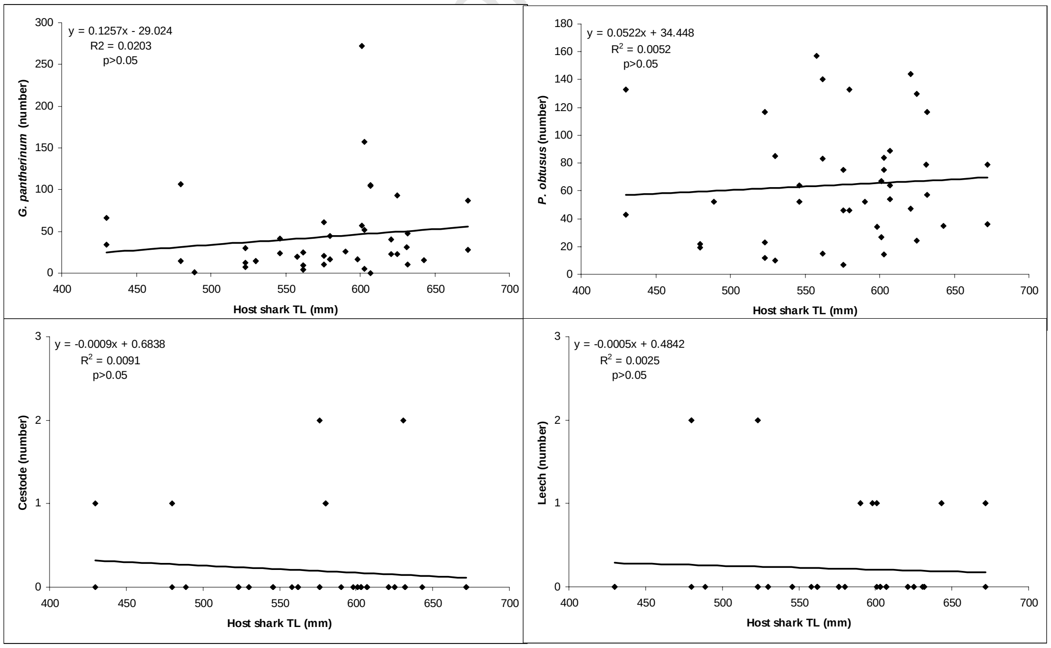

Figure 7.5. Relationship between parasite load and host shark total length (TL) of four species of macroparasite: *Gnathia pantherina*, *Proleptus obtusus*, a cestode (unidentified) and a leech (*Stibarobdella macrothela*?), infecting *Haploblepharus edwardsii* sampled from the zone of exclusive occurrence at De Hoop Nature Reserve. None of the correlations are statistically significant.

The relationship between host size and parasite assemblage was also tested using multivariate analyses. These tests do not assume normality, and also are useful in determining the interactive effects between different parasite species in the community, and host size. ANOSIM, MDS and Cluster results showed that there was no statistically significant relationship among size classes and parasite assemblages of *H. pictus* in the exclusive zone, or *H. edwardsii* in the overlap zone. A significant relationship was shown among the size classes and parasite assemblages of *H. edwardsii* in the exclusive occurrence zone (Global R 0.097, $p<0.05$). However, this test produced a relatively small R-value, and the MDS and Cluster analysis do not display any notable grouping, suggesting that this significant statistic does not reflect a biologically significant relationship (Figure 7.6). As in the univariate tests of parasite load and host shark size class, a significant relationship was shown among the size classes and parasite assemblages of *H. pictus* in the overlap zone (Global R 0.407, $p<0.01$) with a fairly robust R-value and p-value. The MDS plot shows a reasonable grouping pattern, and Cluster analysis grouping of smaller (size classes 2 & 3) and larger (size classes 3 & 4) sharks is clear (Figure 7.6).

SIMPER analyses identify the number of *Gnathia pantherina* and *Proleptus obtusus* as the principal parasite species causing the difference among host size classes for *H. edwardsii* in the exclusive zone. *Perissopus oblongus* and *Proleptus obtusus* are identified as the principal parasite species responsible for the differences among size classes for *H. pictus* in the overlap zone.

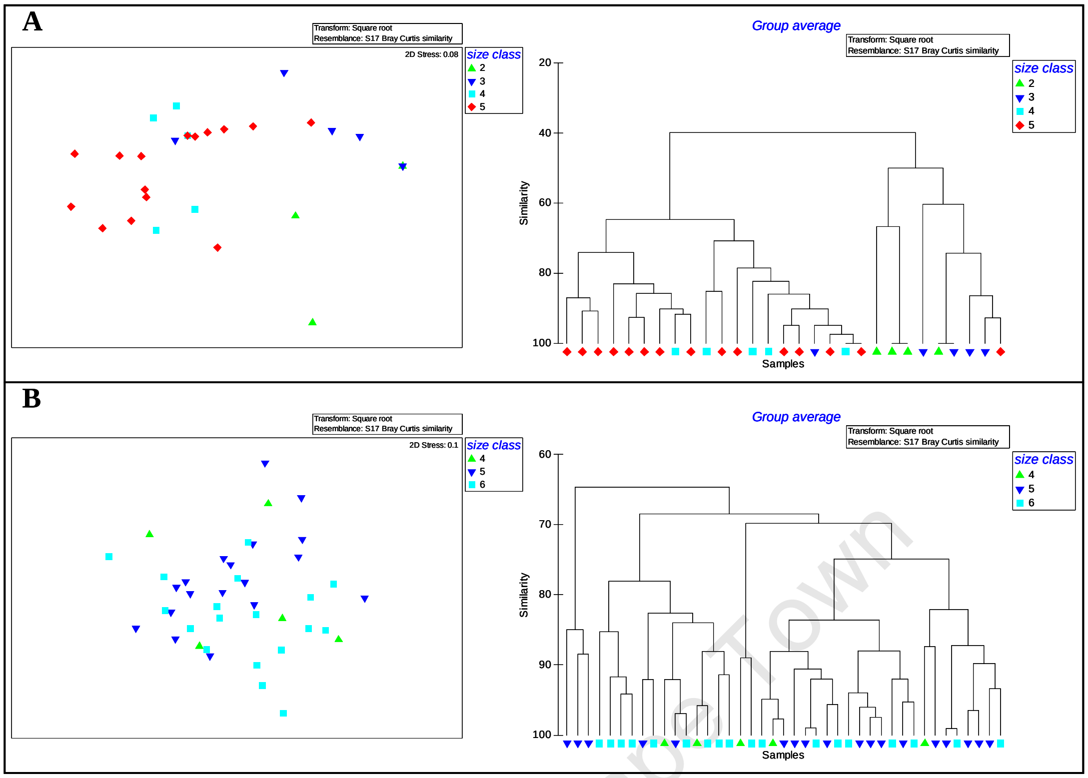

Figure 7.6. Multidimensional scaling plots and Cluster analysis of the relationship between macroparasite assemblage and host shark size. **A.** *Haploblepharus pictus* sampled from False Bay (the overlap occurrence zone). **B.** *Haploblepharus edwardsii* sampled from the De Hoop Nature Reserve (exclusive occurrence zone). Size classes assigned every 100 mm: size class 1 = 100-199 mm, size class 2 = 200-299 mm, etc.

Within each host species, the regional differences in parasite assemblage were tested for, i.e. between the exclusive and overlap occurrence zone. Despite the different length frequency distributions of each host shark species in the sampling zones the lack of relationship between host size and parasite load (in most cases) indicates that this comparison is valid. *Proleptus obtusus* infecting *H. pictus* in the overlap zone seems to have a significant relationship with host size, but this same parasite species infecting the same host does not show this pattern in the exclusive zone, and was therefore considered not to be a confounding factor in the following results.

Parasite assemblages of *H. pictus* sampled from the exclusive occurrence zone were significantly different to those sampled from the overlap zone (ANOSIM: Global R 0.1, p<0.002). SIMPER analysis identified the nematode *P. obtusus* as the leading cause of this difference; however if this parasite is disregarded (as it shows a relationship with host shark size, and size frequency distributions are not equal), SIMPER identifies the copepod *Perissopus oblongus* and the trematode *Probolitrema capense* as the leading cause of differences between the exclusive and overlap zone. Clear grouping can be seen in the MDS plot, supported by Cluster analysis (Figure 7.7).

Parasite assemblages of *H. edwardsii* sampled from the exclusive occurrence zone also showed a significant difference to those sampled from the overlap zone (ANOSIM Global R 0.813, p<0.001). The primary parasite species responsible for this difference was identified by SIMPER analysis as the gnathiid isopod *Gnathia pantherina*, with the nematode *P. obtusus* as a secondary causal species. This grouping is shown clearly in the MDS plot, and supported by Cluster analysis (Figure7.7).

Interspecific difference in parasite assemblages was tested between the two host species, *H. pictus* and *H. edwardsii*, sampled from the same (overlap) geographic zone of False Bay in order to avoid the geographic zones difference confounding the results. ANOSIM, MDS and Cluster results showed that there was no statistically significant relationship between parasite assemblages of *H. pictus* and *H. edwardsii* in the overlap zone. When a sub–sample consisting of all sharks between 350 – 510 mm (of both species) from the overlap zone was selected and tested, in order to compare similar size classes and distributions, still no significant difference was found.

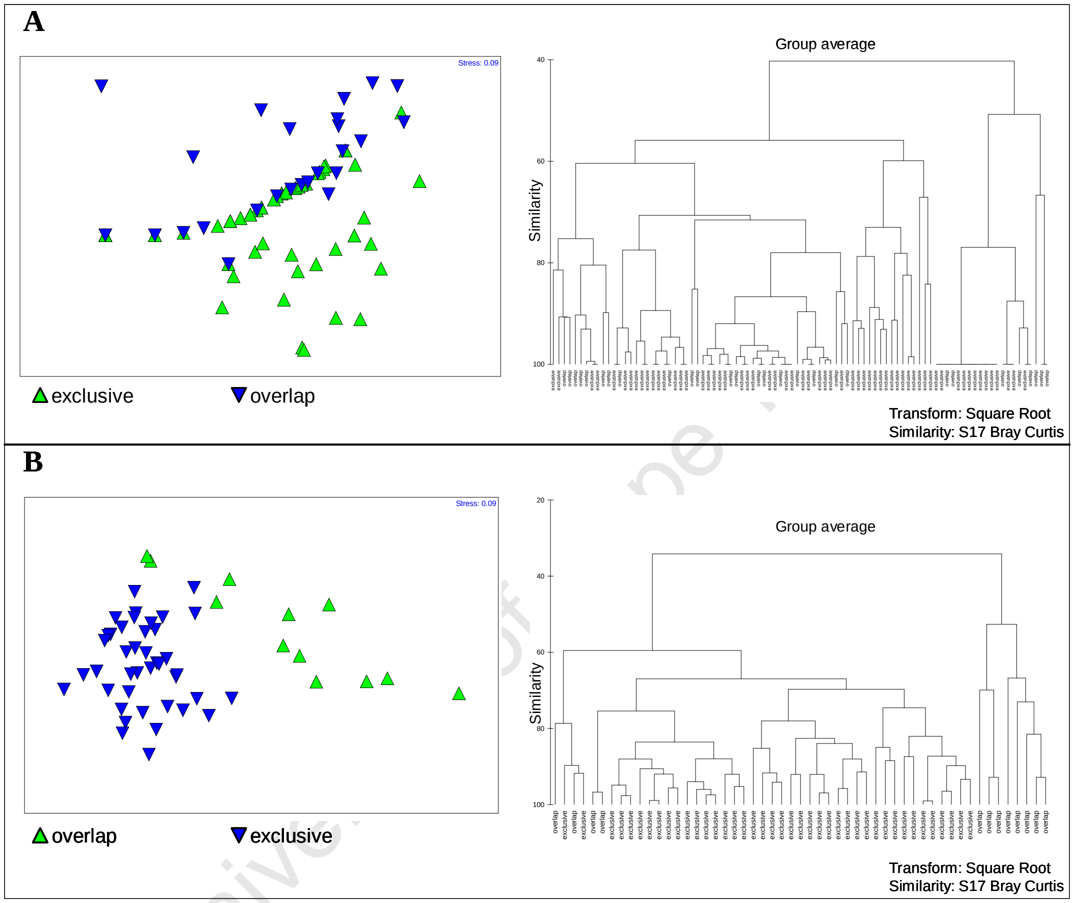

Figure 7.7. Multidimensional scaling plots and Cluster analysis of the relationship between macroparasite assemblage and host shark occurrence zone. **A.** *Haploblepharus pictus* sampled from the West Coast (exclusive occurrence zone) and False Bay (overlap occurrence zone). **B.** *Haploblepharus edwardsii* sampled from False Bay (overlap occurrence zone) and the De Hoop Nature Reserve (exclusive occurrence zone).

7.2.3 Discussion

The structure of parasite communities is influenced by a large number of variables. In order to understand the structure of the community the parasite species present need to be identified and their abundances determined; the inclusion of complementary parameters (such as parasite body size, biomass, species density, etc.) allows an even better description of the community structure (Munoz & Cribb, 2005). Variations in patterns of diversity and richness of parasite infracommunities have also been examined, by taking into account host-related factors such as diet, age, sex, body size and geographic location. Explanations of biological interactions and populations among parasite species have also been considered, such as a spatial concentration resulting from large numbers of individuals, large size and exclusive location (e.g. spiral valve) which leads to the possibility of interaction between species (Sepúlveda *et al*., 2006). Other host-associated factors like density, feeding habits and migratory patterns may affect the distributions of parasite species (Gonzalez *et al*., 2006).

Studies describing or quantifying parasite communities tend to fall into two broad categories: descriptive and mechanistic. Descriptive studies usually comprise comparisons between different populations of hosts, usually addressing some form of similarity (or dissimilarity) between different component or guild communities. Data used are usually presence/absence, or derived data from all sampled infracommunities on one population, compared to presence/absence or derived data from all sampled infracommunities in one or more different populations, or some similar setup. This allows questions such as those of baseline data setup, importance of host size, and other base level research questions to be addressed. In order for thorough descriptive studies to be done the basic data needed are a list of parasites present, a measure and variance of the species richness, and the abundance of each species present. Mechanistic studies, on the other hand, basically refer to how communities are organized in terms of the underlying mechanism, which is not applicable to the current study.

In this study, when individual parasite species were tested, parasite numbers did not generally correlate significantly with host shark size. The exception was the nematode *Proleptus obtusus* in *Haploblepharus pictus* from the overlap occurrence zone, which shows a significant increase in numbers with increasing shark size. When the whole parasite assemblage was tested using multivariate methods, results showed once again that parasite assemblages generally do not have a significant relationship with shark size. The exceptions were parasite assemblages on *H. pictus* from the overlap zone, and *H. edwardsii* from the exclusive occurrence zone, which showed statistically significant relationships between parasite numbers and shark size. In the case of the latter this is not thought to represent a biologically significant relationship, due to the weak ANOSIM test statistic and the lack of grouping evident from graphic representations of similarity analyses.

According to Poulin (1998) there is an absolute maximum number of species available to any parasite infra-, component or supercommunity. This means that we will always see some subset of a theoretical maximum. What determines the number of different parasite species (species richness) in communities? There are a number of potential answers to this question. Firstly, parasite characteristics can be determinants of species richness, e.g. competitive ability, population size and facilitation. Host traits can also determine species richness, for example an increase in host size and age leads to an increase in species richness, given that the hosts are larger targets, have more microhabitats than smaller hosts, eat more, and have had increased exposure time (therefore more infection opportunities). Quantitative and qualitative changes in parasitism are expected with fish growth (Polyanski, 1961) because the relationship between parasites and hosts are strongly influenced by the change in feeding habits of the fish, which in turn are correlated with age.

Correlation has been shown between parasite abundance at the infracommunity level and host size (Morand *et al*., 1999; Alves & Luque, 2001). Weak, but positive, correlations between parasite species richness and fish body size have also been found (Morand *et al*., 1999), and more specifically significant correlations have been shown between number of parasite species present and total fish length, as well as an increasing parasite load with increasing total fish length (Klimpel *et al*., 2003). Larger hosts have higher richness, abundance and biovolume of parasites – possibly as a result of a combination of resources, time and prey.

In general, parasite communities have been considered to be unsaturated by parasite species, and to be without an upper limit in the productivity of the parasites. If this holds true, then parasite communities may be restricted by host body size and parasite body size and the relationship between the energy requirements of both (Munoz & Cribb, 2005).

Given all of the above, the widespread lack of significant relationships between parasite load and community composition (either as individual species or as a subset of the entire assemblage) in this study is both unexpected and contrary to most findings in other studies. Further investigation of the relationship between host size and parasite load will reveal whether this is an artefact of study methodology (i.e. sampling locations, sample size, host shark size range, exclusion of certain taxa, etc.), or a genuine characteristic of South African catshark parasite ecology.

Both *H. pictus* and *H. edwardsii* show significant differences in parasite assemblages between their zones of overlap and their zones of exclusive occurrence. For *H. pictus*, the nematode *P. obtusus* was identified as a principle contributing parasite species. Since this species could potentially be misleading, given the observed correlation between host body size and parasite load, it is notable that when *P. obtusus* was excluded from the analyses then a significant difference was still found. The copepod *Perissopus oblongus* and the digenean *Probolitrema capense* were then primarily responsible for this difference. In the case of *H. edwardsii*, the parasites *Gnathia pantherina* and *Proleptus obtusus* were the principal species identified as the reason for the observed regional differences in parasite assemblage.

Comparative studies can be useful as a way to overcome potential problems, particularly in using guilds of parasites (such as nematode species richness) being compared between different populations of the same host species in different areas, or different host species in the same area. In some community studies, a correlation between prevalence and intensity of parasite infestations within a host species has been reported (Poulin & Rohde, 1997), and this is supported by the findings in the current study for both *H. pictus* and *H. edwardsii*.

Host size, diet composition and habitat use have all been considered useful variables for explaining the variation of parasite niches in both component communities and infracommunities, and the general hypotheses agreed on by most previous studies are that increased species richness for both component and infracommunity levels is attained in fish species that 1) relate to the most heterogeneous habitat (i.e. benthos) and/or 2) reach comparatively larger size (Sepúlveda *et al*., 2006). Both of these are relevant to the host species in this study, being both large fish (as elasmobranchs tend to be) and closely associated with the benthic environment. However, in contrast to many such previous studies the metazoan parasite community of the endemic South African catshark species in this study is relatively species poor.

When the interspecific differences between parasite assemblages of *H. pictus* and *H. edwardsii* from the same geographic area (the overlap zone) were tested, no significant difference was found. The same result was obtained even when a sub–sample of individuals of the same size range was tested (thereby removing any effect of host size). The results of this study, therefore, show that for these two endemic South African catsharks, the geographic zone of occurrence plays more of a role in determining the parasite assemblage composition than host shark species, despite the supposed high degree of host specificity displayed by marine fish parasites.

The most widely used descriptor of the kinds of parasites found in communities is that of host generalists versus host specialists. Frequently these are terms applied to single life history stages, since parasites can differ so monumentally between stages. An additional descriptor, once an appropriate host is reached, is site specialism (akin to resource partitioning). Site specificity has widely been found to transcend host specificity, which is thought to be beneficial in terms of increasing fitness, possibly an adaptive strategy. The diversity of parasites in a community is also dependent on the specific feeding behaviour of the hosts, the availability of both intermediate and final hosts, the depth distribution and the host migrations (Klimpel *et al*., 2003).

All parasitic organisms exhibit various degrees of specialization. There is unequivocal evidence for niche restriction in parasite communities, although the mechanisms behind this are still debated. The five most commonly agreed upon determinants of niche restriction are:

1) By phylogeny/descent, mediated by orientation to some morphological or physiological parameter

2) Mating, both to reinforce reproductive isolation and (according to Rohde, 1982) to enhance or facilitate sexual reproduction

3) Adaptation, this can be specific (which is often morphological e.g. the “lock and key”) and cued physiologically (although some would argue that this is a result, rather than a determinant, of niche restriction [Adamson & Caira, 1994])

4) Predation, such as from cleaning symbioses and

5) Competition, both exploitive from common resource utilization and interference from direct confrontation.

Many studies have concentrated on trying to determine if there is a relationship between species richness in a community and host specialism of the parasites making up the community. Poulin (1997) came up with the most widely-accepted answer to this, which was that a species-poor community is mostly made up of host generalists, whereas a species-rich community is made up of mostly host specialists. Given the relatively species-poor community found in these South African catsharks, and the finding that the geographic occurrence zone seems of more importance in influencing the parasite assemblage composition than host shark size and species, this would seem to hold true and be reinforced by the findings in this study.

CHAPTER 8

SYNTHESIS

The objectives of this thesis were to examine the entire parasite assemblage of three endemic catshark species, *Haploblepharus pictus*, *H. edwardsii* and *Poroderma africanum*, on the west and south coasts of South Africa, and to explore the ecological applications thereof. In order to meet these objectives the following key questions were asked (Chapter 1):

i. What parasites species are found on the three host catshark species?

ii. Of these parasite species, which are known from South African catsharks, which are known from other South African sharks, which are known internationally, and which are new to science?

iii. Within each host species, does the parasite assemblage differ over the area sampled?

iv. For areas where host shark species co-occur, does the parasite assemblage differ between host species?

v. Can the whole parasite assemblage of individual sharks be used to detect these differences?

These were answered in the following chapters: Chapter 3 dealt with those species of parasite already known from South African sharks. Chapter 4 described a trypanosome species, new to science, from the blood of South African catsharks. Two species of intraerythrocytic haemogregarine, new to science, were described from South African catsharks in Chapter 5. An already described but poorly known digenean trematode was fully redescribed and a taxonomic revision recommended in Chapter 6. Chapter 7 discussed the whole parasite assemblages of the host sharks, summarised the information presented in the preceding chapters, and investigated the use of the assemblage as a biological indicator.

8.1 Taxonomic Contributions

This thesis presents the first comprehensive description of the parasite assemblages of South African catsharks.

During this investigation, three species of parasite already known from South African sharks were encountered (Chapter 3). The physalopteroid nematode *Proleptus obtusus* was found in the stomachs of 100 % of sharks examined, from all sampling areas. Detailed prevalence and infestation rates were determined, parasite distribution records were amended, and *H*. *pictus* and *P*. *africanum* reported as new host records. Further scanning electron micrographs (SEM) were also made available for this *P*. *obtusus*.

Catsharks *H*. *pictus* and *H*. *edwardsii* were found to host pandarid copepods *Perissopus* (*Achtheinus*) *oblongus* on their skin and fins. Prevalence and infestation rates were determined, and new host records added. A brief, detailed redescription of both male and female copepods was provided, and the first SEM made available for this species. Some aspects of its functional morphology: attachment of the female to the host shark, and attachment of the male to the female, were also discussed and illustrated through the use of SEM.

The gnathiid isopod *Gnathia pantherina* was found on the gills of *H. edwardsii* and *P. africanum* from De Hoop Nature Reserve. *P. africanum* was a new host record for this isopod. Further SEM were made available for this species, and its potential as a vector for protozoan blood parasites infecting the host catsharks discussed. The pathogenic potential of high *G. pantherina* infestations was also considered, as this is the most comprehensive host sampling to be done in this area, allowing more accurate prevalence and infestation rates to be determined.

Three new species of parasitic protozoan were discovered in the blood of 100 % of catsharks sampled, from all collection sites (Chapter 4). All three species were new to science. *Trypanosoma* sp. A was found in the blood plasma of the sharks, and described as a new species based on its morphometrics, morphological features, geographical location and host endemicity. This was the first trypanosome to be described from Southern African elasmobranchs. *Haemogregarina* sp. A and *Haemogregarina* sp. B were found in the blood cells of all host sharks (Chapter 5). They were described as new species, and as separate species from each other, due to their staining properties, morphology, morphometrics, and cell ultrastructure through the use of transmission electron microscopy (TEM). These were the first haemogregarines to be described from South African elasmobranchs. A brief investigation of an ectoparasitic leech species (found on the skin of infected host sharks) as the potential vector of either of the haemogregarine species did not reveal any developmental stages of the haemogregarines.

A digenean trematode, *Probolitrema richiardii*, was found parasitizing the body cavity of *H. pictus* from the west coast of South Africa (Chapter 6). This is a new host record. This trematode, although already described, was very poorly known. A detailed redescription was presented, including morphometrics, the first SEM for this species, detailed morphology, information on its prevalence and infestation rate, and a structural assessment of the tegument. This is by far the most intensive sampling of this parasite to date, with sample numbers an order of magnitude greater than any prior study. Due to a number of factors discussed in Chapter 6, it was recommended that the prior name of this digenean, *Probolitrema capense*, be resurrected and that the species no longer be considered synonymous with *P. richiardii*.

8.2 Ecological Applications

Using the information from Chapters 3-6, a summary of the infection sites, prevalence and infestation rates of the parasites was detailed, for each of the three host shark species, and an overview of the parasitic fauna on the three host catshark species, incorporating the standard indicative parasitic indices, was presented (Chapter 7). These parasite assemblage data were then used to apply some ecological and community study techniques (Chapter 7) and the following hypotheses were tested:

H01: There is no relationship between either parasite load or assemblage and host catshark size.

H02: There is no difference in parasite assemblage on one host species between its zone of exclusive occurrence and the overlap zone**.**

H03: The host catshark species does not affect the species composition of the parasite assemblage.

The results indicate that, in general, neither parasite load nor assemblage correlated with host body size. There was a widespread lack of significant relationships between parasite load and community composition. *H. pictus* and *H. edwardsii* showed significant differences in parasite assemblages between their zones of overlap and their zones of exclusive occurrence. It was also found that for *H. pictus* and *H. edwardsii*, the geographic zone of occurrence plays more of a role in determining the parasite assemblage composition than the host shark species, despite the supposed high degree of host specificity displayed by marine fish parasites. This is a good example of ecological specificity taking precedence over phylogenetic specificity, although the two host species are congeneric. In conclusion, these South African catsharks show a relatively species-poor parasite community and geographic occurrence zone seems of more importance in influencing the parasite assemblage composition than host shark size and species.

8.3 Conclusions and Future Research

This thesis is the first examination of marine fish parasitology from the point of view of the hosts in South Africa. In other words, it is the first study where the entire parasite assemblage of specific host fish was examined, incorporating both macro- and micro-parasites. This has a number of unique advantages. Firstly, the catsharks as parasite hosts are thoroughly explored. Importantly for our knowledge of the marine biodiversity of South Africa's coastline, and particularly the under-studied marine invertebrates, several species new to science have been described, thorough redescriptions have also been supplied for those species that were very poorly known, and additional biological information supplied about known species. Secondly, the knowledge of the whole parasite assemblage allows it to be applied as an indicator of host shark ecology. This means that the parasites, whilst themselves intrinsically interesting and important ecologically, can also be used as a tool to further understand the biology and ecology of the host species. The techniques developed in the course of this study are applicable to future studies on the parasite assemblages of other elasmobranch species, including economically important shark species. The expanded knowledge of elasmobranch parasite taxonomy and biodiversity, and ecological applications developed during this thesis, will facilitate these types of future studies. Thirdly, the information gathered spans a whole range of different taxonomic groupings, up to and including different phylum levels, allowing the author to gain an understanding and competence in the taxonomy of a diverse range of invertebrate phyla, particularly those of significance in fish parasitology.

Opportunities for future research in this field are numerous, but the following are recommended as priorities:

1. Elucidating the detailed life cycles, including intermediate and definitive hosts, time intervals and moulting periods, for *Prolepus obtusus*, *Perissopus oblongus*, and *Probolitrema capense*.
2. Elucidating the detailed life cycles, including all host stages and vectors, for *Trypanosoma* sp. A, *Haemogregarina* sp. A and *H*. sp. B.
3. An investigation into the identity of the ectoparasitic leech species observed during this study. This was tentatively identified as juvenile *Stibarobdella macrothela*, but histology revealed the presence of developed gonads.

4. Thorough sampling to obtain more specimens of the very few cestodes observed in the spiral intestine of host sharks, and an investigation into the identity(ies) thereof.
5. Studies on the parasite assemblages of commercially important South African elasmobranchs (e.g. soupfin or vaalhaai *Galeorhinus galeus*; hound sharks, *Mustelus mustelus*; blue sharks, *Prionace glauca*; mako sharks *Isurus oxyrinchus*), and the application of techniques, using the whole parasite assemblage, developed in the course of this study.

REFERENCES

Abdel-Nasser, A.H., 2006. Light and transmission electron microscopic studies of a haemogregarine in naturally infected fan-footed gecko (*Ptyodactylus hasselquistii*). *Parasitology Research*, **98**, 468-471.

Adamson, M.L. & Caira, J.N., 1994. Evolutionary factors influencing the nature of parasite specificity. *Parasitology*, **109** (suppl.), S85–S95.

Alves, D.R. & Luque, J.L., 2001. Community ecology of the metazoan parasites of white croaker, *Micropogonias furnieri* (Osteichthyes: Sciaenidae), from the coastal zone of the state of Rio de Janeiro, Brazil. *Memórias do Instituto Oswaldo Cruz*, **96**, 145-153.

Aragort, W., Alvarez, M.F., Leiro, J.L. & Sanmartin, M.L., 2005. Blood protozoans in elasmobranchs of the family Rajidae from Galicia (NW Spain). *Diseases of Aquatic Organisms*, **65**, 63–68.

Bacigalupo, J. & De La Plaza, N., 1948. Presencia de tripanosomas en las rayas de Mar del Plata. *Revista de la Sociedad Argentina de Biologia*, **24**, 269-274.

Bancroft, J.D. & Cook, H.C., 1994. *Manual of Histological Techniques and Their Diagnostic Application*. Edinburgh; New York: Churchill-Livingstone.

Barber, D.L., Mills Westermann, J.E. & Storoz, P., 1987. *Haemogregarina nototheniae* n. sp., from the blood of Antarctic nototheniids. *Systematic Parasitology*, **10**, 135-147.

Barnard, K.H., 1955. South African Parasitic Copepoda. *Annals of the South African Museum*, **41**, 223-312.

Baylis, H.A., 1933. On the nematode genus *Proleptus*. *Annals and Magazine of Natural History*, Ser. 10, **12**, 325-335.

Becker, C.D. & Overstreet, R.M., 1979. Haematozoa of marine fishes from the northern Gulf of Mexico. *Journal of Fish Diseases*, **2**, 469-479.

Benz, G.W., 1981. Observations on the attachment scheme of the parasitic copepod *Pandarus satyrus* (Copepoda: Pandaridae). *Journal of Parasitology*, **67**, 966-967.

Benz, G.W., 1992. How *Pandarus* species (Copepoda: Pandaridae) attach to their shark hosts. *Journal of Parasitology*, **78**, 368-370.

Benz, G.W., Mollet, H.F., Ebert, D.A., Davis, C.R. & Van Sommeran, S.R., 2003. Five species of parasitic copepods (Siphonostomatoida) from the body surface of a white shark captured in Morro Bay, California. *Pacific Science*, **57**, 39-43.

Bozzola, J.J. & Russell, L.D., 1999. *Electron Microscopy: Principles and Techniques for Biologists*. Boston, Toronto, London, Singapore: Jones and Bartlett Publishers.

Branch, G.M., Griffiths, C.L., Branch, M.L. & Beckley, L.E., 1995. *Two Oceans: a Guide to the Marine Life of Southern Africa*. David Philip Publishers, Claremont, South Africa.

Bray, R.A., 1984. Some helminth parasites of marine fishes and cephalopods of South Africa: Aspidogastrea and the digenean families Bucephalidae, Haplosplanchnidae, Mesometridae and Fellodistomidae. *Journal of Natural History*, **18**, 271-292.

Bray, R.A., 1986. Some helminth parasites of marine fishes of South Africa: families Enenteridae, Opistholebetidae and Pleorchiidae (Digenea). *Journal of Natural History*, **20**, 471-488.

Bray, R.A., 1987. Some helminth parasites of marine fishes of South Africa: family Opecoelidae (Digenea). *Journal of Natural History*, **21**, 1049-1075.

Bullard, S.A. & Dippenaar, S.M., 2003. *Branchotenthes robinoverstreeti* n.gen. and n. sp. (Monogenea: Hexabothriidae) from the gill filaments of the Bowmouth Guitarfish, *Rhina ancylostoma* (Rhynchobatidae), in the Indian Ocean. *Journal of Parasitology*, **89**, 595-601.

Bullard, S.A., Dippenaar, S.M., Hoffmayer, E.R. & Benz, G., 2004. New locality records for *Dermophthirius carcharhini* (Monogenea: Microbothriidae) and *Dermophthirius maccullumi* and a list of hosts and localities for species of *Dermophthirius*. *Comparative Parasitology*, **71**, 78-80.

Burreson, E.M., 1989. Haematozoa of fishes from Heron I., Australia, with the description of two new Species of *Trypanosoma*. *Australian Journal of Zoology*, **37**, 15-23.

Bush, A.O. & Holmes, J.C., 1986. Intestinal parasites of lesser scaup ducks: Patterns of association. *Canadian Journal of Zoology*, **64**, 132-141.

Bush, A.O., Fernández, J.C., Esch, G.W. & Seed, R.J., 2001. *Parasitism: the Diversity and Ecology of Animal Parasites*. Cambridge U.K.: Cambridge University Press.

Caira, J.N. & Healy, C.J., 2004. Elasmobranchs as hosts of metazoan parasites. In: *Biology of Sharks and their Relatives*. Carrier, J.C., Musick, J.A. & Heithaus, M.R. (eds.). Boca Raton, London, New York and Washington, D.C.: CRC Press LLC.

Campbell, R.A., 2008. Family Gorgoderidae Looss, 1899. In: *Keys to the Trematoda*. Gibson, D.I., Jones, A. & Bray, R.A. (eds.). London, U.K.: CAB International and The Natural History Museum.

Chambers, A.C. & Dick, T.A., 2005. Trophic structure of one deep-sea benthic fish community in the eastern Canadian Arctic: application of food, parasites and multivariate analysis. *Environmental Biology of Fishes*, **74**, 365-378.

Christison, K.W. & Amin, O.M., 2005. *Neoechinorhynchus* (*Neoechinorhynchus*) *dorsovaginatus* n. sp. (Acanthocephala: Neoechinorhynchidae) from the dusky kob *Argyrosomus japonicus* (Sciaenidae) on the southern coast of South Africa. *Systematic Parasitology*, **61**, 173-179.

Coetzee, M.L., Smit, N.J., Grutter, A.S. & Davies, A.J., 2008. A new gnathiid (Crustacea: Isopoda) parasitizing two species of requiem sharks from Lizard Island, Great Barrier Reef, Australia. *Journal of Parasitology*, **94**, 608-615.

Coetzee, M.L., Smit, N.J., Grutter, A.S. & Davies, A.J., 2009. *Gnathia trimaculata* n. sp. (Crustacea: Isopoda: Gnathiidae), an ectoparasite found parasitizing requiem sharks from off Lizard Island, Great Barrier Reef, Australia. *Systematic Parasitology*, **72**, 97-112.

Compagno, L.J.V., 1987. Kinds of Sharks. In: *Sharks*. Stevens, J.D. (Ed.). New York: Facts on File Publications.

Compagno, L.J.V., 1999. Systematics and Body Form. In: *Sharks, Skates and Rays: the Biology of Elasmobranch Fishes*. Hamlett, W.C. (Ed.). Baltimore: Johns Hopkins University Press.

Compagno, L.J.V., Ebert, D.A. & Smale, M.J., 1989. *Guide to the Sharks and Rays of Southern Africa*. Cape Town: Struik Publishers.

Cook, C.A., 2008. Biodiversity and systematics of apicomplexan parasites infecting South African leopard and hinged tortoises. MSc dissertation, University of Johannesburg.

Cressey, R., 1967. Revision of the family Pandaridae (Copepoda: Caligoida). *Proceedings of the United States National Museum*, **121**, 3-133.

Cribb, T.H., Bray, R.A. & Littlewood, D.T.J., 2001. The nature and evolution of the association among digeneans, molluscs and fishes. *International Journal for Parasitology*, **31**, 997-1011.

Curran, S.S., Blend, C.K. & Overstreet, R.M., 2003. *Anaporrhutum euzeti* sp. N. (Gorgoderidae: Anaporrhutinae) from rays in the Gulf of California, Mexico. In *Taxonomie, Écologie et Évolution des Métazoaires Parasites*. Tome, I., Combes, C. & Jourdane, J. (eds). Perpignan, France: PUP.

Dainty, A.M., 2002. Biology and ecology of four catshark species in the southwestern Cape, South Africa. MSc dissertation, University of Cape Town.

Davies, A.J., 1995. The biology of fish haemogregarines. *Advances in Parasitology*, **36**, 117-203.

Davies, A.J. & Johnston, M.R.L., 2000. The biology of some intraerythrocytic parasites of fishes, amphibians and reptiles. *Advances in Parasitology*, **45**, 1-107.

Davies, A.J. & Smit, N.J., 2001. The life cycle of *Haemogregarina bigemina* (Adeleina: Haemogregarinidae) in South African hosts. *Folia Parasitologica*, **48**, 169-177.

Davies, A.J., Smit, N.J., Hayes, P.M., Seddon, A.M. & Wertheim, D., 2004. H*aemogregarina bigemina* (Protozoa: Apicomplexa: Adeleorina) – past, present and future. *Folia Parasitologica*, **51**, 99-108.

Diniz, J.A., Silva, E.O., de Souza, W. & Lainson, R., 2002. Some observations on the fine structure of trophozoites of the haemogregarine *Cyrilia lignieresi* (Adeleina: Haemogregarinidae) in erythrocytes of the fish *Synbranchus marmoratus* (Synbranchidae). *Parasitology Research*, **88**, 593-597.

Dippenaar, S.M. & Olivier, P.A.S., 1999. New morphological information of the parasitic copepod *Kroyeria dispar* Wilson, 1935 (Copepoda: Kroyeriidae) from the east coast of South Africa. *South African Journal of Zoology*, **34** (3), 125-129.

Dippenaar, S.M., Olivier, P.A.S. & Benz, G.W., 2001. *Kroyeria sphyrnae* Rangnekar, 1957 (Copepoda, Siphonostomatoida, Kroyeriidae): First description of the male, supplementary remarks on the female, a new geographic record for the species, and a key to *Kroyeria* males. *Crustaceana*, **74**, 883-894.

Dippenaar, S.M., 2004. Reported siphonostomatoid copepods parasitic on marine fishes of South Africa. *Crustaceana*, **77**, 1281-1328.

Dippenaar, S.M., Olivier, P.A.S. & Benz, G.W., 2004. *Schistobrachia jordaanae* n. sp. (Copepoda: Siphonostomatoida: Lernaeopodidae) from gill filaments of a Diamond Ray (*Gymnura natalensis*) captured in the Indian Ocean and a key to the species of *Schistobrachia*, *Dendrapta* and *Brianella*. *Journal of Parasitology*, **90**, 481-484.

Dippenaar, S.M. & Jordaan, B.P., 2006. *Nesippus orientalis* Heller, 1868 (Pandaridae: Siphonostomatoida): descriptions of the adult, young and immature females, a first description of the male and aspects of their functional morphology. *Systematic Parasitology*, **65**, 27-41.

Etchegoin, J.A., Ivanov, V.A. & Timi, J.T., 2001. Resurrection of *Perissopus galeorhini* (Yamaguti, 1936), with notes on the genus *Perissopus* Steenstrup & Lütken, 1861 (Copepoda: Pandaridae) parasitic on sharks. *Systematic Parasitology*, **50**, 31-39.

Dujardin, F., 1845. *Histoire Naturelle des Helminthes ou Vers Intestinaux*. Paris: Librairie Encyclopédique de Roret.

Galigher, A.E. & Kozloff, E.N., 1971. *Essentials of Practical Microtechnique* (2nd edition). Philadelphia: Lea & Febiger.

Gibson, D.I., 1976. Monogenea and Digenea of fishes. *Discovery Reports*, **36**, 115-266.

Gibson, D.I. & Bray, R.A., 1977. The Azygiidae, Hirudinellidae, Ptychogonimidae, Sclerodistomidae and Syncoeliidae of fishes from the north-east Atlantic. *Bulletin of the British Museum of Natural History (Zoology)*, **32**, 167-245.

Gibson, D.I., Jones, A. & Bray, R.A. (eds.), 2003. *Keys to the Trematoda*. London, U.K.: CAB International and The Natural History Museum.

Gilbert, P.W., Mathewson, R.F. & Rall, D.P., 1967. *Sharks, Skates and Rays*. Baltimore: Johns Hopkins University Press.

González, M.T., Barrientos, C. & Moreno, C.A., 2006. Biogeographical patterns in endoparasite communities of a marine fish (*Sebastes capensis* Gmelin) with extended range in the Southern Hemisphere. *Journal of Biogeography*, **33**, 1086-1095.

Grabda, J., 1991. *Marine Fish Parasitology: an Outline*. Warszawa: PWN – Polish Scientific Publishers.

Grobler, N.J., van As, J.G. & Olivier, P.A.S., 2002. Description of the previously unknown male of *Caligus mortis* Kensley, 1970 (Copepoda: Caligidae), parasite of intertidal fish from South Africa. *Folia Parasitologica*, **49**, 131-136.

Grobler, N.J., van As, J.G. & Olivier, P.A.S., 2003a. Additional morphological information on two species of *Caligus* (Copepoda: Caligidae) parasitic on South African marine and estuarine fish. *African Zoology*, **38**, 139-143.

Grobler, N.J., Christison, K.W., Olivier, P.A.S. & Van As, J.G., 2003b. Observations on the development of *Udonella caligorum* Johnston, 1835 (Monogenea: Polyonchoinea) on a parasitic copepod species of *Caligus* (Copepoda: Caligidae) collected from Lake St Lucia, South Africa. *African Zoology*, **38**, 393-396.

Grobler, N.J., Van As, J.G. & Olivier, P.A.S., 2004. New morphological information on the parasitic copepods *Caligus epinepheli* Yamaguti, 1936 and *Caligus rotundigenitalis* Yu, 1933 (Copepoda, Caligidae) from South Africa. *Crustaceana*, **77**, 187-196.

Hadfield, K.A. & Smit, N.J., 2008. Description of a new gnathiid, *Afrignathia multicavea* gen. et sp. n. (Crustacea: Isopoda: Gnathiidae), from South Africa. *African Zoology*, **43**, 81-89.

Hadfield, K.A., Smit, N.J. & Oldewage, A., 2008. A new species of gnathiid isopod, *Gnathia pilosus* sp. nov. (Crustacea, Isopoda, Gnathiidae) from the East Coast of South Africa. *Zootaxa*, **1894**, 23-41.

Hayes, P.M., Smit, N.J., Seddon, A.M., Wertheim, D.F. & Davies, A.J., 2006. A new fish haemogregarine from South Africa and its suspected dual transmission with trypanosomes by a marine leech. *Folia Parasitologica*, **53**, 241-248.

Hayes, P.M., Smit, N.J., & Davies, A.J., 2007. Pathology associated with parasitic juvenile gnathiids feeding on the puffadder shyshark, *Haploblepharus edwardsii* (Voight). *Journal of Fish Diseases*, **30**, 55-58.

Heemstra, P. & Heemstra, E., 2004. *Coastal Fishes of Southern Africa*. Grahamstown: South African Institute for Aquatic Biodiversity and National Inquiry Service Centre.

Hemmingsen, W., Jansen, P.A. & MacKenzie, K., 2005. Crabs, leeches and trypanosomes: an unholy trinity? *Marine Pollution Bulletin*, **50**, 336-339.

Heupel, M.R. & Bennett, M.B., 1999. The occurrence, distribution and pathology associated with gnathiid isopod larvae infecting the epaulette shark, *Hemiscyllium ocellatum*. *International Journal for Parasitology*, **29**, 321-330.

Homes, J.C. & Price, P.W., 1986. Communities of parasites. In: *Community Ecology: Patterns and Processes*. Anderson, D.J. & Kikkawa, J. (eds). Oxford: Blackwell Scientific Publications.

Honma, Y. & Chiba, A., 1991. Pathological changes on the branchial wall of stingrays, *Dasyatis* spp., associated with the presence of juvenile gnathiids (Isopoda, Crustacea). *Gyobyo Kenkyu*, **26**, 9-16.

Honma, Y., Tsunaki, S., Chiba, A. & Ho, J., 1991. Histological studies on the juvenile gnathiids (Isopoda, Crustacea) parasitic on the branchial wall of the stingray, *Dasyatis akajei*, in the Sea of Japan. *Report of the Sado Marine Biological Station, Niigata University*, **21**, 37-47.

Johnston, T.H., 1934. Some Australian anaporrhutine trematodes. *Transactions of the Royal Society of South Australia*, **58**, 139-148.

Jones, S.R.M. & Woo, P.T.K., 1991. Development and infectivity of *Trypanosoma phaleri* in leech and fish hosts. *Canadian Journal of Zoology*, **69**, 1522-1529.

Kabata, Z., 1979. *Parasitic Copepods of British Fishes*. London: The Ray Society.

Karlsbakk, E., Haugen, E. & Nylund, A., 2005. Morphology and aspects of growth of a trypanosome transmitted by the marine leech *Johanssonia arctica* (Piscicolidae) from Northern Norway. *Folia Parasitologica*, **52**, 209-215.

Kensley, B. & Grindley, J.R., 1973. South African parasitic Copepoda. *Annals of the South African Museum*, **62**, 69-130.

Khan, R.A., 1972. Developmental stages of *Haemogregarina delagei* Laveran and Mesnil in an elasmobranch, *Raja radiate* Donovan. *Canadian Journal of Zoology*, **50**, 906-907.

Khan, R.A. & Lacey, D., 1986. Effect of concurrent infections of *Lernaeocera branchialis* (Copepoda) and *Trypanosoma murmanensis* (Protozoa) on Atlantic cod, *Gadus morhua*. *Journal of Wildlife Diseases*, **22**, 201-208.

Khan, R.A., Barrett, M. & Murphy, J., 1980. Blood parasites of fish from the northwestern Atlantic Ocean. *Canadian Journal of Zoology*, **58**, 770-781.

Kirmse, P., 1979. Redescription of the life cycle of *Haemogregarina simondi* (Laveran and Mesnil, 1901) in its vertebrate host the marine fish *Solea solea* (Linnaeus). *Zeitschrift für Parasitenkunde*, **59**, 141-150.

Klimpel, S., Seehagen, A. & Palm, H.W., 2003. Metazoan parasites and feeding behaviour of four small-sized fish species from the central North Sea. *Parasitology Research*, **91**, 290-297.

Laird, M., 1951. Studies on the trypanosomes of New Zealand fish. *Proceedings of the Zoological Society of London*, **121**, 285-309.

Laird, M. & Bullock, W., 1969. Marine fish haematozoa from New Brunswick and New England. *Journal of the Fisheries Research Board of Canada*, **26**, 1075-1102.

*Laveran, A., 1908. Sur une haemogregarine, un trypanosome et un spirille trouves dans le sang d’un requin. *Bulletin de la Société de Pathologie Exotique*, **1**, 148-150.

Laveran, A. & Mesnil, F., 1902. Des trypanosomes des poissons. *Archiv für Protistenkunde*, **1**, 496-498.

Lechanteur, Y.A.R.G. & Griffiths, C.L., 2002. Diets of common suprabenthic reef fish in False Bay, Cape Town. *African Zoology*, **38**, 213-227.

*Linstow, O., 1901. Beobachtungeng an Helminthen des Senkenbergischen Naturhistorischen Museums des Breslauer zoologischen Instituts und anderen. *Archiv für Mikroskopie, Anatomie und Entwicklungsgestchichte*, **58**, 182-198.

*Lloyd, J.H., 1920. Some observations on the structure and life-history of the common nematode of the dogfish (*Scyllium canicula*). *Proceedings of the Zoological Society of London*, **1920**, 449-456.

Lom, J. & Dykova, I., 1992. Protozoan parasites of fishes. *Developments in Aquaculture and Fisheries Science*, **26**, 25-119.

*Looss, A., 1902. Ueber neue und bekannte Trematoden aus Seeschildkröten. Nebst Erörterungen zur Systematik und Nomenclatur. Zoologischen Jahrbüchern. *Abtheilung für Systematik, Geographie und Biologie der Thiere*, **16**, 411-894 + 21-31 pls.

*Lopez, C., 1888. Un Distoma probabilmente nuovo. *Atti della Società Toscana di Scienze Naturali Pisa*, *processi verbali*, **6**, 137-138.

MacKenzie, K., 1999. Parasites as pollution indicators in marine ecosystems: a proposed early warning system. *Marine Pollution Bulletin*, **38**, 955-959.

Mackerras, M.J. & Mackerras, I.M., 1961. The haematozoa of Australian frogs and fish. *Australian Journal of Zoology*, **9**, 123-139.

Markell, E.K., 1953a. *Nagmia floridensis*, n. sp., an Anaporrhutine trematode from the coelom of the sting ray *Amphotistius sabinus*. *Journal of Parasitology*, **39**, 45-51.

Markell, E.K., 1953b. Further observations on the anatomy of the gorgoderid trematode *Probolitrema californiense*. *Transactions of the American Microscopical Society*, **72**, 68-77.

Markell, E.K., 1956. *Probolitrema mexicana*, n. sp., an anaporrhutine trematode from elasmobranchs off Baja California. *Journal of Parasitology*, **42**, 56-59

Mann, B.Q. (Ed.), 2000. *Southern African Marine Linefish Status Reports. Oceanographic Research Institute, Special Publication*, **7**. Durban: South African Association for Marine Biological Research.

McKiernan, J.P., Grutter, A.S. & Davies, A.J., 2005. Reproductive and feeding ecology of parasitic gnathiid isopods of epaulette sharks (*Hemiscyllium ocellatum*) with consideration of their role in the transmission of a haemogregarine. *International Journal for Parasitology*, **35**, 19-27.

Minchin, E.A. & Woodcock, H.M., 1910. Observations on certain blood parasites of fishes occurring at Rovigno. *Quarterly Journal of Microscope Science*, **55**, 113-154.

Molyneux, D.H., 1977. Vector relationships in the Trypanosomatidae. *Advances in Parasitology*, **15**, 1-82.

Morand, S., Poulin, R., Rohde, K. & Hayward, C., 1999. Aggregation and species coexistence of ectoparasites of marine fishes. *International Journal for Parasitology*, **29**, 663-672.

Moravec, F., Van As, J.G. & Dykova, I., 2002. *Proleptus obtusus* Dujardin, 1845 (Nematoda: Physalopteridae) from the puffadder shyshark *Haploblepharus edwarsii* (Scyliorhinidae) from off South Africa. *Systematic Parasitology*, **53**, 169-173

Morillas, J., George-Nascimento, M., Valeria, H. & Khan, R.A., 1987. *Trypanosoma humboldti* n. sp. from the Chilean Catshark, *Schroederichthys chilensis* (Guichenot, 1848). *Journal of Protozoology*, **34**, 342-344.

Müller, J.F., 1925. Some new features of nematode morphology in *Proleptus obtusus* Dujardin. *Journal of Parasitology*, **12**, 84-90.

Muñoz, G. & Cribb, T.H., 2005. Infracommunity structure of parasites of *Hemigymnus melapterus* (Pisces: Labridae) from Lizard Island, Australia: the importance of habitat and parasite body size. *Journal of Parasitology*, **91**, 38-44.

Neumann, R.D., 1909. Studien uber protozoische Parasitien im Blut von Meeresfischen. *Zeitschrift für Hygiene und Infectionskrankheiten*, **63**, 1-112.

*Ofenheim, E.von, 1900. Ueber eine neue Distomidengattung. *Zeitschrift für Naturwissenschafting*, **73**, 146-186.

Oldewage, W.H., 1992. Description of *Perissopus oblongus* (Wilson, 1908) (Copepoda, Pandaridae) from Southern African sharks with a first description of the male. *Crustaceana*, **63**, 44-50.

Oldewage, W.H., 1993. Three species of piscine parasitic copepods from Southern African coastal waters. *South African Journal of Zoology*, **28**, 113-121.

Payne, A.I.L. & Crawford, R.J.M. (Eds.), 1989. *Oceans of Life off Southern Africa*. Vlaeberg: Vlaeberg Publishers CC.

Petter, A.J. & Radujković, B.M., 1989. Parasites des poissons marins du Montenegro: nematodes. *Acta Adriatica*, **30**, 195-236.

Polyanski, Y.I., 1961. Ecology of parasites of marine fishes. In: Dogiel, V.A., Petrushevski, K.G. & Polyanski, Y.I. (eds), *Parasitology of Fishes*. Edinburgh: Oliver & Boyd.

Poulin, R., 1997. Species richness of parasite assemblages: evolution and patterns. *Annual Review of Ecology and Systematics*, **28**, 341-358.

Poulin, R., 1998. *Evolutionary Ecology of Parasites: from Individuals to Communities*. London: Chapman & Hall.

Poulin,R. & Rhode, K., 1997. Comparing the richness of metazoan ectoparasite communities of marine fishes: controlling for host phylogeny. *Oecologia*, **110**, 278-283.

Presnell, J.K., Schreibman, M.P. & Humason, G.L., 1997. *Humason's Animal Tissue Techniques*. Baltimore: Johns Hopkins University Press.

Pulsford, A., 1984. Preliminary studies on trypanosomes from the dogfish, *Scyliorhinus canicula* L. *Journal of Fish Biology*, **24**, 671-682.

Reed, C.C., Basson, L., van As, L.L. & Dykova, I., 2007. Four new myxozoans (Myxosporea: Bivalvulida) from intertidal fishes along the south coast of Africa. *Folia Parasitologica*, **54**, 283-292.

Rhode, K., 1982. *Ecology of Marine Parasites*. St Lucia: University of Queensland Press.

Roberts, L.S. & Janovy, J., 2000. *Schmidt and Roberts' Foundations of Parasitology* 6th Edition. Singapore: McGraw-Hill Book Co.

Rodrigues, H.O., Varela, M.C., Rodrigues, S.S. & Cristafaro, R., 1975. Novas contribuicão para o estudo dos nematódeos de peixes do oceano Atlantico – costa continental Portuguesa e costa do Norte da África. *Memórias do Instituto Oswaldo Cruz*, **73**, 127-134.

Rokicki, J. & Bychawska, D., 1991. Parasitic copepods of Carcharhinidae and Sphyridae (Elasmobranchia) from the Atlantic Ocean. *Journal of Natural History*, **25**, 1439-1448.

Sanmartín, M.L., Alvarez, M.F., Peris, D., Iglesias, R. & Leiro, J., 2000. Parasite communitry study of the undulate ray *Raja undulata* in the Ría of Muros (Galicia, northwest Spain). *Aquaculture*, **184**, 189-201.

Saunders, D.C., 1958. The occurrence of *Haemogregarina bigemina* Laveran and Mesnil, and *H. dasyatis*, n. sp. in marine fish from Bimini, Bahamas, B.W.I. *Transactions of the American Microscopic Society*, **77**, 402-412.

Sepúlveda, F., Marin, S.L. & Carvajal, J., 2006. Metazoan parasites in wild fish and farmed salmon from aquaculture sites in southern Chile. *Aquaculture*, **235**, 89-100.

*Seurat, L.G., 1919. Sur la morphologie du *Proleptus obtusus* Duj. (Acuariidae). *Bulletin du Muséum National d'Histoire Naturelle, Paris*, **25**, 166-172.

Siddall, M.E., 1995. Phylogeny of Adeleid blood parasites with a partial systematic revision of the Haemogregarine Complex. *Journal of Eukaryotic Microbiology*, **42**, 116-125.

Siddall, M.E. & Desser, S.S., 1990. Gametogenesis and sporogonic development of *Haemogregarina balli* (Apicomplexa: Adeleina: Haemogregarinidae) in the leech *Placobdella ornata*. *Journal of Protozoology*, **37**, 511-520.

Siddall, M.E. & Desser, S.S., 1992. Ultrastructure of gametogenesis and sporogony of *Haemogregarina* (sensu lato) *myoxochephali* (Apicomplexa: Adeleina) in the marine leech *Malmania scorpii*. *Journal of Protozoology*, **39**, 545-554.

Siddall, M.E. & Desser, S.S., 1993. Cytopathological changes induced by *Haemogregrina myoxocephali* in its fish host and leech vector. *Journal of Parasitology*, **79**, 297-301.

Siddall, M.E. & Desser, S.S., 2001. Developmental stages of *Haemogregarina delagei* in the leech *Oxytonostoma typica*. *Canadian Journal of Zoology*, **79**, 1897-2001.

Sindermann, C.J., 1990. *Principal Diseases of Marine Fish and Shellfish* Vol. 1*, Diseases of Marine Fish*. New York: Academic Press.

*Skryabin, K.I. & Sobolev, A.A., 1964. [*Spirurata of animals and man and the diseases caused by them*. Part 2. *Physalopteroidea*]. *Osnovy Nematodologii*. Moscow: Nauka, **12**.

Smit, N.J., 2000. The biology of gnathiid isopod parasites and their role as vectors of fish blood parasites in South Africa. PhD thesis, University of the Orange Free State, Bloemfontein.

Smit, N.J. & Davies, A.J., 2001. An encapsulated haemogregarine from the evileye pufferfish in South Africa. *Journal of the Marine Biological Association of the United Kingdom*, **81**, 751-754.

Smit, N.J. & Basson, L., 2002. *Gnathia pantherina* sp. n. (Crustacea: Isopoda: Gnathiidae), a temporary ectoparasite of some elasmobranch species from southern Africa. *Folia Parasitologica*, **49**, 137-151.

Smit, N.J., Eiras, J.C., Ranzani-Paiva, M.J.T. & Davies, A.J., 2002. A *Desseria* species from flathead mullet in South Africa. *Journal of the Marine Biological Association of the United Kingdom*, **82**, 4143.1-4143.2.

Smit, N.J., Van As, J.G. & Davies, A.J., 2003a. Taxonomic re-evaluation of the South African fish haemogregarine, *Desseria fragilis*. *Journal of Parasitology*, **89**, 151-153.

Smit, N.J., Basson, L. & Van As, J.G., 2003b. Life cycle of the temporary fish parasite, *Gnathia africana* (Crustacea: Isopoda: Gnathiidae). *Folia Parasitologica*, **50**, 135-142.

Smit, N.J. & Davies, A.J., 2004. The curious life-style of the parasitic stages of gnathiid isopods. *Advances in Parasitology*, **58**, 289-391.

Smit, N.J. & Davies, A.J., 2005. Intraerythrocytic merogony in the development of *Haemogregarina koppiensis* (Apicomplexa: Adeleorina: Haemogregarinidae) *Folia Parasitologica*, **52**, 277-278.

Smit, N.J. & Davies, A.J., 2006. A new haemogregarine from *Zeus capensis* from the South and West coasts of South Africa. *Journal of the Marine Biological Association of the United Kingdom*, **86**, 1477-1480.

Smith, M.M. & Heemstra, P.C., 1986. *Smith's Sea Fishes*. Cape Town: Struik Publishers.

Specian, R.D., Ubelaker, J.E. & Dailey, M.D., 1975. *Neoleptus* gen. n. and a revision of the genus *Proleptus* Dujardin, 1845. *Proceedings of the Helminthological Society of Washington*, **42**, 14-21.

Stevens, J.R. & Gibson, W., 1999. The molecular evolution of trypanosomes. *Parasitology Today*, **15**, 432-437.

Stunkard, H.W., 1935. A new trematode, *Probolitrema californiense*, from the coelom of the sting ray *Myliobatis californicus*. *Journal of Parasitology*, **21**, 353-364.

Turpie, J.K., Beckley, L.E. & Katua, S.M., 2000. Biogeography and the election of priority areas for conservation of South African coastal fishes. *Biological Conservation*, **92**, 59-72.

Vaughan, D.B., Chisholm, L.A. & Christison, K.W., 2008. Overview of South African *Dendromonocotyle* (Monogenea: Monocotylidae), with descriptions of two new species from stingrays (Dasyatidae) kept in public aquaria. *Zootaxa*, **1826**, 26-44.

*Von Prowazek, S., 1910. Parasitische protozoen aus Japan, gesammelt von Herrn Dr. Mine in Fukuoka. *Archiv fur Schiffs- und TropenHygiene*, **14**, 296.

Wenyon, C.M., 1926. *Protozoology: A Manual for Medical Men, Veterinarians and Zoologists*, Vol. 1. Bailliere, Tindall and Cox, London.

Williams, H.H., MacKenzie, K. & McCarthy, A.M., 1992. Parasites as biological indicators of the population biology, migrations, diet, and phylogenetics of fish. *Reviews in Fish Biology and Fisheries*, **2**, 144-176.

Wilson, C.B., 1908. North American parasitic copepods. A list of those found upon the fishes of the Pacific coast, with descriptions of new genera and species. *Proceedings of the United States National Museum*, **35**, 131-181.

Wilson, C.B., 1912. Descriptions of new species of parasitic copepods in the collections of the U.S. National Museum. *Proceedings of the United States National Museum*, **42**, 233-243.

*Woolcock, V., 1935. Digenetic parasites from some Australian fishes. *Parasitology*, **27**, 309-331

Yamaguti, S., 1936. Parasitic copepods from fishes of Japan, 3. *Caligoida*, **2**, 1-21.

Zar, J.H., 1996. *Biostatistical Analysis*. New Jersey: Prentice-Hall.

* Not seen in original